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(21) 出願番号	特願2003-509084 (P2003-509084)	(71) 出願人	501243030
(86) (22) 出願日	平成14年7月1日 (2002.7.1)		ザ ボード オブ トラスティーズ オブ
(85) 翻訳文提出日	平成15年12月25日 (2003.12.25)		ザ レランド スタンフォード ジュニア
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(31) 優先権主張番号	60/302,344		カミーノ レアル 1 7 0 5
(32) 優先日	平成13年6月29日 (2001.6.29)	(74) 代理人	100078282
(33) 優先権主張国	米国 (US)		弁理士 山本 秀策
		(74) 代理人	100062409
			弁理士 安村 高明
		(74) 代理人	100113413
			弁理士 森下 夏樹

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(54) 【発明の名称】 T細胞調節遺伝子およびその使用方法

(57) 【要約】

免疫機能の調節および細胞の生存に関連するタンパク質の、遺伝子座および対応するファミリーを提供する。これらの遺伝子は、保存された I g V ドメインおよびムチンドメインを有する、細胞表面タンパク質をコードする。T I Mファミリーを含有する遺伝子座は、一般的に、喘息を含む免疫不全に関連する。さらに、このT I M遺伝子ファミリーは、ヒトの第5染色体の領域内に位置付けられ、これは、悪性腫瘍および骨髄形成異常症候群において、共通して欠失される。遺伝子配列における多型は、気道の過剰応答性およびアレルギー性炎症の発達、ならびにT細胞の I L - 4 および I L - 1 3 の産生に関連する。これらのタンパク質は、ヒトA型肝炎細胞レセプターの h H A V c r - 1 を含む。

【特許請求の範囲】

【請求項 1】

哺乳動物の T I M 遺伝子配列を含む、単離された核酸分子。

【請求項 2】

前記哺乳動物の T I M 遺伝子配列が、T I M-1、T I M-3 および T I M-4 の対立遺伝子 2、3、4、5 および 6 からなる群より選択されるヒト配列である、請求項 1 に記載の単離された核酸分子。

【請求項 3】

前記哺乳動物の T I M 遺伝子配列が、T I M-1、T I M-2、T I M-3 および T I M-4 からなる群より選択されるマウス配列である、請求項 1 に記載の単離された核酸分子 10。

【請求項 4】

配列番号 2、4、6、8、10、12、14、16、18、20、22、24、26、28、30、32、34、または 36 のうちのいずれ 1 つに記載される、少なくとも 20 ヌクレオチドの連続配列を含む、単離された核酸。

【請求項 5】

配列番号 2、4、6、8、10、12、14、16、18、20、22、24、26、28、30、32、34、または 36 のうちのいずれ 1 つに記載される配列を含む、単離された核酸。

【請求項 6】

T I M 遺伝子多型の検出のためのプローブを含む、請求項 1 に記載の単離された核酸分子 20。

【請求項 7】

請求項 6 に記載のプローブの 2 つ以上を含む、オリゴヌクレオチドのアレイ。

【請求項 8】

請求項 1 に記載の核酸組成物を含む細胞。

【請求項 9】

少なくとも 50 重量%のタンパク質を含む精製ポリペプチド組成物であって、該タンパク質が、請求項 1 に記載の核酸またはそのフラグメントの産物として存在する、組成物。

【請求項 10】

前記ポリペプチドが、配列番号 1、3、5、7、9、11、13、15、17、19、21、23、25、27、29、31、33、および 35 に記載の配列を含む、請求項 9 に記載の精製ポリペプチド。 30

【請求項 11】

哺乳動物の T I M ポリペプチドに特異的な、抗体。

【請求項 12】

個体において喘息に対する素因を検出するための方法であって、該方法が、以下：少なくとも 1 つの素因性の T I M の多型の存在について該個体を分析する工程であって、ここで、該素因性の多型の存在が、該喘息に対する罹患率の増大を示す、工程、を包含する、方法。 40

【請求項 13】

前記分析工程が、前記個体のゲノム D N A または m R N A と、請求項 6 に記載の 1 つまたは複数のプローブとの間の特異的な結合の検出を包含する、請求項 12 に記載の方法。

【請求項 14】

前記分析工程が、前記個体由来の細胞またはタンパク質と、請求項 11 に記載の抗体との間の特異的な結合の検出を包含する、請求項 12 に記載の方法。

【請求項 15】

T I M 遺伝子のノックアウトを含む、T I M 遺伝子機能についての非ヒトトランスジェニック動物モデル。

【請求項 16】

T I M機能を調節する、生物学的に活性な薬剤についてのスクリーニングの方法であって、該方法が、以下：

以下のいずれか1つと、生物学的に活性な薬剤の候補を混ぜる工程：

- (a) 哺乳動物のT I Mポリペプチド；
 - (b) 哺乳動物のT I Mポリペプチドをコードする核酸を含有する、細胞；または
 - (c) T I M遺伝子機能についての非ヒトトランスジェニック動物モデル；および
- T I M機能に対する該薬剤の効果を決定する工程、
を包含する、方法。

【請求項17】

前記生物学的に活性な薬剤が、T I Mの発現を下方制御または上方制御する、請求項16 10
に記載の方法。

【請求項18】

前記生物学的に活性な薬剤が、前記T I Mポリペプチドの活性を阻害するかまたは上昇させる、請求項16に記載の方法。

【請求項19】

個体において免疫不全を診断または段階付けするための方法であって、該方法が、以下：
少なくとも1つの素因性のT I Mの多型の存在について該個体を分析する工程であって、
ここで、該素因性の多型の存在が、該免疫不全に対する罹患率の増大を示す、工程、
を包含する、方法。

【請求項20】

個体において癌を診断または段階付けするための方法であって、該方法が、以下：
少なくとも1つの素因性のT I Mの多型の存在について該個体を分析する工程であって、
ここで、該素因性の多型の存在が、癌治療に対する耐性の増大を示す、工程、
を包含する、方法。

【請求項21】

個体において癌を診断または段階付けするための方法であって、該方法が、以下：
少なくとも1つの素因性のT I M-1発現プロファイルの存在について該個体を分析する
工程であって、ここで、該T I M-1の発現プロファイルが、癌治療に対する耐性の増大
を示す、工程、
を包含する、方法。

【請求項22】

個体において癌を診断または段階付けするための方法であって、該方法が、以下：
少なくとも1つの素因性のT I M-4発現プロファイルの存在について該個体を分析する
工程；ここで、該T I M-4の発現プロファイルが、癌治療に対する耐性の増大を示す、
工程、
を包含する、方法。

【請求項23】

個体において免疫学的障害を処置するための方法であって、該方法が、以下：
T I Mポリペプチドの機能を調節する薬剤を、該個体に投与する工程、
を包含する、方法。

【請求項24】

前記T I Mポリペプチドが、T I M-1またはT I M-4である、請求項23に記載の方法。

【請求項25】

前記薬剤が、T I M-1に特異的な抗体または抗体フラグメントである、請求項24に記載の方法。

【請求項26】

前記薬剤が、T I M-1機能を阻害する化合物である、請求項24に記載の方法。

【請求項27】

前記免疫学的障害が喘息である、請求項24に記載の方法。

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【請求項 28】

前記免疫学的障害がアレルギーである、請求項 24 に記載の方法。

【請求項 29】

前記免疫学的障害が湿疹である、請求項 24 に記載の方法。

【請求項 30】

前記免疫学的障害が自己免疫疾患である、請求項 24 に記載の方法。

【請求項 31】

前記薬剤が、TIM-4 に特異的な抗体または抗体フラグメントである、請求項 23 に記載の方法。

【請求項 32】

前記薬剤が、TIM-4 の機能を阻害する化合物である、請求項 30 に記載の方法。

【請求項 33】

個体において悪性腫瘍を処置するための方法であって、該方法が、以下：
TIMポリペプチドの機能を調節する薬剤を、該個体に投与する工程、
を包含する、方法。

【請求項 34】

前記TIMポリペプチドがTIM-1 である、請求項 33 に記載の方法。

【請求項 35】

前記薬剤が、TIM-1 に特異的な抗体または抗体フラグメントである、請求項 34 に記載の方法。

【請求項 36】

前記薬剤が、TIM-1 の機能を阻害する化合物であり、そして化学療法剤または放射線治療の効果を増強する、請求項 35 に記載の方法。

【請求項 37】

前記TIMポリペプチドがTIM-4 である、請求項 33 に記載の方法。

【請求項 38】

前記薬剤が、TIM-4 に特異的な抗体または抗体フラグメントである、請求項 37 に記載の方法。

【請求項 39】

前記薬剤が、TIM-4 の機能を阻害する化合物であり、そして化学療法剤または放射線治療の効果を増強する、請求項 38 に記載の方法。

【発明の詳細な説明】

【背景技術】

【0001】

(発明の背景)

免疫系の驚異的な複雑性は、その最大の威力である。10¹² - 10¹⁴ の潜在的な抗体特異性、様々な調節細胞およびエフェクター細胞間の精密な相互作用、MHC 抗原によるT細胞応答の制限；これら全てが宿主の感染性因子および外来性と考えられる他の抗原に対して効率的に応答する能力に寄与する。しかし、この多様性はその欠点を有する。誤りが起こる：応答の標的が正常な自己タンパク質であることが判明し得る；炎症性応答が誤調節される；そして正常な応答が、移植片および移植細胞に対して望ましくなく向けられる。これらの状況下で、その系の複雑性は診断および治療を非常に困難にする。

【0002】

免疫応答の間にCD4⁺ T細胞によって産生されるサイトカインのプロフィールが、免疫応答の結果を発生および調節するエフェクター機能の性質を決定する。Th1-優位の応答におけるIL-2 およびIFN- γ の産生は、激しい細胞媒介性免疫、IgG2a の誘導およびIgE 合成の阻害、および細胞内病原体に対する抵抗性と関連する。対照的に、Th2-優位の応答におけるIL-4、IL-5、およびIL-10 の産生は、体液性免疫および自己免疫病理からの保護と関連する。アレルゲン特異的CD4⁺ T細胞によるTh2-サイトカインの過剰産生は、アレルギー疾患および喘息の発生を引き起こし得、一

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方 T h 1 細胞は、様々な炎症誘発性疾患と関連していた。

【0003】

免疫関連疾患に対する1つのアプローチは、免疫療法である。免疫療法は、適当に用いた場合に有効であることが証明され、そして免疫学的介入の進歩は有効性をさらに改善すると希望される。代替のアプローチは、サイトカインを使用して免疫応答をシフトさせようと試みた。マクロファージおよび樹状細胞によって産生されるヘテロダイマーのサイトカイン、I L - 1 2 は、未処置およびメモリー C D 4 ⁺ T 細胞において、T h 1 サイトカイン合成の発生を強力に推進する。I L - 1 3 および I L - 4 のような他のサイトカインが、T 細胞の T h 2 型への分化と関連していた。

【0004】

喘息、アレルギー性鼻炎、およびアトピー性皮膚炎を含むアトピーは、遺伝的に感受性の個体において、環境的に誘発された免疫応答の結果として起こる、複雑な形質である。全てのアトピー性疾患の罹患率は、過去20年間の間に産業化諸国において劇的に増加した。喘息は小児期の最もよくある慢性疾患であり、そして合衆国において1500万人以上に影響を与え、1年あたり110億ドルを越える直接治療コストをもたらす。疫学的研究が、喘息罹患率の増加は、産業化社会における衛生状態の変化および感染（例えば結核またはA型肝炎）の頻度低下に起因することを示唆した。しかし、喘息罹患率の増加を引き起こす特定の分子的経路、および喘息感受性を与える遺伝的多型はあまり理解されていない。

【0005】

喘息の発現は、非相加的な方法でお互いに相互作用する、複数の環境的および遺伝的因子によって影響され、喘息感受性遺伝子の同定を複雑にしている。喘息感受性は、いくつかの染色体領域と連結されたが、分解能は5-10cMより低く、そこには通常数百の候補遺伝子が存在する。さらに、どの単一遺伝子における遺伝的変異の効果も、おそらく喘息の病因全体では中程度の効果しか有さないもので、そして遺伝子-遺伝子および遺伝子-環境相互作用が分析を混乱させるので、ポジショナルクローニングに対して応答性の領域に推定感受性遺伝子を位置決めすることは、精密化が困難であることが判明した。それにもかかわらず、喘息感受性は第5染色体、第6染色体、第11染色体、第14染色体、および第12染色体に連結した。これらのうち、染色体5q23-35が、それはI L - 9、I L - 1 2 p 4 0、β-アドレナリンレセプター、およびI L - 4、I L - 5およびI L - 1 3 の遺伝子を含むI L - 4 サイトカインクラスターを含む多くの候補遺伝子（11、12、13、14、15、16、17、18）を含むので、最も注目を集めた。しかし、5qの連結領域の大きなサイズがその分析を複雑にし、そしてこの部位からの喘息遺伝子はまだ決定的に同定されていない。

【0006】

（関連する出版物）

ヒトA型肝炎ウイルス細胞レセプターの遺伝配列は、Genbank、登録番号XM__011327において見出し得る。関連する配列が、Genbank、登録番号BAB55044において提供される。Monneyら（2002）Nature 415:436は、T h 1 細胞に発現する細胞表面分子を記載する。米国特許第5721351号、同第6204371号、同第6288218号は、マウスT I M - 3 対立遺伝子に対応する配列に関連する。

【発明の開示】

【課題を解決するための手段】

【0007】

（発明の要旨）

免疫機能および細胞の生存に関連するポリペプチドをコードする遺伝子ファミリーの遺伝配列が提供される。これらの遺伝子は、本明細書中でT細胞免疫グロブリンドメインタンパク質およびムチンドメイン（T I M）タンパク質と呼ぶ、保存されたI g Vおよびムチンドメインを有する細胞表面分子をコードする。T I Mファミリーを含む遺伝子座は、喘

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息を含む免疫機能不全と遺伝的に関連する。さらに、TIM遺伝子ファミリーは、悪性腫瘍および脊髄形成異常症候群において通常欠失しているヒト第5染色体の領域に位置している。TIM-1、TIM-3およびTIM-4において多型が同定され、それはTh1/Th2分化および気道過剰応答性(AHR)と関連し得る。

【0008】

核酸組成物を使用してコードされたタンパク質を産生し、それを機能的研究のために、治療薬として、および関連する生理学的経路の研究において採用し得る。核酸、抗体などを含むTIM特異的結合因子は、アトピーおよび喘息に対する遺伝的感受性を決定する診断薬として、そして癌治療に対する腫瘍抵抗性を評価する診断薬として有用である。TIM阻害薬は、免疫機能不全、および悪性腫瘍を含む細胞生存の障害の処置において治療薬として有用である。

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【0009】

(実施形態の詳細な説明)

喘息に対する感受性を含む、免疫機能に関連する遺伝配列が提供される。マウスTim-1、Tim-2、Tim-3およびTim-4の配列、およびヒトの対応する配列が、本明細書中で提供される。主要な多型の配列も提供される。遺伝配列は、欠失を含むこれらの多型は、スプライシング改変体ではなく、真の多型であることを示す。TIM-1およびTIM-3配列の改変体は、気道過剰応答性およびアレルギー性T細胞応答と関連しており、そして他の改変体はこれらの応答に対する保護に関連する。

【0010】

TIM遺伝子ファミリーメンバーの細胞外ドメインは、2つのドメイン(IgVドメインおよびムチンドメイン)を含む。このIg/ムチン構造はMAdCAM(粘膜addressin細胞接着分子)においても見出され、これは2つのIgドメインおよび1つのムチンドメインを含む；しかし、TIMとMAdCAMとの間には低いレベルの相同性しか存在しない。同程度の相同性が、TIM-1およびTOSO(NP_005440.1 GI:4885641)、TIM-1のように活性化T細胞に発現し、そしてT細胞をFasによるアポトーシスから保護するタンパク質の間にも存在する。

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【0011】

T細胞は遺伝子のTIMファミリーを発現し、それはCD4T細胞の分化を決定的に調節する。Th1細胞は優先的にTIM-3タンパク質を発現し、一方Th2細胞は選択的にTIM-1タンパク質を発現する。TIM-1は、気道過剰応答性と連結し、そしてTIM-3は自己免疫疾患と連結し、従って分化するリンパ細胞における発現パターンおよびリンパ細胞におけるTIM-1発現の動態は、これら分子の機能を反映する。

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【0012】

本発明の別の局面において、TIM遺伝子座における遺伝子型を決定することによって、個体の喘息およびアトピー性疾患の発生に対する感受性を決定する方法が提供される。スクリーニングは、例えば、本明細書中で提供された、または他の方法で決定されたTIM-1対立遺伝子、TIM-3対立遺伝子またはTIM-4対立遺伝子のいずれか1つにおける多型を分析し得る。そのような多型をスクリーニングする方法、例えばSSCP分析、サイズ多型等が提供される。

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【0013】

本発明の別の局面において、Tim遺伝子またはポリペプチド機能を調節する生物学的に活性な薬剤をスクリーニングする方法が提供され、ここでその方法は、生物学的に活性な候補因子を、以下のもののいずれか1つと組み合わせる工程：(a) Timポリペプチド；(b) Timポリペプチドをコードする核酸を含む細胞；または(c)以下の1つを含むTim遺伝子機能の非ヒトトランスジェニック動物モデル：(i) Tim遺伝子のノックアウト；(ii) 外来性および安定に遺伝するTim遺伝子配列；または(iii) リポーター遺伝子に作動可能に連結したTimプロモーター配列；および当該因子のTim機能に対する効果を決定することを含む。

【0014】

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T I Mポリペプチドの活性を、免疫機能を指示するために調節し得る。T I M-1はT h 2細胞に優先的に発現し、そしてT I M-1活性を調節する薬剤は、アレルギー、喘息等を含むT h 2に関連する疾患の治療に有用である。T I M-3はT h 1細胞において優先的に発現し、そしてT I M-3活性を調節する薬剤は、自己免疫疾患、移植片拒絶等を含む炎症誘発性免疫疾患の治療に有用である。

【0015】

(目的の状態)

アトピー性疾患は、遺伝的に素因のある個体において、環境的に誘発された免疫応答の結果として発現する複雑な遺伝的形質である。アトピー性または非アトピー性の個体はどちらも同じ環境因子にさらされるが、アトピー性個体を非アトピー性個体と区別する遺伝的差異が、ある個体においてアトピー性疾患を引き起こし、気管、皮膚または胃腸管におけるアレルギー性炎症、および上昇した血清I g E、好酸球増加、およびぜん鳴、くしゃみ、またはじんましの症状によって明らかになる。それに加えて、アレルギー性炎症応答は、高レベルのI L-4、I L-5、I L-9およびI L-13を産生するT h 2リンパ球の存在によって特徴付けられ、それは好酸球、肥満細胞、好塩基球およびI g Eを産生するB細胞の増殖、分化、および／または補充を増強する。

【0016】

目的のアレルゲンは、イチゴ、ピーナッツ、乳タンパク質、卵白等のような食物に見出される抗原を含む。他の目的のアレルゲンは、草の花粉、動物の鱗屑、イエダニの糞等のような、様々な空気中で運ばれる抗原を含む。分子的にクローニングされたアレルゲンは、Dermatophagoides pteronyssinus (Der P1) ; ライグラス花粉由来のLol p1-V ; jumper ant Myrmecia pilosula由来の毒液 ; Apis melliferaハチ毒液ホスホリパーゼA2 (PLA₂) および抗原5S ; スズメバチVespula maculifronsおよびクロスズメバチDolichovespula maculata由来のホスホリパーゼを含む多くの昆虫毒液 ; カバ花粉、ブタクサ花粉、Parol (Parietaria officinalisの主要なアレルゲン)、および交差応答性アレルゲンParj1 (Parietaria judaica由来)を含む多くの花粉タンパク質、およびOlea europaea、Artemisia sp.、gramineae等を含む他の大気中花粉を含む。他の目的のアレルゲンは、吸血節足動物、例えばカ (Anopheles sp.、Aedes sp.、Culiseta sp.、Culex sp.) ; ハエ (Phlebotomus sp.、Culicoides sp.) 特にブユ、メクラアブおよびヌカカ ; ダニ (Dermacenter sp.、Ornithodoros sp.、Otobius sp.) ; ノミ、例えばXenopsylla、PulexおよびCtenocephalides felis felis属を含むSiphonaptera目を含む、Dipteraによって引き起こされるアレルギー性皮膚炎を引き起こすものである。特異的アレルゲンは、多糖、脂肪酸部分、タンパク質等であり得る。

【0017】

多くの相互作用／上位アトピー遺伝子における多型は、アレルギー性障害への感受性を増強させると考えられる。ゲノム範囲での走査が、アレルギーおよび喘息の様々なパラメーターを特定の遺伝子の多型DNAマーカー、通常DNAの反復配列 (ジー、トリ、およびテトラヌクレオチドリピートを含むマイクロサテライト) と連結させることによって、責任遺伝子を同定しようとした。これらの研究は、アトピーの病因に関与する可能性があるとしていくつかの染色体領域を同定したが、分解能は5-10 cMにすぎず、そこには典型的には数百の候補遺伝子が存在する。それにもかかわらず、2つ以上のこれらゲノム範囲での走査研究によって、喘息感受性が、染色体5q23-31、染色体6p21、染色体11q13、および染色体12q (9-13) と連結された。

【0018】

喘息は、本明細書中で定義されるように、ある期間にわたる個体における可逆的な気流の

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制限である。喘息は、気道壁における好酸球、肥満細胞、好塩基球、およびCD25⁺Tリンパ球のような細胞の存在によって特徴付けられる。様々な伝達および生物学的エフェクター性質を有するサイトカインの活性に起因して、これらの細胞間には密接な相互作用が存在する。ケモカインは細胞を炎症部位に引き寄せ、そしてサイトカインはそれらを活性化して、炎症および粘膜への損傷を引き起こす。過程の慢性化と共に、基底膜の肥厚および繊維症のような二次的な変化が起こる。その疾患は、様々な刺激に対する気道過剰応答性の増加および気道の炎症によって特徴付けられる。喘息と診断された患者は、一般的にある時間にわたってぜん鳴、喘息発作、およびメタコリンチャレンジに対する陽性応答、すなわち約4mg/ml以下のメタコリンチャレンジに対してPC20を含む複数の徴候を有する。診断ガイドラインを、例えばNational Asthma Education Program Expert Panel Guidelines for Diagnosis and Management of Asthma、National Institutes of Health、1991、Pub. No. 91-3042において見出し得る。

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【0019】

喘息、アレルギー性鼻炎（枯草熱）、アトピー性皮膚炎（湿疹）および食物アレルギーは、同じ家族内で起こる疾患であり、共通の遺伝的メカニズムを示唆する。これらのアトピー性疾患はきわめて罹患率が高く、一般人口の20-40%に影響し、そして主要な公衆衛生問題となっている。これら疾患の経済的コストは莫大である。喘息単独で、1996年の推定健康管理コストは140億ドルであった。それに加えて、全てのアトピー性疾患の罹患率は、まだ明らかでない理由のために、過去20年間の間に産業化諸国において劇的に増加した。数字が最も正確である、産業化諸国における喘息の罹患率は、1982年から倍増し、そして2020年までに罹患率が再び倍増すると予測される。

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【0020】

Th1型T細胞に関連する炎症誘発性疾患は、多発性硬化症、慢性関節リウマチ、I型糖尿病等のような自己免疫疾患を含む。（RA）は世界人口の0.8%に影響する、慢性自己免疫炎症性滑膜炎である。RAの現在の治療は、免疫機能を非特異的に抑制または調節する治療薬を利用する。最近開発されたTNF α アンタゴニストを含むそのような治療は、根本的に治癒力のあるものではなく、そして疾患の活性は治療の中断後すぐに戻る。全身性免疫抑制または調節を引き起こさない、根本的に治癒力のある治療の非常に大きな臨床的ニーズが存在する。

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【0021】

IFN γ を分泌し得るミエリン自己応答性T細胞の定量的増加が、MSおよびEAの病因に関連し、MS患者の末梢血における自己免疫誘導剤／ヘルパーTリンパ球が、MS患者における脱髄過程を開始および／または調節し得ることを示唆する。その明白な疾患は、筋肉の虚弱、腹部反射の喪失、視覚の欠損、および感覚異常と関連する。前駆症状の期間中、リンパ球の脳脊髄液への浸透、炎症および脱髄が存在する。

【0022】

IDDMは、インスリン分泌 β 細胞の破壊および明らかな高血糖を引き起こす、細胞媒介性自己免疫疾患である。Tリンパ球がランゲルハンス島に侵入し、そしてインスリン産生 β 細胞を特異的に破壊する。 β 細胞の潤渇により、血液中のグルコースレベルを調節できなくなる。疾患の進行を、家族歴および遺伝的分析によって感受性であると診断された個体においてモニターし得る。最も重要な遺伝的効果は、主要組織適合性遺伝子座（IDDM1）の遺伝子で見られるが、インスリン遺伝子領域（IDDM2）を含む他の遺伝子座も、疾患との連結を示す（Daviesら、前出およびKennedyら（1995）Nature Genetics 9:293-298を参照のこと）。

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【0023】

（遺伝子ファミリー）

提供されるTIMファミリー遺伝子およびそのフラグメント、コードするタンパク質、ゲノム調節領域、および特異的抗体は、喘息を発現するまたは抵抗する素因のある個体の同

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定に、および予防的および治療的目的のためにインビボで遺伝子活性を調節するために有用である。コードされたタンパク質は、特異的抗体を惹起する免疫原として、タンパク質の変化した形式を含む、活性または発現を模倣または調節する組成物の薬剤スクリーニングにおいて、および治療薬として有用である。

【0024】

TIMファミリー遺伝子は、介在遺伝子なしに、TIM-4、TIM-1、TIM-3の順番で、ヒト第5染色体上でお互いにすぐ隣接している。ヒト第5染色体のこのセグメントは、悪性腫瘍および脊髄形成異常症候群におけるような形成異常細胞集団でよく欠失する(Boultonら(1997) Genomics 45:88-98を参照のこと)。第5染色体、第12染色体、および第19染色体上にTIM偽遺伝子が存在する。TIM-4以外の各TIMタンパク質は、明らかな予測されるチロシンシグナル伝達モチーフを含む。TIM-1の細胞質領域は、2つのチロシン残基を含み、そして高度に保存されたチロシンキナーゼリン酸化モチーフ、RAEDNIYを含む。拡大領域、SRAEDNIYIVEDRPは、予測されるItkリン酸化およびEGFレセプターリン酸化部位を含む。

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【0025】

マウスTim1遺伝子は、305アミノ酸の膜タンパク質をコードする。TIM-1の細胞質領域は、2つのチロシン残基を含み、そして高度に保存されたチロシンキナーゼリン酸化モチーフ、RAEDNIYを含む。TIM-1のムチンドメインは、複数のO-結合グリコシル化部位を有し、そして免疫グロブリンドメインに2つのN-結合グリコシル化部位が見出される。

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【0026】

同様の305アミノ酸膜タンパク質であるマウスTIM-2は、マウスTIM-1に対して64%の同一性、ラットKIM-1に対して60%の同一性、そしてhHAVcr-1に対して32%の同一性を有する。TIM-1と同様、TIM-2は2つの細胞外N-結合グリコシル化部位および、多くのO-結合グリコシル化部位を有するセリン、スレオニンリッチなムチンドメインを有する。TIM-2はまた、細胞内チロシンキナーゼリン酸化モチーフ、RTRCEDQVYを有する。

【0027】

Tim3は、マウスにおいて281アミノ酸の膜タンパク質、そしてヒトにおいて301アミノ酸のタンパク質をコードし、それらは複数の細胞外グリコシル化部位および細胞内チロシンリン酸化モチーフを有する、同様の膜貫通糖タンパク質構造を有する。TIM-3においてムチンドメインはTIM-1およびTIM-2におけるほど顕著ではないが、T細胞に発現したTIM-3はAPCのリガンドと相互作用し、そしてAPC活性化を変化させる。TIM-3は4つのN-結合および5つのO-結合グリコシル化部位を有し、TIM-3は、TIM-1およびTIM-2と同様、多くグリコシル化されており、そして抗原提示細胞のような他の細胞に存在するリガンドと相互作用し得ることを示唆する。

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【0028】

Tim4は、マウスにおいて344アミノ酸のタンパク質、そしてヒトにおいて378アミノ酸のタンパク質をコードする。予測されるTIM-4はまた、高度に保存されたシステイン残基を有するIgV様ドメイン、スレオニンリッチなムチンドメイン、および短い細胞内テイルを有し、他のTIMタンパク質の一般的な膜糖タンパク質構造モチーフを共有する。

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【0029】

マウス配列の多型を、BALB/cおよびHBA/DBA株に関して配列表で提供する。TIM-1において、これらの多型は3つのアミノ酸の差異およびHBA/DBAにおける15個のアミノ酸欠失をコードする。7つの予測されるアミノ酸の差異がTIM-3で同定された。TIM-1およびTIM-4における多型は、シグナルおよびムチン様ドメインに位置し、一方TIM-3で同定された多型はIgドメインに集中している。

【0030】

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ヒト *T i m 1* のコード領域における改変体を、配列表および図 8 で提供する。変異は、65% の染色体で観察される挿入（多型 1 と名称をつける）、157 *i n s M T T T V P*、および 65% の染色体で観察される欠失（多型 5）、187 Δ *T h r* を含む。他の多型は、T140A（多型 7）；V161A（多型 2）；V167I（多型 3）；T172A（多型 4）；N258D（多型 6）である。多型 4 は、40% の染色体で観察され、そして他の多型はそれぞれ $\leq 5\%$ の染色体で観察された。これら変異のほとんどは（2-6）、最初のムチンをコードするエキソンであるエキソン 3 に位置し、そして全ての改変体はゲノムレベルで起こり、そしてスプライシング改変体ではない。*T i m 1* と喘息感受性との間の関連は、ダニ感受性小児喘息の、*T i m 1* から約 0.5 メガベースであるマーカー、D5S820 に対する有意な連結（平均 LOD スコア = 4.8）の報告によってさらに支持される。 10

【0031】

ヒト組織において、4.4 kb の *T I M-1* mRNA がほとんど全ての組織に存在するが、ほとんどにおいてかすかなものである。5.5 kb のバンドが結腸および肝臓で観察された。7.5 kb のバンドが脾臓、胸腺、および末梢血白血球で観察され、そしてより小さい 4.4 kb のバンドがいくつかの臓器で観察された。*T I M-1* mRNA は、異なる細胞集団において、代わりの（*a l t e r n a t e*）5' 非翻訳領域とともに発現する。低酸素および虚血が、上皮細胞において *T I M-1* の発現を誘導し、そして照射が *T I M* 遺伝子ファミリー mRNA の発現を誘導する。*T I M* 遺伝子は腫瘍標本において発現している。ヒト *T I M-4* mRNA は、神経膠芽腫組織において発現し、そしてそれはまた分裂促進物質で刺激した、または照射末梢血単球において検出される。 20

【0032】

1 つの局面において、本発明は、*T I M-1* タンパク質、*T I M-2* タンパク質、*T I M-3* タンパク質もしくは *T I M-4* タンパク質、またはそれらのホモログもしくは改変体をコードする配列を含む、天然に存在する染色体以外の単離核酸分子を提供し、その改変体は気道過剰応答性およびアレルギー性 T 細胞応答に対する感受性と関連し得る。その核酸を、同一源または異種由来の宿主細胞において発現するために、ベクターおよび／または調節配列に作動可能に連結し得る。そのような宿主細胞は、コードされたタンパク質の産生に有用であり得る。本発明の別の局面において、*T I M-1* タンパク質、*T I M-2* タンパク質、*T I M-3* タンパク質もしくは *T I M-4* タンパク質、またはそれらのホモ 30
ログもしくは改変体の精製ポリペプチドが提供され、その改変体は気道過剰応答性およびアレルギー性 T 細胞応答に対する感受性と関連し得る。別の局面において、*T I M-1* ポリペプチド、*T I M-2* ポリペプチド、*T I M-3* ポリペプチドもしくは *T I M-4* ポリペプチドに結合する抗体または他の特異的結合メンバーが提供される。

【0033】

ヒトおよびマウス *T I M* 配列の配列が、以下のように配列表で提供される：

【0034】

【表 1】

配列番号	名前	長さ	型
1	BALB/c TIM-1	305	タンパク質
2	BALB/c TIM-1	918	DNA
3	ES-HBA TIM-1	282	タンパク質
4	ES-HBA TIM-1	849	DNA
5	BALB/c TIM-2	305	タンパク質
6	BALB/c TIM-1	958	DNA
7	ES-HBA TIM-2	305	タンパク質
8	ES-HBA TIM-2	958	DNA
9	BALB/c TIM-3	281	タンパク質
10	BALB/c TIM-3	2725	DNA
11	ES-HBA TIM-3	281	タンパク質
12	ES-HBA TIM-3	862	DNA
13	BALB/c TIM-4	345	タンパク質
14	BALB/c TIM-4	1032	DNA
15	ES-HBA TIM-4	345	タンパク質
16	ES-HBA TIM-4	1032	DNA
17	hTIM-1 対立遺伝子 1	359	タンパク質
18	hTIM-1 対立遺伝子 1	1080	DNA
19	hTIM-1 対立遺伝子 2	359	タンパク質
20	hTIM-1 対立遺伝子 2	1080	DNA
21	hTIM-1 対立遺伝子 3	365	タンパク質
22	hTIM-1 対立遺伝子 3	1098	DNA
23	hTIM-1 対立遺伝子 4	359	タンパク質
24	hTIM-1 対立遺伝子 4	1079	DNA
25	hTIM-1 対立遺伝子 5	364	タンパク質
26	hTIM-1 対立遺伝子 5	1095	DNA
27	hTIM-1 対立遺伝子 6	364	タンパク質
28	hTIM-1 対立遺伝子 6	1099	DNA
29	hTIM-3 対立遺伝子 1	301	タンパク質
30	hTIM-3 対立遺伝子 1	1116	DNA
31	hTIM-3 対立遺伝子 2	301	タンパク質
32	hTIM-3 対立遺伝子 2	1116	DNA
33	hTIM-4 対立遺伝子 1	378	タンパク質
34	hTIM-4 対立遺伝子 1	1156	DNA
35	hTIM-4 対立遺伝子 2	378	タンパク質
36	hTIM-4 対立遺伝子 2	1156	DNA

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T i mポリペプチドをコードするDNA配列は、cDNAまたはゲノムDNAあるいはそのフラグメントであり得る。プローブ、ポリペプチドの産生等のための目的のフラグメントは、1つ以上の多型残基を含み得る。T i m遺伝子という用語は、特定のT i mポリペプチド、イントロンのいずれか1つをコードするオープンリーディングフレーム、ならびにコード領域を越えて約1 kbまでの、しかしあるいはどちらかの方向にさらに遠くまで、発現の調節に関与する隣接する5'および3'非コードヌクレオチド配列を意味することが意図される。その遺伝子を、染色体外での維持または宿主への組込みのために適切なベクターに導入し得る。

【0035】

いくつかの実施形態において、T i m遺伝子配列は、配列表に示されるようなヒトT I M-1対立遺伝子1以外、および／またはマウスT I M-3 D B A対立遺伝子以外である。

【0036】

本明細書中で使用される「cDNA」という用語は、ネイティブの成熟mRNA種で見出される配列エレメントの配置を共有する全ての核酸を含むよう意図され、ここで配列エレメントはエキソンならびに3'および5'非コード領域である。通常mRNA種は連続的なエキソンを有し、間にあるイントロンは核RNAスプライシングによって除去されてT

i mタンパク質をコードする連続的なオープンリーディングフレームを作製する。

【0037】

目的のゲノム配列は、列挙した配列で規定されたような、天然染色体において通常存在する全てのイントロンを含む、開始コドンおよび停止コドンの間に存在する核酸を含む。それはさらに、成熟mRNAで見出される3'および5'非翻訳領域を含み得る。それはさらに、約1kb、しかしあるいはより長い、転写領域の5'または3'末端のいずれかの隣接したゲノムDNAを含む、プロモーター、エンハンサー等のような、特異的転写配列および翻訳調節配列を含み得る。ゲノムDNAを100kbpまたはより小さいフラグメントとして単離し得る；そして本質的に側面の染色体配列を含まない。

【0038】

5'領域の配列、ならびにさらに5'上流配列および3'下流配列を、エンハンサー結合部位を含むプロモーターエレメントのために利用し得、それはTim遺伝子が発現する組織における発現を提供する。組織特異的発現は、発現パターンを決定するために、およびネイティブの発現パターンを模倣するプロモーターを提供するために有用である。プロモーター領域に天然に存在する多型は、天然の発現における変異、特に疾患と関連し得るものを決定するために有用である。あるいは、突然変異をプロモーター領域に導入して、実験的に定義されたシステムにおいて変化した発現の効果を決定し得る。転写因子の結合に関与する特異的DNAモチーフを同定する方法、例えば公知の結合モチーフに対する配列類似性、ゲル遅延研究等が、当該分野で公知である。例えば、Blackwellら(1995) Mol Med 1:194-205; Mortlockら(1996) Genome Res. 6:327-33; ならびにJoulinおよびRichard-Foy(1995) Eur J Biochem 232:620-626を参照のこと。

【0039】

調節配列を使用して、特に異なる組織または発達段階におけるTIM発現の転写または翻訳調節に必要なシス作用配列を同定し得、そしてTIM発現を調節または媒介するシス作用配列およびトランス作用因子を同定し得る。そのような転写調節領域または翻訳調節領域を、野生型または変化したTIMまたは他の目的のタンパク質の、培養細胞、または胚、胎児組織もしくは成人組織における発現を促進するために、および遺伝子治療のためにTIM遺伝子に操作可能に連結し得る。

【0040】

本発明の核酸組成物は、本ポリペプチドの全てまたは一部をコードし得る。伝統的な方法によってオリゴヌクレオチドを化学的に合成することによって、制限酵素消化によって、PCR増幅等によって、DNA配列のフラグメントを得ることができる。ほとんどの場合、DNAフラグメントは少なくとも15nt、通常少なくとも18nt、より通常には少なくとも約50ntである。そのような小さいDNAフラグメントは、PCR、ハイブリダイゼーションスクリーニング等のプライマーとして有用である。より大きなDNAフラグメント、すなわち100ntより大きなものは、コードされたポリペプチドの産生に有用である。PCRのような増幅反応で使用するために、1対のプライマーを使用する。プライマー配列の正確な組成は、本発明に決定的にはないが、ほとんどの適用に関して、プライマーは、当該分野で公知であるように、ストリンジェントな条件下で本配列にハイブリダイズする。少なくとも約50nt、好ましくは少なくとも約100ntの増幅産物を産生する1対のプライマーを選択することが好ましい。プライマー配列を選択するためのアルゴリズムは一般的に公知であり、そして市販のソフトウェアパッケージで入手可能である。増幅プライマーはDNAの相補鎖にハイブリダイズし、そしてお互いに向かって初回刺激する。

【0041】

TIM遺伝子は、実質的な純度で、一般的にインタクトな哺乳動物染色体以外として単離され、そして得られる。通常、DNAは、TIM配列またはそのフラグメントを含まない他の核酸配列を実質的に含まないで得られ、一般的に少なくとも約50%、通常少なくとも約90%の純度であり、そして典型的には「組換え体」であり、すなわち天然に存在す

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る染色体で通常関連しない1つ以上のヌクレオチドに隣接する。

【0042】

DNA配列を様々な方法で使用する。それらを、TIM関連遺伝子を同定するプローブとして使用し得る。哺乳動物ホモログは本配列と実質的な配列類似性、すなわち本DNA配列のヌクレオチド配列と少なくとも75%、通常少なくとも90%、より通常には少なくとも95%の配列同一性を有する。配列類似性を、保存されたモチーフ、コード領域、隣接領域等のような、より大きな配列のサブセットであり得る参照配列に基づいて計算する。参照配列は通常、少なくとも約18nt長、より通常には少なくとも約30nt長であり、そして比較される完全な配列まで伸長し得る。Altshulら(1990) J Mol Biol 215:403-10で記載されたBLASTのような、配列分析のためのアルゴリズムが、当該分野で公知である。 10

【0043】

配列類似性を有する核酸を、低いストリンジェンシー条件下例えば50℃で10×SSC(0.9Mの生理食塩水/0.09Mのクエン酸ナトリウム)でのハイブリダイゼーションによって検出し、そして55℃で1×SSCでの洗浄にかけた場合に結合したままにする。配列同一性をストリンジェントな条件下、例えば50℃またはより高く、および0.1×SSC(9mMの生理食塩水/0.9mMのクエン酸ナトリウム)でのハイブリダイゼーションによって決定し得る。プローブ、特にDNA配列の標識プローブを使用することによって、ホモログまたは関連遺伝子を単離し得る。相同的遺伝子の供給源はあらゆる種、例えば霊長類種、特にヒト；ラットおよびマウスのようなげっ歯類、イヌ、ネコ、ウシ、ヒツジ、ウマ、酵母、Drosophila、Caenorhabditis等であり得る。 20

【0044】

そのDNAをまた、生物学的標本における遺伝子の発現を同定するために使用し得る。ゲノムDNAまたはRNAのような特定のヌクレオチド配列の存在に関して細胞をプローブする方法は、文献においてよく確立されており、そしてここで詳述する必要はない。mRNAを細胞サンプルから単離する。mRNAを、逆転写酵素を用いてRT-PCRによって増幅し、相補的DNA鎖を形成し得、続いて本DNA配列に特異的なプライマーを用いたポリメラーゼ連鎖反応増幅を行なう。あるいは、mRNAサンプルをゲル電気泳動によって分離し、適当な支持体、例えばニトロセルロース、ナイロン等に移動させて、そして次いでプローブとして本DNAのフラグメントを用いてプローブする。オリゴヌクレオチドライゲーションアッセイ、インサイチュハイブリダイゼーション、および固体チップ上に整列したDNAプローブへのハイブリダイゼーションのような他の技術も有用であり得る。本配列にハイブリダイズするmRNAの検出は、サンプルにおけるTIM遺伝子の発現を示す。 30

【0045】

本核酸配列を、特にそれらを細胞内で使用する場合、例えば遺伝子切断等のために核酸切断薬剤(例えば鉄またはクロムのようなキレート化金属)を結合させることによって、多くの目的のために修飾し得る。

【0046】

隣接プロモーター領域およびコード領域を含むTIM遺伝子座の配列を、当該分野で公知の様々な方法で突然変異させて、プロモーターの強さ、コードされるタンパク質の配列等における標的化変化を産生し得る。DNA配列またはそのような変異の産物は、本明細書中で提供される配列と本質的に同様である、すなわち少なくともそれぞれ1ヌクレオチドまたはアミノ酸が異なる、そして少なくとも2つ、しかし約10以下のヌクレオチドまたはアミノ酸が異なり得る。配列変化は、置換、挿入または欠失であり得る。欠失はさらに、ドメインまたはエキソンの欠失のような、より大きな変化を含み得る。他の目的の修飾は、例えばFLAGシステム、HA等を用いたエピトプタグ化を含む。細胞レベル下での局在の研究のために、グリーン蛍光タンパク質(GFP)との融合タンパク質を使用し得る。そのような変異遺伝子を使用して、TIMポリペプチドの構造-機能相関を研究し 40 50

得るかまたはその機能もしくは調節に影響を与えるタンパク質の性質を変化させ得る。例えば、構成的に活性な転写因子、または転写を活性化することなくTIM DNA標的部位に結合するドミナントネガティブに活性なタンパク質を、この方法で作成し得る。

【0047】

クローニングされた遺伝子のインビトロ突然変異生成技術は公知である。変異を調査するプロトコルの例は、Gust inら、Biotechniques 14:22 (1993); Barany、Gene 37:111-23 (1985); Colicelliら、Mol Gen Genet 199:537-9 (1985); およびPrentkiら、Gene 29:303-13 (1984) において見出され得る。部位特異的突然変異生成の方法は、Sambrookら、Molecular Cloning: A Laboratory Manual、CSH Press 1989 15.3-15.108頁; Weinerら、Gene 126:35-41 (1993); Sayersら、Biotechniques 13:592-6 (1992); JonesおよびWinistorfer、Biotechniques 12:528-30 (1992); Bartonら、Nucleic Acids Res 18:7349-55 (1990); MarottiおよびTomich、Gene Anal Tech 6:67-70 (1989); およびZhu Anal Biochem 177:120-4 (1989) において見出され得る。

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【0048】

アレイは、1つのサンプルにおいて多くのポリヌクレオチドをアッセイし得るハイスループット技術を提供する。本発明の1つの局面において、1つ以上のTIM遺伝子、タンパク質、または抗体を含む、好ましくはこれらの配列全てを含むアレイを構築し、そのアレイはさらにT細胞、単球等で上方制御または下方制御されることが公知の他の配列を含み得る。この技術を、差次的発現、または遺伝子型同定を試験する道具として使用し得る。ポリヌクレオチドプローブを、二次元マトリックスまたは結合したプローブを有するアレイに、基質（例えばガラス、ニトロセルロース等）に配置することによって、アレイを作成し得る。プローブを、共有結合または、疎水性相互作用のような非特異的相互作用のいずれかによって基質に結合し得る。アレイの構築技術およびそれらのアレイを使用する方法は、例えばSchenaら (1996) Proc Natl Acad Sci US A 93(20):10614-9; Schenaら (1995) Science 270(5235):467-70; Shal onら (1996) Genome Res. 6(7):639-45; USPN5, 807, 522、EP799897; WO97/29212; WO97/27317; EP785280; WO97/02357; USPN5, 593, 839; USPN5, 578, 832; EP728520; USPN5, 599, 695; EP721016; USPN5, 556, 752; WO95/22058; およびUSPN5, 631, 734に記載されている。

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【0049】

アレイにおいて利用されるプローブは、様々な型であり得、そして例えば比較的短い長さの（例えば20マーまたは25マー）合成プローブ、cDNA（全長または遺伝子のフラグメント）、増幅DNA、DNAのフラグメント（例えば制限酵素によって産生される）および逆転写DNAを含み得る。あつらえたアレイおよび一般的なアレイの両方を、差次的発現レベルの検出に利用し得る。あつらえたアレイを、特定の前もって選択したmRNA遺伝子配列の部分配列またはそれらから調製した増幅産物にハイブリダイズするプローブを用いて調製し得る。

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【0050】

アレイを、例えば遺伝子の差次的発現を調査するために、および遺伝子機能を決定するために使用し得る。例えば、アレイをTIM遺伝子における差次的発現、または多型配列の発現を検出するために使用し得る。アレイの例示的な使用が、例えばPappalardoら (1998) Sem. Radiation Oncol. 8:217; およびRamsey (1998) Nature Biotechnol. 16:40においてさらに

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記載されている。さらに、アレイを用いた検出方法の多くのバリエーションが、当該分野の技術の範囲内および本発明の範囲内である。例えば、プローブを固体支持体に固定化するよりも、試験サンプルを固体支持体に固定化し得、それを次いでプローブと接触させる。発現分析におけるマイクロアレイの使用に関するさらなる議論は、例えばDugganら、Nature Genetics Supplement 21:10-14 (1999) ; Bowtell、Nature Genetics Supplement 21:25-32 (1999) ; BrownおよびBotstein、Nature Genetics Supplement 21:33-37 (1999) ; Coleら、Nature Genetics Supplement 21:38-41 (1999) ; DebouckおよびGoodfellow、Nature Genetics Supplement 21:48-50 (1999) ; Bassett, Jr. ら、Nature Genetics Supplement 21:51-55 (1999) ; およびChakravarti、Nature Genetics Supplement 21:56-60 (1999) において見出され得る。

【0051】

薬理遺伝学は、個人の遺伝子型およびその個人の治療薬に対する代謝または反応能力の間の連関である。代謝または標的感受性における差異が、薬物の生物活性投与量および血液濃度の間の関係を変化させることによって、重篤な毒性または治療の失敗を引き起こし得る。過去数年間の間に、多くの研究が代謝酵素または薬物標的における多型と応答および毒性両方との間に強い関係を確立した。これらの関係を使用して、治療投与量を個別化し得る。

【0052】

多型対立遺伝子の遺伝子型決定を使用して、個人が特定の治療レジメンによく応答するかどうかを評価する。多型配列をまた、薬物スクリーニングアッセイにおいて使用して、候補治療剤の投与量および特異性を決定する。候補TIM多型を標的治療でスクリーニングして、喘息治療における有効性に影響があるかどうかを決定する。薬物スクリーニングアッセイを、上記で記載したように行なう。典型的には2つまたはそれ以上の異なる配列多型を、治療への応答に関して試験する。

【0053】

本遺伝子を、完全なTIMタンパク質、またはそのポリペプチドフラグメント、特に機能的ドメイン；結合部位等に対応するフラグメントを、および本ポリペプチドの他のタンパク質またはその一部との融合を含んで、合成するために採用し得る。発現のために、誘導的または構成的であり得る、転写開始領域および翻訳開始領域を提供する、発現カセットを使用し得、ここでコード領域は転写開始領域ならびに転写停止領域および翻訳停止領域の転写調節下に作動可能に連結される。発現宿主で機能的な様々な転写開始領域を使用し得る。

【0054】

TIMポリペプチドのフラグメントである、特に目的のポリペプチドは、TIMポリペプチドの特定のドメインを含み、ここでドメインは例えば細胞外ドメイン、または細胞外ドメイン中のドメイン：ムチンドメインおよび／もしくはIgドメインを含み得る。ドメインはまた、細胞質ドメイン、例えばチロシンキナーゼリン酸化モチーフ、RAEDNIY、または拡大領域、SRAEDNIYIVEDRPを含むフラグメントを含み得る。可溶性スプライス改変体によってコードされるポリペプチドもまた目的のものである。Igドメインの配列は以下のとおりである：ヒトTIM-1 Igドメイン、配列番号第17、19、21、23、25、27番、残基21-126；ヒトTIM-3 Igドメイン、配列番号第29および31番、残基22-131；ヒトTIM-4 Igドメイン、配列番号第33および35番、残基25-133；マウスTIM-1 Igドメイン、配列番号第1および3番、残基21-129；マウスTIM-2 Igドメイン、配列番号第7番、残基22-128；マウスTIM-3 Igドメイン、BALB/c対立遺伝子、配列番号第9番、残基22-132；マウスTIM-3 Igドメイン、DBA/2対立遺

伝子、配列番号第11番、残基22-132；マウスTIM-4 Igドメイン、配列番号第13および15番、残基25-135。

【0055】

機能的に等価なポリペプチドが有用であり得、ここで等価なポリペプチドは、サイレントな変化を引き起こす、アミノ酸残基の欠失、付加、または置換を含み得、従って経路遺伝子産物に対して異なって発現される機能的に等価なものを産生する。アミノ酸置換を、関与する残基の極性、荷電、可溶性、疎水性、親水性、および／または両親媒性の性質における類似性に基づいて行ない得る。本明細書中で使用される「機能的等価物」は、TIM遺伝子によってコードされるポリペプチドと本質的に同様のインビボ活性を示し得るタンパク質を指す。

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【0056】

ポリペプチドを、発現の目的によって、伝統的な方法によって原核生物または真核生物で発現し得る。タンパク質の大規模産生のために、E. coli、B. subtilis、S. cerevisiaeのような単細胞有機体、または脊椎動物、特に哺乳動物のようなより高等な有機体の細胞、例えばCOS7細胞を、発現宿主細胞として使用し得る。多くの状況において、TIM遺伝子を哺乳動物細胞で発現することが望ましくあり得、ここでTIM遺伝子は、天然のフォールディングおよび翻訳後修飾から利益を受ける。特異的ペプチドエпитープ、ドメイン等を含む小さいペプチドをまた、研究室で合成し得、ここでペプチドは通常少なくとも約8アミノ酸の長さ、より通常には少なくとも約20アミノ酸の長さ、完全なドメインまで、そして全長タンパク質である。ペプチドは、タンパク質の多型領域を含み得る。融合タンパク質も含まれ、ここでTIMタンパク質の全てまたはフラグメントを、異種由来のポリペプチド、例えばグリーン蛍光タンパク質、抗体Fc領域、ポリヒスチジン等に融合する。

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【0057】

哺乳動物宿主細胞において、レトロウイルス、レンチウイルス、アデノウイルス、アデノ随伴ウイルス等を含む、多くのウイルスに基づく発現システムを使用し得る。アデノウイルスを発現ベクターとして使用する場合には、目的のコード配列をアデノウイルス転写／翻訳調節コントロール複合体、例えば後期プロモーターおよび3つに分かれた(tripartite)リーダー配列にライゲーションし得る。このキメラ遺伝子を次いでインビトロまたはインビボ組換えによってアデノウイルスゲノムに挿入し得る。ウイルスゲノムの重要でない領域(例えばE1領域またはE3領域)における挿入は、生存可能であり、そして感染宿主において差次的に発現されるタンパク質または経路遺伝子タンパク質を発現し得る組換えウイルスを生じる。

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【0058】

遺伝子の効率的な翻訳のために、特異的な開始シグナルも必要であり得る。これらのシグナルは、ATG開始コドンおよび隣接する配列を含む。それ自身の開始コドンおよび隣接配列を含む完全な遺伝子が、適切な発現ベクターに挿入される場合には、さらなる翻訳制御シグナルは必要でないかもしれない。しかし、配列をコードする遺伝子の一部しか挿入されない場合には、外来性の翻訳制御シグナルを提供しなければならない。これらの外来性翻訳制御シグナルおよび開始コドンは、天然および合成どちらも、様々な起源であり得る。発現の効率は、適切な転写エンハンサーエレメント、転写ターミネーター等を含むことによって増強され得る。

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【0059】

発現宿主を使用することによって、ポリペプチドを大量に入手可能なら、ポリペプチドを伝統的な方法によって単離および精製し得る。発現宿主の溶解物を調製し得、そして溶解物をHPLC、排除クロマトグラフィー、ゲル電気泳動、アフィニティークロマトグラフィー、または他の精製技術を用いて精製し得る。精製ポリペプチドは、一般的には少なくとも約80%純粋、好ましくは少なくとも約90%純粋、そして100%純粋までおよび100%純粋を含み得る。純粋は、他のタンパク質、および細胞の破片を含まないことを意味する。

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【0060】

ポリペプチドを、直接的または間接的いずれかで標識し得る。^{1 2 5} Iのような放射性同位体；基質に曝露した場合に検出可能な比色定量シグナルまたは光を産生する酵素標識システム；および蛍光標識を含むがこれに限らない、様々な適当な標識システムのいずれかを使用し得る。間接的な標識は、目的のポリペプチドに特異的に結合する、標識抗体のようなタンパク質の使用を含む。そのような抗体は、ポリクローナル、モノクローナル、キメラ、一本鎖、F a bフラグメントおよびF a b発現ライブラリーによって産生されるフラグメントを含むがこれに限らない。

【0061】

（特異的結合メンバー）

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本明細書中で使用される場合、「特異的結合メンバー」または「結合メンバー」という用語は、特異的な結合対、すなわち2つの分子、通常2つの異なる分子のメンバーを指し、ここで分子の1つ（すなわち第1の特異的結合メンバー）が、化学的または物理的手段で他の分子（すなわち第2の特異的結合メンバー）に特異的に結合する。特異的結合対の相補的なメンバーは、リガンドおよびレセプター；またはレセプターおよびカウンターレセプター（c o u n t e r - r e c e p t o r）と呼ばれることもある。本発明の目的のために、例えばアッセイが公知の結合対の結合を妨害する化合物を検出することに向けられる場合、2つの結合メンバーはお互いに結合することが公知であり得る。あるいは、目的の化合物の結合パートナーであることが予想される候補化合物を使用し得る。

【0062】

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目的の特異的結合対は、炭水化物およびレクチン；相補的ヌクレオチド配列；ペプチドリガンドおよびレセプター；エフェクターおよびレセプター分子；ホルモンおよびホルモン結合タンパク質；酵素補助因子および酵素；酵素インヒビターおよび酵素；脂質および脂質結合タンパク質等を含む。特異的結合対は、もとの特異的結合メンバーのアナログ、誘導体、およびフラグメントを含み得る。例えば、レセプターおよびリガンド対は、ペプチドフラグメント、化学的に合成したペプチド模倣物、標識タンパク質、誘導体化タンパク質等を含み得る。

【0063】

好ましい実施形態において、特異的結合メンバーは抗体である。「抗体」または「抗体部分」という用語は、エピトープに適合およびエピトープを認識する特異的な形状を有する任意のポリペプチド鎖を含む分子構造を含むように意図され、ここで1つ以上の非共有結合相互作用が、分子構造とエピトープとの間の複合体を安定化する。T I Mタンパク質の1つに特異的に結合する抗体を、抗T I Mと呼ぶ。原型の抗体分子は免疫グロブリンであり、そして全ての供給源、例えばヒト、げっ歯類、ウサギ、ウシ、ヒツジ、ブタ、イヌ、他の哺乳動物、ニワトリ、他の鳥類等由来の、全ての型の免疫グロブリン、I g G、I g M、I g A、I g E、I g D等が、「抗体」であると考えられる。本発明で利用される抗体は、ポリクローナル抗体であり得るが、細胞培養によってまたは組換え的に複製し得、そしてその抗原性を抑制するために修飾し得るので、モノクローナル抗体が好ましい。

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【0064】

ポリクローナル抗体を、標準的なプロトコルによって、産生動物にポリペプチドまたはインビボで発現したc D N Aであり得る抗原性組成物を注射することによって産生し得る。例えばH a r l o wおよびL a n e、A n t i b o d i e s : A L a b o r a t o r y M a n u a l、C o l d S p r i n g H a r b o r L a b o r a t o r y、1988を参照のこと。タンパク質全体、またはタンパク質のより大きな部分を利用する場合、産生動物をタンパク質および適切なアジュバント（例えばフロイント、完全フロイント、水中油型エマルジョン等）で免疫することによって抗体を産生し得る。より小さいペプチドを利用する場合、ペプチドをより大きな分子に結合して免疫刺激性の結合物を作成することが有利である。そのような利用のために市販で入手可能な、よく利用される結合タンパク質は、ウシ血清アルブミン（B S A）およびカサガイヘモシアニン（K L H）を含む。多型残基のような特定のエピトープに対して抗体を産生するために、全長配列由来の

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ペプチドを利用し得る。免疫原を、好ましくは前もって決定した1回またはそれ以上の追加免疫を含むスケジュールによって、動物宿主に注射し、そして動物は定期的に採血される。次いでポリクローナル抗体をそのような抗血清から、例えば適切な固体支持体に結合したポリペプチドを用いたアフィニティークロマトグラフィーによって精製し得る。

【0065】

あるいは、モノクローナル抗体に関しては、接種動物の脾臓由来のような、刺激免疫細胞を単離することによって、ハイブリドーマを形成し得る。次いでこれらの細胞を、骨髓腫細胞または形質転換細胞のような不死化細胞と融合し、それらを細胞培養中で無限に複製し得、それによって不死の、免疫グロブリン分泌細胞株を産生する。利用される不死細胞株は、好ましくはある栄養素の利用に必要な酵素に欠損があるものが選択される。多くのそのような細胞株（骨髓腫など）が当業者に公知であり、そして例えばチミジンキナーゼ（TK）またはヒポキサンチン-グアニンホスホリボシルトランスフェラーゼ（HGPRT）を含む。これらの欠損は、それらの例えばヒポキサンチンアミノプテリンチミジン培地（HAT）上で増殖する能力によって融合細胞を選択することを可能にする。

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【0066】

好ましくは、利用される不死融合パートナーは、免疫グロブリンを分泌しない株由来である。得られた融合細胞、またはハイブリドーマを、非融合細胞ではなく融合細胞の生存を可能にする条件下で培養し、そしてできたコロニーを、望ましいモノクローナル抗体の産生に関してスクリーニングする。大量の抗体を産生するために、そのような抗体を産生するコロニーをクローニング、発展、および増殖させる。KohlerおよびMilstein、1975 Nature 256:495（その開示は本明細書中で参考として援用される）を参照のこと。

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【0067】

次いで、クローンをマウスの腹腔に注射し、そこから腹水を回収することによって、分泌ハイブリドーマ由来の大量のモノクローナル抗体を産生し得る。好ましくはプリスタン、または他の腫瘍プロモーターで初回刺激され、そして化学的にまたは照射によって免疫抑制されたマウスは、当該分野で公知の様々な適当なかぶのいずれかであり得る。腹水をマウスから回収し、そしてモノクローナル抗体をそこから、例えばCMセファロースカラムまたは他のクロマトグラフィー手段によって精製する。あるいは、ハイブリドーマをインビトロで、または懸濁培養として培養し得る。バッチ、連続培養、または他の適当な培養処理を利用し得る。次いでモノクローナル抗体を培養培地または上清から回収する。

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【0068】

それに加えて、抗体または抗原結合フラグメントを、遺伝的操作によって産生し得る。この技術において、標準的なハイブリドーマ手順と同様、抗体産生細胞を望ましい抗原または免疫原で感作する。免疫脾臓細胞またはハイブリドーマから単離されたメッセンジャーRNAを鋳型として使用して、PCR増幅を用いてcDNAを作成する。それぞれ最初の抗原特異性を維持する、1つの重鎖遺伝子および1つの軽鎖遺伝子を含むベクターのライブラリーを、増幅免疫グロブリンcDNAの適当な部分を発現ベクターへ挿入することによって産生する。コンビナトリアルライブラリーを、重鎖遺伝子ライブラリーと軽鎖遺伝子ライブラリーを組み合わせることによって構築する。これは、重鎖および軽鎖を同時発現するクローンのライブラリーを生ずる（Fabフラグメントまたは抗体分子の抗原結合フラグメントに類似する）。これらの遺伝子を有するベクターを、宿主（例えば細菌、昆虫細胞、哺乳動物細胞、または他の適当なタンパク質産生宿主細胞）に同時トランスフェクトする。抗体遺伝子の合成がトランスフェクトされた宿主で誘導されると、重鎖および軽鎖タンパク質は自己集合して、抗原または免疫原を用いたスクリーニングによって検出され得る活性な抗体を産生する。

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【0069】

キメラ抗体を、主にヒトドメインを有する抗体を産生するために、マウス（または他の動物由来の）ハイブリドーマクローンから得たマウス可変軽鎖領域および重鎖領域（VKおよびVH）を、ヒト定常軽鎖領域および重鎖領域と組み合わせることによる組換え手段によ

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って作成し得る。そのようなキメラ抗体の産生は当該分野で周知であり、そして標準的な手法で達成し得る（例えばその全体が本明細書中で参考として援用される、米国特許第5,624,659号によって記載されたように）。ヒト化抗体を、さらによりヒト様の免疫グロブリンドメインを含み、そして動物由来抗体の相補性決定領域のみを含むように、遺伝子工学的に操作する。これは、モノクローナル抗体可変領域の超可変ループの配列を注意深く調査し、そしてそれらをヒト抗体鎖の構造に適合させることによって達成される。表面的には（facially）複雑であるが、その過程は実際には単純である。例えば、その全体が本明細書中で参考として援用される、米国特許第6,187,287号を参照のこと。

【0070】

あるいは、ポリクローナル抗体またはモノクローナル抗体を、ヒト免疫グロブリンを産生するように遺伝的に変化させた動物から産生し得る。そのような動物を産生する、およびそこから抗体を得る技術は、その全体が本明細書中で参考として援用される、米国特許第6,162,963号および同第6,150,584号に記載されている。

【0071】

あるいは、一本鎖抗体（下記に記載されるようなFv）を、ヒト可変領域を含むファージライブラリーから産生し得る。米国特許第6,174,708号を参照のこと。一本鎖抗毒素、LMB-7 [B3 (Fv) - PE38] のクモ膜下投与は、ラットモデルにおいて癌性髄膜炎を治療することが示された。Proc Natl. Acad. Sci USA 92, 2765-9、それらはその全体が本明細書中で参考として援用される。

【0072】

免疫グロブリン全体（またはその組換え対応物）に加えて、エピトープ結合部位を含む免疫グロブリンフラグメント（例えばFab'、F(ab')₂、または他のフラグメント）が、本発明において抗体分子として有用である。そのような抗体フラグメントを、ペプシン、パパイニン、または他のプロテアーゼ切断によって免疫グロブリン全体から産生し得る。「フラグメント」または最低限の免疫グロブリンを、組換え免疫グロブリン技術を利用して設計し得る。例えば、本発明において使用する「Fv」免疫グロブリンを、可変軽鎖領域を、可変重鎖領域にペプチドリンカー（例えばポリグリシンまたはアルファヘリックスまたはベータシートモチーフを形成しない別の配列）を介して結合することによって産生し得る。

【0073】

Fvフラグメントは、可変重鎖ドメイン（V_H）および可変軽鎖ドメイン（V_L）のヘテロダイマーである。例えば、IgG全体に存在する重鎖および軽鎖ドメインのヘテロダイマーを、ジスルフィド結合によって連結する。V_HおよびV_Lがペプチドリンカーによって連結された組換えFvは、典型的には安定である。これらは、特異性および親和性を維持することが見出された、そして腫瘍の画像化および腫瘍治療のための組換え抗毒素を作成するのに有用であることが見出された一本鎖Fvである。しかし、研究者らは、いくつかの一本鎖Fvは抗原に対して減少した親和性を有し、そしてペプチドリンカーは結合を妨害し得ることを発見した。その全体が本明細書中で参考として援用される、米国特許第6,147,203号において記載されたように、V_HおよびV_L領域間のジスルフィド結合の安定化を含む、改善したFvも作成された。これら最低限の抗体のいずれかを、本発明において利用し得、そしてHAMA反応を避けるためにヒト化されたものが、本発明の実施形態において使用するのに好ましい。

【0074】

それに加えて、化学的リンカー、蛍光色素、酵素、基質、化学発光部分等のような検出可能な部分またはストレプトアビジン、アビジン、もしくはビオチン等のような特異的結合部分を加えた、誘導体化免疫グロブリンを、本発明の方法および組成物において利用し得る。便利さのために、「抗体」または「抗体部分」という用語を、全体を通して一般的に脳腫瘍タンパク質標的のエピトープに特異的に結合する分子を指すために使用するが、その用語は、上記に記載したような、全ての免疫グロブリン、誘導体、フラグメント、組換

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えまたは遺伝子操作免疫グロブリン、および修飾免疫グロブリンを含む。

【0075】

候補抗体を、あらゆる適当な標準的な方法によって、活性に関して試験し得る。最初のスクリーニングとして、抗体を免疫原に対する結合に関して試験し得る。2番目のスクリーニングとして、抗体を、対立遺伝子間、およびTIMファミリーメンバー間の交差応答性に関してスクリーニングし得、そしてTIM機能の阻害活性に関して試験し得る。これらのスクリーニングに関して、候補抗体を検出のために標識し得る。TIMタンパク質の生物学的活性を変化させる抗体を、機能的形式でアッセイし得る。

【0076】

(診断)

T i M多型に関連する喘息またはアトピーの診断を、患者由来のあらゆる簡便なサンプル、例えば生検材料、血液サンプル、頬からの擦過等の、タンパク質、DNAまたはRNA配列および／またはハイブリダイゼーション分析によって行なう。TIMと関連し得る喘息を有する患者由来の核酸サンプルを、TIMにおける素因を与える多型の存在に関して分析する。典型的な患者の遺伝子型は、少なくとも1つの染色体上に、少なくとも1つの素因を与える変異を有する。遺伝子産物の活性または発現に影響を与え、そして喘息に対する増加した感受性を与える多型TIM配列の存在は、素因を与える多型と考えられる。喘息中性配列と比較して、素因を与える多型の存在に関してDNAまたはmRNAを分析することによって、個人をスクリーニングする。目的の特異的な配列は、臨床的気管支過剰応答性を引き起こす、または他の方法で喘息と関連するあらゆる多型を含み、コード領域配列、スプライシングに影響を与えるイントロン配列、またはタンパク質の活性および発現に影響を与えるプロモーターもしくはエンハンサー配列における挿入、置換および欠失を含むがこれに限らない。特定のTIM多型の例を、実施例で提供する。

【0077】

スクリーニングはまた、タンパク質の機能的特徴または抗原性特徴に基づき得る。TIMタンパク質において、素因を与える多型を検出するように設計されたイムノアッセイを、スクリーニングで使用し得る。多くの多様な変異が特定の疾患表現型を引き起こす場合、機能的タンパク質アッセイが、有効なスクリーニング手段であることが示された。

【0078】

T I Mコード領域またはコントロール領域における候補配列多型が疾患と関連するかどうかを決定するために、生化学的研究を行ない得る。例えば、TIMの発現に影響を与えるプロモーター配列またはエンハンサー配列の変化は、喘息に対する素因を与え得る。候補異型対立遺伝子の発現レベルを、当該分野で公知の様々な方法によって、正常対立遺伝子の発現レベルと比較する。プロモーターまたはエンハンサーの強さを決定する方法は、発現した天然タンパク質の定量；簡便な定量を提供するβ-ガラクトシダーゼ、ルシフェラーゼ、クロラムフェニコールアセチルトランスフェラーゼ等のようなりポーター遺伝子と共に異型コントロールエレメントをベクターに挿入すること、等を含む。コードされたTIMタンパク質の活性を、野生型タンパク質との比較によって決定し得る。

【0079】

特定の配列の存在に関して核酸を分析する多くの方法が利用可能である。大量のDNAが入手可能である場合、ゲノムDNAを直接使用する。あるいは、目的の領域を適当なベクターにクローニングして、そして分析のために十分な量を増殖させる。気管細胞のような、TIM遺伝子を発現する細胞を、mRNAの供給源として使用し得、それを直接分析し得るか、または分析のためにcDNAに逆転写し得る。核酸を、ポリメラーゼ連鎖反応(PCR)のような従来技術によって増幅して、分析に十分な量を提供し得る。ポリメラーゼ連鎖反応の使用はS a i k iら(1985) Science 239:487に記載されており、そして現在の技術の概説を、S a m b r o o kら、M o l e c u l a r C l o n i n g : A L a b o r a t o r y M a n u a l、C S H P r e s s 1989、14.2-14.33頁において見出し得る。増幅をまた、多型に特異的なプライマーを使用することによって、多型が存在するかどうかを決定するために使用し得る。ある

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いは、多型を検出する手段としてオリゴヌクレオチドライゲーションを利用する、様々な方法が当該分野で公知であり、例えばRileyら(1990) N. A. R. 18:2887-2890; およびDeLahuntlyら(1996) Am. J. Hum. Genet. 58:1239-1246を参照のこと。

【0080】

検出可能な標識を増幅反応に含み得る。適当な標識としては、蛍光色素、例えばフルオレセインイソチオシアネート(FITC)、ローダミン、テキサスレッド、フィコエリトリン、アロフィコシアニン、6-カルボキシフルオレセイン(6-FAM)、2', 7'-ジメトキシ-4', 5'-ジクロロ-6-カルボキシフルオレセイン(JOE)、6-カルボキシ-X-ローダミン(ROX)、6-カルボキシ-2', 4', 7', 4, 7, -ヘキサクロフルオレセイン(HEX)、5-カルボキシフルオレセイン(5-FAM)またはN, N, N', N'-テトラメチル-6-カルボキシローダミン(TAMRA)、放射活性標識(例えば³²P、³⁵S、³H)等が挙げられる。標識は2段階システムであり得、ここで増幅DNAを高親和性結合パートナー(例えばアビジン、特異的抗体等)を有する、ビオチン、ハプテン等に結合し、ここで結合パートナーが検出可能な標識に結合する。標識を、1つまたは両方のプライマーに結合し得る。あるいは、増幅産物に標識を組み込むために、増幅に使用するヌクレオチドのプールを標識する。

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【0081】

サンプル核酸、例えば増幅またはクローン化フラグメントを、当該分野で公知の多くの方法の1つによって分析する。核酸を、ジデオキシまたは他の方法によって配列決定し得、そして塩基配列を中性TIM配列と比較する。サザンブロット、ドットブロット等による、異型配列とのハイブリダイゼーションもまた、その存在を決定するために使用し得る。US5, 445, 934またはWO95/35505において記載されたように、固体支持体に固定化されたオリゴヌクレオチドプローブのアレイに対するコントロールおよび異型配列のハイブリダイゼーションパターンも、異型配列の存在を検出する手段として使用し得る。一本鎖高次構造多型(SSCP)分析、変性剤濃度勾配ゲル電気泳動(DGGE)、ミスマッチ切断検出、およびゲルマトリックスにおけるヘテロ二本鎖分析を使用して、電気泳動移動度の変化としてDNA配列変異によって産生される高次構造の変化を検出する。あるいは、多型が制限エンドヌクレアーゼの認識部位を産生または破壊する場合(制限フラグメント長多型、RFLP)、サンプルをエンドヌクレアーゼで消化し、そして産物をサイズ分画して、フラグメントが消化されたかどうかを決定する。分画を、ゲル電気泳動またはキャピラリー電気泳動(特にアクリルアミドまたはアガロースゲル)によって行なう。

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【0082】

US5, 445, 934またはWO95/35505において記載されたように、固体支持体に固定化されたオリゴヌクレオチドプローブのアレイに対するコントロールおよび異型配列のハイブリダイゼーションパターンを、異型配列の存在を検出する手段として使用し得る。本発明の1つの実施形態において、オリゴヌクレオチドのアレイが提供され、ここでアレイの別々の位置が、TIM遺伝子座のmRNAまたはゲノムDNAの少なくとも一部と相補的である。そのようなアレイは一連のオリゴヌクレオチドを含み得、それらの各々は、核酸(例えばTIM遺伝子座由来のmRNA、cDNA、ゲノムDNA等)に特異的にハイブリダイズし得る。

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【0083】

TIM多型に特異的な抗体を、スクリーニングイムノアッセイに使用し得る。中性TIMの減少または増加、および/または喘息関連多型の存在は、喘息がTIMに関連することを示す。TIM関連喘息を有すると疑われる患者からサンプルを採取する。本明細書中で使用される場合、サンプルとしては、気管洗浄液、血液、脳脊髄液、涙、唾液、リンパ液、透析液等のような生物学的液体; 臓器または組織培養由来の液体; および生理学的組織から抽出された液体が挙げられる。そのような液体の誘導体および画分もその用語に含まれる。生検サンプル、例えば気管擦過等が特に興味深い。サンプル中の細胞数は、一般的

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に少なくとも約 10^3 、通常少なくとも 10^4 、より通常には少なくとも約 10^5 である。固体組織の場合には細胞を分離し得るか、または組織切片を分析し得る。あるいは細胞の溶解物を調製し得る。

【0084】

診断を、多くの方法によって行ない得る。異なる方法は全て、TIMに素因を与える多型を有すると疑われる患者の細胞における、正常TIMまたは異常TIMの欠如または存在または変化した量を決定する。例えば、検出は、従来の方法に従って行なわれる、細胞または組織学的切片の染色を利用し得る。目的の抗体を細胞サンプルに加え、そしてエピトープへの結合を可能にするのに十分な時間、通常少なくとも約10分間インキュベートする。抗体を、放射性同位体、酵素、蛍光剤、化学発光剤、または直接検出のための他の標識で標識し得る。あるいは、2次抗体または試薬を使用して、シグナルを増幅する。そのような試薬は、当該分野で周知である。例えば、一次抗体をビオチンに結合し、2次試薬として西洋ワサビペルオキシダーゼ結合アビジンを加え得る。最終的な検出は、ペルオキシダーゼの存在下で色を変化する基質を使用する。抗体結合の欠如または存在を、分離細胞のフローサイトメトリー、顕微鏡、ラジオグラフィー、シンチレーションカウンター等を含む様々な方法によって決定し得る。

【0085】

診断の代替方法は、溶解物中における抗体とTIMとの間の結合のインビトロ検出に依存する。サンプルまたはその画分におけるTIM結合の濃度の測定を、様々な特異的アッセイによって達成し得る。従来のサンドイッチ型アッセイを使用し得る。例えば、サンドイッチアッセイは、まずTIM特異的抗体を不溶性表面または支持体に結合し得る。TIM特異的抗体が試薬および本発明の方法全体と適合性がある限り、結合の特定の方式は重要ではない。TIM特異的抗体はプレートに共有結合的にまたは非共有結合的に、好ましくは非共有結合的に結合し得る。

【0086】

他のイムノアッセイが当該分野で公知であり、そして診断薬としての使用を見出し得る。オークターロニープレートは、抗体結合の簡単な決定を提供する。ウェスタンブロットを、タンパク質ゲルまたはフィルター上のタンパク質スポットに対して、望ましいようにTIMに特異的な検出システムを用いて、サンドイッチアッセイに関して記載したような標識方法を簡便に用いて、行ない得る。

【0087】

TIM遺伝子は、例えば発現の発達および組織特異的パターンの決定において、TIM発現を分析するために、およびインビトロおよびインビボで発現を調節するために、有用である。遺伝子の導入に有用なベクターとしては、プラスミドおよびウイルスベクターが挙げられる。特に関心のあるのは、哺乳動物細胞において一時的にまたは安定に維持される、レトロウイルスベクター（例えばマロニルマウス白血病ウイルスおよび改変ヒト免疫不全ウイルス；アデノウイルスベクター等）である。細胞ゲノムへの遺伝子のトランスフェクションおよび／または組込みのために、広く様々なベクターを採用し得る。あるいは、マイクロインジェクションは、遺伝子の適当な宿主細胞への導入のために融合等を利用し得る。例えば、Dhawanら（1991）*Science* 254:1509-1512およびSmithら（1990）*Molecular and Cellular Biology* 3268-3271を参照のこと。

【0088】

発現ベクターは、機能的mRNAを産生するように指向された転写開始領域を有する。天然の転写開始領域または外来性転写開始領域を利用し得る。プロモーターを、インビトロで組換え方法によって、または配列の染色体への相同的組込みの結果として導入し得る。 β -アクチンプロモーター、SV40初期および後期プロモーター、ヒトサイトメガロウイルスプロモーター、レトロウイルスLTR、メタロチオネイン応答要素（MRE）、テトラサイクリン誘導性プロモーター構築物等を含む、多くの強力なプロモーターが、当該分野で公知である。

【0089】

発現ベクターは一般的に、核酸配列の挿入を提供するために、プロモーター配列の近くに位置する簡便な制限部位を有する。転写開始領域、標的遺伝子またはそのフラグメント、および転写終止領域を含む転写カセットを調製し得る。転写カセットを、様々なベクター（例えばプラスミド；レトロウイルス（例えばレンチウイルス）；アデノウイルス；等）に導入し得、ここでベクターは細胞内で、一時的にまたは安定に、通常少なくとも約1日間、より通常には少なくとも約数日から数週間の間、維持され得る。

【0090】

アンチセンス分子を使用して、細胞内でTIMの発現を下方制御する。アンチセンス試薬は、アンチセンスオリゴヌクレオチド（ODN）、特に天然核酸から化学的修飾を有する合成ODN、またはそのようなアンチセンス分子をRNAとして発現する核酸構築物であり得る。アンチセンス配列は、標的遺伝子のmRNAに相補的であり、そして標的遺伝子産物の発現を阻害する。アンチセンス分子は、様々なメカニズムによって、例えば翻訳のために利用可能なmRNAの量を減少させることによって、RNAアーゼHの活性化によって、または空間的障害によって、遺伝子発現を阻害する。1つの、または組み合わせのアンチセンス分子を投与し得、ここで組み合わせは複数の異なる配列を含み得る。

【0091】

アンチセンス分子を、適当なベクターにおいて標的遺伝子配列の全てまたは一部を発現することによって産生し得、ここで転写開始は、アンチセンス鎖がRNA分子として産生されるように指向される。あるいは、アンチセンス分子は合成オリゴヌクレオチドである。アンチセンスオリゴヌクレオチドは、一般的に少なくとも約7ヌクレオチド長、通常少なくとも約12ヌクレオチド長、より通常には少なくとも約20ヌクレオチド長であり、かつ約500ヌクレオチド長より短く、通常約50ヌクレオチド長より短く、より通常には約35ヌクレオチド長より短く、ここで長さは阻害の効率、交差応答性の欠如を含む特異性、等によって支配される。7～8塩基長の短いオリゴヌクレオチドが、遺伝子発現の強力かつ選択的なインヒビターであり得ることが見出された（Wagnerら（1996）Nature Biotechnology 14：840-844を参照のこと）。

【0092】

（トランスジェニック動物）

本発明の核酸を使用して、遺伝的に改変された非ヒト動物、または細胞株における部位特異的遺伝子改変を産生し得る。用語「トランスジェニック」は、TIM遺伝子活性の欠失または他のノックアウトを有する、宿主細胞において安定に遺伝する外来性TIM遺伝子を有する、またはリポーター遺伝子に操作可能に連結した外来性TIMプロモーターを有する、遺伝的に修飾された動物を含むように意図される。トランスジェニック動物を、相同的組換えによって作成し得、ここでTIM遺伝子座は変化する。あるいは、核酸構築物をゲノム中に無作為に組み込む。安定な組込みのためのベクターは、プラスミド、レトロウイルスおよび他の動物ウイルス、YAC、等を含む。トランスジェニック哺乳動物（例えばウシ、ブタ、ヤギ、ウマ等）および特にげっ歯類（例えばラット、マウス等）に特に関心がある。

【0093】

「ノックアウト」動物は、遺伝的に操作して、内因性TIM機能を本質的に減少させるか、または排除する。「ノックアウト」を達成するために、異なるアプローチを使用し得る。天然TIMホモログの全てまたは一部の染色体欠失を誘導し得る。非コード領域、特にプロモーター領域、3'調節配列、エンハンサーの欠失、またはTIM遺伝子の発現を活性化する遺伝子の欠失。機能的ノックアウトもまた、天然TIM遺伝子の発現をブロックするアンチセンス構築物を導入することによって達成し得る（例えば、LiおよびCohen（1996）Cell 85：319-329を参照のこと）。

【0094】

外来性TIM遺伝子を有するトランスジェニック動物を作成し得る。外来性遺伝子は、通常動物宿主とは異なる種由来であるか、またはそうでなければコード配列または非コード

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配列で変化している。導入遺伝子は、野生型遺伝子、天然に存在する多型、または遺伝的に操作した配列（例えばコードまたは非コード領域に欠失、置換または挿入を有する以前に記載したもの）であり得る。導入配列は、TIMポリペプチドをコードし得るか、またはリポーター遺伝子に操作可能に連結したTIMプロモーターを利用し得る。導入遺伝子がコード配列である場合、それは通常構成的または誘導的であり得るプロモーター、および宿主動物における発現に必要な他の調節配列に操作可能に連結される。

【0095】

目的の特異的構築物としては、TIM発現をブロックするアンチセンスTIM、ドミナントネガティブTIM変異体の発現、およびTIM遺伝子の過剰発現が挙げられるがこれに限らない。lacZのような検出可能なマーカーを、TIM遺伝子座に導入し得、ここでTIM発現のアップレギュレーションは、表現型にいて容易に検出される変化を引き起こす。

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【0096】

TIM機能および調節の研究において、改変細胞または動物が有用である。例えば、候補薬剤の喘息に対する効果を決定するために、機能研究、薬剤スクリーニング等において動物を使用し得る。TIM遺伝子において一連の小さな欠失および／または置換を作成して、DNA結合、転写調節等における異なるエキソンの役割を決定し得る。それが通常産生されない細胞においてTIMタンパク質の発現を提供することによって、細胞の動きに変化を誘導し得る。これらの動物はまた、喘息の遺伝モデル（例えば優性対劣性）；異なる対立遺伝子の相対的効果、およびTIMとゲノムの他の場所にある他の喘息遺伝子との間の相乗的効果を調査するのに有用である。

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【0097】

相同的組換えのためのDNA構築物は、望ましい遺伝的改変を有するTIM遺伝子の少なくとも一部を含み、そして標的遺伝子座と相同的な領域を含む。無作為な組込みのためのDNA構築物は、組換えを媒介する相同的な領域を含む必要はない。簡便に、ポジティブおよびネガティブセレクションのためにマーカーを含む。相同的組換えによって標的化遺伝子改変を有する細胞を産生する方法は、当該分野で公知である。哺乳動物細胞をトランスフェクトする様々な技術に関して、Keownら（1990）Methods in Enzymology 185: 527-537を参照のこと。

【0098】

本発明の動物モデルと組み合わせて、薬剤スクリーニングを行ない得る。多くの哺乳動物遺伝子が、酵母およびより下等な動物にホモログを有する。そのようなホモログの生理的役割および他のタンパク質との相互作用の研究は、生物学的機能の理解を促進し得る。遺伝的相補性に基づくモデルシステムに加えて、酵母は、Chienら（1991）P. N. A. S. 88: 9578-9582において記載される、ツーハイブリッドシステムによって、タンパク質-タンパク質相互作用を研究する強力な手段であることが示された。ツーハイブリッドシステム分析は、TIMタンパク質による転写活性化を調査するために特に関心がある。

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【0099】

（化合物スクリーニング）

TIMに結合する、TIMの作用を調節または模倣するリガンドまたは基質を同定し得る。調査の領域は、免疫障害、喘息、癌、虚血-再灌流障害、およびストレスに対する細胞反応に関連する他の疾患の処置の開発である。薬剤スクリーニングは、影響された細胞におけるTIM機能の阻害、置換、または増強を提供する薬剤を同定する。例えば、TIM機能を逆転または阻害する薬剤は、Th2サイトカインのレベルを減少することによって、喘息における気管支応答性を減少し得、そしてTIMインヒビターは、アポトーシスを誘導する放射線処置および化学療法剤処置の効果を増強することによって、癌治療に対する腫瘍の感受性を増強し得る。特に関心があるのは、ヒト細胞に対して低い毒性を有する薬剤のスクリーニングアッセイである。この目的のために、標識インビトロタンパク質-タンパク質結合アッセイ、タンパク質-DNA結合アッセイ、電気泳動移動度シフトアッ

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セイ、タンパク質結合のイムノアッセイ等を含む、広範な様々なアッセイを使用し得る。精製タンパク質をまた、3次元結晶構造の決定のために使用し得、それを分子間相互作用、転写調節等のモデル化に使用し得る。

【0100】

本明細書中で使用される場合、用語「薬剤」は、シグナルチロシンキナーゼインヒビター、またはインテグリン結合部位のペプチドインヒビターのような、TIMの生理学的機能を変化させるまたは模倣する能力を有する、任意の分子（例えばタンパク質または医薬）を記載する。一般的に、複数のアッセイ混合物を、異なる薬剤濃度で並行して走らせ、様々な濃度に対する差次的反応を得る。典型的には、これら濃度のうち1つはネガティブコントロール（すなわち濃度ゼロまたは検出レベル以下の濃度）として供する。

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【0101】

候補薬剤は、多くの化学的クラスを含むが、典型的にはそれらは有機分子、好ましくは50以上および約2,500ダルトン以下の分子量を有する、小さい有機化合物である。候補薬剤は、タンパク質との構造的相互作用、特に水素結合に必要な官能基を含み、そして典型的には少なくとも1つのアミン基、カルボニル基、ヒドロキシル基、またはカルボキシル基、好ましくは少なくとも2つの化学官能基を含む。候補薬剤は、多くの場合1つまたはそれ以上の上記の官能基で置換された、環状炭素または複素環構造および／または芳香族または多環芳香族構造を含む。候補薬剤はまた、ペプチド、サッカライド、脂肪酸、ステロイド、プリン、ピリミジン、その誘導体、構造的アナログまたは組み合わせを含むがこれに限らない生物分子中に見出される。

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【0102】

候補薬剤を、合成または天然化合物のライブラリーを含む広範な様々な供給源から得る。例えば、広範な多様な有機化合物および生物分子の無作為および指向合成のために、無作為化オリゴヌクレオチドおよびオリゴペプチドの発現を含む、多くの手段が利用可能である。あるいは、細菌、真菌、植物、および動物抽出物形式の天然化合物のライブラリーが入手可能または容易に産生される。さらに、天然または合成的に産生されたライブラリーおよび化合物を、従来の化学的、物理的、および生化学的手段によって容易に改変し、そしてコンビナトリアルライブラリーを産生するために使用し得る。公知の医薬品に、アシル化、アルキル化、エステル化、アミド化等の指向性または無作為化学的修飾を行なって、構造的アナログを産生し得る。

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【0103】

スクリーニングアッセイが結合アッセイである場合、1つまたはそれ以上の分子を標識に結合し得、ここで標識は直接または間接的に検出可能なシグナルを提供し得る。様々な標識としては、放射性同位元素、蛍光剤、化学発光剤、酵素、特異的結合分子、粒子（例えば磁気粒子）等が挙げられる。特異的結合分子は、ビオチンおよびストレプトアビジン、ジゴキシンおよび抗ジゴキシン等のような対を含む。特異的結合メンバーに関しては、相補的メンバーを通常、公知の手順に従って、検出を提供する分子で標識する。

【0104】

様々な他の試薬をスクリーニングアッセイに含み得る。これらは、塩、中性タンパク質、例えばアルブミン、界面活性剤等のような試薬を含み、それらは最適なタンパク質-タンパク質結合を促進するおよび／または非特異的またはバックグラウンド相互作用を減少するために使用される。プロテアーゼインヒビター、ヌクレアーゼインヒビター、抗菌剤、等のような、アッセイの効率を改善する試薬を使用し得る。成分の混合物を、必要な結合を提供するいずれかの順序で加える。インキュベーションを任意の適切な温度、典型的には4℃～40℃の間で行なう。インキュベーション時間を、最適な活性のために選択するが、迅速なハイスループットスクリーニングを促進するために最適化し得る。典型的には0.1時間～1時間の間が十分である。

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【0105】

目的の他のアッセイは、TIM機能を模倣する薬剤を検出する。例えば、候補薬剤を、機能的TIMを欠く細胞に加え、そして機能的アッセイにおいてTIMを再生する能力に関

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してスクリーニングする。

【0106】

(治療方法)

TIM遺伝子またはタンパク質の活性を調節する薬剤（特にポリペプチドの活性または遺伝子の発現を阻害または上方制御する薬剤）は、治療または予防的介入点を提供する。発現を直接調節する薬剤（例えば発現ベクター、標的ポリペプチドに特異的なアンチセンス）；およびタンパク質に作用する薬剤（例えば特異的抗体およびそのアナログ、触媒活性をブロックする小さい有機分子）等を含む、多くの薬剤が、この活性を調節するのに有用である。

【0107】

核酸を特定の細胞に選択的に伝達するための方法を設計し得る。そのような細胞の例としては、T細胞等が挙げられる。目的の細胞に発現ベクターを選択的に発現する、特定の処置方法を設計する。神経細胞における選択的発現を達成する1つの技術は、コード配列を、主に免疫系細胞で活性なプロモーター（例えばIL-2プロモーター、T細胞抗原レセプタープロモーター等）に操作可能に連結することである。あるいは、またはそれに加えて、核酸を、核酸を目的の細胞に標的化する薬剤と共に投与し得る。例えば、核酸を、細胞表面抗原に特異的に結合する抗体と共に投与し得る。リポソームを利用する場合、エンドサイトーシスに関連する細胞表面膜タンパク質に結合する基質を、リポソームに結合して、リポソームを神経細胞に標的化し、そして取り込みを促進し得る。

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【0108】

アンチセンス分子を使用して、細胞における発現を下方制御し得る。アンチセンス試薬は、アンチセンスオリゴヌクレオチド(ODN)（特に天然核酸から化学的修飾を有する合成ODN）またはそのようなアンチセンス分子をRNAとして発現する核酸構築物であり得る。アンチセンス配列は、標的遺伝子のmRNAに相補的であり、そして標的遺伝子産物の発現を阻害する。アンチセンス分子は、様々なメカニズムによって、例えば翻訳のために利用可能なmRNAの量を減少することによって、RNAアーゼHの活性化、または空間的障害を介して、遺伝子発現を阻害する。アンチセンス分子を1つまたは組み合わせて投与し得、ここで組み合わせは複数の異なる配列を含み得る。

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【0109】

アンチセンス分子を、適当なベクターにおける、標的遺伝子配列の全てまたは一部の発現によって産生し得、ここで転写開始は、アンチセンス鎖がRNA分子として産生されるように指向される。あるいは、アンチセンス分子は合成オリゴヌクレオチドである。アンチセンスオリゴヌクレオチドは、一般的に少なくとも約7ヌクレオチド長、通常少なくとも約12ヌクレオチド長、より通常には少なくとも約20ヌクレオチド長であり、そして約500ヌクレオチド長より短い、通常約50ヌクレオチド長より短い、より通常には約35ヌクレオチド長より短く、ここで長さは阻害の効率、交差応答性の欠如を含む特異性等によって支配される。7～8塩基長の短いオリゴヌクレオチドが、遺伝子発現の強力かつ選択的なインヒビターであり得ることが見出された(Wagnerら(1996) Nature Biotechnology 14:840-844を参照のこと)。

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【0110】

内因性センス鎖mRNA配列の特異的領域を、アンチセンス配列と相補的になるように選択する。オリゴヌクレオチドの特異的配列の選択は、経験的な方法を使用し得、ここでいくつかの候補配列を、インビトロまたは動物モデルにおいて、標的遺伝子の発現阻害に関してアッセイする。配列の組み合わせも使用し得、ここでmRNA配列のいくつかの領域をアンチセンス相補性のために選択する。

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【0111】

アンチセンスオリゴヌクレオチドを、当該分野で公知の方法によって化学的に合成し得る(Wagnerら(1993)前出、およびMilliganら、前出を参照のこと)。好ましいオリゴヌクレオチドは、その細胞内安定性および結合親和性を増加させるために、天然ホスホジエステル構造から化学的に改変される。多くのそのような改変が文献にお

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いて記載され、それはバックボーン、糖または複素環塩基の化学を変化させる。

【0112】

バックボーン化学の有用な変化は、ホスホロチオエート；架橋していない酸素がどちらも硫黄で置換されたホスホロジチオエート；ホスホラミダイト；アルキルホスホトリエステルおよびボラノホスフェート（boranophosphates）である。アキラルなホスフェート誘導体としては、3'-O'-5'-S-ホスホロチオエート、3'-S-5'-O-ホスホロチオエート、3'-CH₂-5'-O-ホスホネート、および3'-NH-5'-O-ホスホロアミダート（phosphoramidate）が挙げられる。ペプチド核酸は、リボースホスホジエステルバックボーン全体を、ペプチド結合で置換する。安定性および親和性を増強するために、糖の改変も使用する。デオキシリボースのα-アノマーを使用し得、ここで塩基は、天然β-アノマーに関して転位する。リボース糖の2'-OHを、2'-O-メチルまたは2'-O-アシル糖を形成するように変化させ得、それは親和性を損なうことなく分解に対する抵抗性を提供する。複素環塩基の改変は、適当な塩基対形成を維持しなければならない。いくつかの有用な置換は、デオキシチミジンのためにデオキシウリジン；デオキシシチジンのために5-メチル-2'-デオキシシチジンおよび5-プロモ-2'-デオキシシチジンを含む。5-プロピニル-2'-デオキシウリジンおよび5-プロピニル-2'-デオキシシチジンは、それぞれデオキシチミジンおよびデオキシシチジンを置換した場合に、親和性および生物学的活性を増加させることが示された。

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【0113】

所望の薬理学的活性を有する化合物を、生理学的に受容可能なキャリア中で、TIM機能の欠損に起因する喘息の処置のために宿主に投与し得る。その化合物をまた、TIM機能を増強するために使用し得る。治療薬を、経口、局所的、非経口的（例えば皮下）腹腔内、ウイルス感染に従って、血管内等の様々な方法で、に投与し得る。吸入処置が特に興味深い。導入の方法によって、その化合物を様々な方法で処方し得る。処方中の治療的に活性な化合物の濃度は、約0.1～100重量%で変化し得る。

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【0114】

薬学的組成物を、顆粒、錠剤、丸剤、坐剤、カプセル、懸濁液、軟膏、ローション等のような、様々な形式で調製し得る。経口および局所使用のために適当な薬学的等級の有機または無機キャリアおよび／または希釈剤を使用して、治療的に活性な化合物を含む組成物を作成し得る。当該分野で公知の希釈剤としては、水性媒体、植物油および動物油ならびに脂肪が挙げられる。安定化剤、湿潤剤および乳化剤、浸透圧を変化させる塩、または適当なpH値を生じさせる緩衝液、および皮膚浸透増強剤を、補助剤として使用し得る。

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【0115】

本発明は、変化し得るので、特定の方法論、プロトコール、細胞株、動物種または属、および記載された試薬に制限されないことが理解される。本明細書中で使用される用語は、特定の実施形態を記載する目的のためのみであり、そして添付の請求によってのみ制限される、本発明の範囲を制限することを意図しないことが理解される。

【0116】

本明細書中で使用される場合、単数形「a」、「and」および「the」は、文脈が明らかに他に規定しなければ、複数の指示対象を含む。従って、例えば「細胞（a cell）」への言及は、複数のそのような細胞を含み、そして「配列（the array）」への言及は、1つまたはそれ以上のアレイおよび当業者に公知のその等価物を含む等である。本明細書中で使用される全ての技術的および科学的用語は、明らかに他に示されなければ、本発明が属する当業者に通常理解されるのと同じ意味を有する。

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【0117】

本明細書中で言及された全ての出版物は、例えばここで記載される発明と関連して使用され得る、その出版物で記載された、細胞株、構築物および方法論を記載および開示する目的のために、本明細書中で参考文献に組み込まれる。上記および本文全体で議論された出版物は、本出願の提出日より前にそれらが開示されたためだけに提供される。本明細書中

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で何物も、以前の発明によって発明者がそのような開示に先立つ権利がないという承認として解釈されない。

【0118】

以下の実施例は、本発明の作成および使用方法の完全な開示および記載を当業者に提供するために述べられ、そして本発明として考えられるものの範囲を制限することを意図しない。使用された数（例えば量、温度、濃度等）に関して、正確さを保証するために努力がなされたが、いくらかの実験誤差およびずれは許されるべきである。他に示されなければ、割合は重量による割合、分子量は平均分子量、温度は摂氏、そして圧は大気またはその付近である。

【0119】

（実験）

喘息感受性遺伝子に関してヒト5q23-35領域を分析するために、発明者らはいくつかの潜在的な利点を提供するマウスモデルを利用した。環境の変化を調節し得、複数の表現型を同時に試験し得、そして同系交配株をアレルゲンで感作して、ヒト喘息の基本的な特徴である気道過剰応答性（AHR）を発現し得る。発明者らは、ヒト染色体5qに相同的な小さな染色体領域でのみ異なる、類遺伝子性の同系交配マウス株を利用し、それによってこの領域を、領域外の遺伝的変異の非存在下で研究することを可能にした。ポジショナルクローニングは、T細胞膜タンパク質（Tim）をコードする新規遺伝子ファミリー、TIM-1、TIM-2、TIM-3、TIM-4、TIM-5、TIM-6、およびTIM-7を明らかにし、このうちTIM-1、TIM-3およびTIM-4の主要な配列異型が、Taprと完全に同時分離する。

【0120】

IL-4産生および気道過剰応答性は、HBAマウスで減少される。発明者らは、個々のDBA/2染色体から受け継がれた別々のゲノム間隔を有する、BALB/cゲノムバックグラウンドで産生された類遺伝子性マウスを調査した。BALB/cマウスは、Th2に偏った、増強したAHRを伴うアトピーに似た免疫反応を発現し、一方DBA/2マウスは、AHRの発現に対して保護する、減少されたIL-4反応を発現する。これら類遺伝子性株のいくつかを、抑制されたTh2応答性に関してスクリーニングすることによって、発明者らは1つの類遺伝子性株、C.D2-Es-Hba（HBA）を同定し、これはヒト5q23-35に相同的な、DBA/2マウスから受け継がれた第11染色体の部分を含んでいた。図1aは、免疫コントロールBALB/cマウスからのリンパ節細胞が、予測される通り、高レベルのIL-4を産生することを示し、Th2に偏った免疫反応を発現するBALB/cマウスの傾向を確認した。対照的に、HBAマウス由来のリンパ節細胞は、DBA/2マウスで観察されるものと同様の、有意に低いレベルのIL-4を産生した。それに加えて、HBAマウスはBALB/cマウスと比較して、有意に少ないIL-13およびIL-10、そしていくらか低いレベルのIL-5を産生し、一方IFN-γの産生は図1bに示すように増加した。これらの結果は、ヒト5q23-35との保存されたシンテニーの大きな領域を有する、HBA第11染色体のDBA/2由来の領域は、抗原特異的IL-4、IL-13、およびIL-10産生を減少し、IFN-γ産生を増強し、そしてBALB/cサイトカイン表現型をDBA/2サイトカイン表現型へ転換する遺伝子を含むことを示した。

【0121】

HBAマウスを、Th2に偏った免疫反応と関連する、抗原誘発性気道過剰応答性（AHR）を発現する能力に関して調査した。アレルゲンによる感作およびチャレンジ時に、コントロールBALB/cマウスは高いAHRを発現し、一方同様に免疫したHBA類遺伝子性マウスは、DBA/2マウス同様、メタコリンに反応して通常の気道応答性を示した（図1c）。集合的に、これらの結果は、第11染色体上の単一の遺伝子座における遺伝的変異が、Th2サイトカイン産生およびAHRの両方を調節することを強く示唆し、従って発明者らは、HBAマウスにおける関連する遺伝的決定基を、単一遺伝子座、T細胞および気道表現型レギュレーター（Tapr）と仮に呼ぶ。

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【0122】

本出願人らは (BALB/c × HBA) F1 マウスもまた調査し、それは BALB/c マウス同様、高レベルの IL-4、IL-13、および IL-10 を産生し (図 1 a および 1 b)、そして上昇した抗原誘発 AHR を発現した (図 1 c)。これらの結果は、第 11 染色体の DBA/2 対立遺伝子が、IL-4 合成を調節する他の遺伝子と独立して、劣性な様式で IL-4 産生および AHR を低減したことを示す。対照的に、(BALB/c × DBA/2) F1 マウスは、低レベルの IL-4 を産生し、そして免疫化に対して通常の気道応答性を有しており (図 1)、DBA ゲノムの他の領域由来の遺伝子座も IL-4 産生および抗原誘発 AHR を調節すること、そして DBA/2 対立遺伝子は、全体として優性な様式で IL-4 産生および AHR を制限するように機能することを示す。これらの結果は、アトピー性形質の多遺伝子、複雑な性質を強調し、そして喘息表現型にもまた影響を与える複数の上位遺伝子からの干渉なしに、単一遺伝子座を単離および特徴づけるために、類遺伝子性株を使用する潜在的な利点を示す。

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【0123】

AHR および IL-4 応答性を調節する遺伝子座、Tapr の遺伝的マッピング。以前に、HBA マウスの類遺伝子性領域が、2 つの第 11 染色体マーカー、ヘモグロビン-α2 (hba-α2) およびエステラーゼ-3 (es-3) 遺伝子座を含む、36 のゲノム範囲のマーカーで詳細に描写された。第 11 染色体の外側、HBA ゲノムは、BALB/c から受け継がれた。DBA/2 および BALB/c マウス間で多型であることが公知の 25 個の単純配列長多型 (SSLP) マーカーを用いたより正確な分析が、HBA マウスは DBA/2 から第 11 染色体の 2 つの部分を受け継いだことを示した (図 2、左カラム)。近位領域は、染色体 5 q 23-35 と相同性を有する 20 cM の部分を含んでおり、それはヒト喘息連鎖研究で関係した遺伝子座を、喘息のマウスモデルで同定し得る可能性を提供した。

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【0124】

TH2-AHR 調節遺伝子座、Tapr をより高い分解能でマッピングするために、(BALB/c × HBA) F1 マウスを、HBA マウスと戻し交配して N2 動物を産生した。この戻し交配アプローチを用いて、HBA 親によって寄与される対立遺伝子のセットを前もって決定し、そして F1 親によって寄与される対立遺伝子のセットを遺伝子型決定によって評価し得る。従って、類遺伝子性領域に情報を得られるハプロタイプを産生する組換えの発生を、N2 マウスにおいて検出し得、そして Tapr の類遺伝子性間隔における遺伝子座への連鎖を評価するために使用し得る。Tapr の劣性性質のために、本出願人らは、これら戻し交配由来の N2 マウスを試験して、HBA Tapr 表現型を与えるのに十分な HBA 由来遺伝子の最低限のホモ接合体領域を同定した。2,000 を超える N2 動物を産生し、そして遺伝子型決定した。SSLP マーカーを用いて、本出願人らは情報を得られる組換え発生を有する N2 マウスを選択し、そしてこれらの N2 マウスを、カサガイヘモシアニン (KLH) による免疫化に反応して IL-4 を産生する能力に関して表現型決定した。この一次分析において、本出願人らは、関連する遺伝子座が、近位類遺伝子性領域、D11Mit135 と D11Mit260 との間に存在することを決定した。より高い分解能で Tapr をマッピングするために、22 のさらなるマーカーを同定し、そして関心のある領域で 0.1-1 cM の分解能を提供するために利用した。

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【0125】

数ヶ月にわたって行なわれた IL-4 サイトカイン分析の結果を正確に比較するために、各実験の IL-4 指数を各 N2 マウスに関して生成し、(B-x/B-H)、ここで B = BALB/c マウス由来の細胞による IL-4 産生、H = HBA マウス由来の細胞による IL-4 産生、そして x = 評価される N2 マウス由来の細胞による IL-4 産生である。IL-4 の高い濃度 (BALB/c 様) は、0 に近い指数値で表され、そして IL-4 の低い濃度 (HBA 様) は、1.0 に近い指数値で表される。“B” および “H” 値は、本出願人らが試験した、情報を得られる組換えを有する 3-6 匹の N2 マウスのグループそれぞれに関して、3-5 匹のコントロールマウスで確立された。指数値は、二方式分布に

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なり（図3a）、そこで非組換えHBA遺伝子型を有するN2マウスに関連する表現型指数は、非組換え（BALB/c×HBA）F1遺伝子型を有するN2マウスに関連する表現型指数よりも有意に高かった（ $P < 0.0001$ 、対の学生のt-試験）。

【0126】

独特の遺伝子型を有するマウスに関して、これは連鎖分析において重要であるので、本出願人らは数ヶ月を費やして、サイトカイン産生およびAHRの単回測定が適当であることを保証した。まず、関心のある組換えを有するN2マウスをそれぞれ試験すると同時に、本出願人らは、遺伝子型が厳密にHBAまたはF1（BALB×HBA）である、各“組換えN2”の“非組換え”同胞も試験した。さらに、本出願人らは、その特定のN2遺伝子型を有する個々のマウスをより多く有するために、関心のある組換えを有するN2マウスのいくらかを、HBAマウスに戻し交配することによって、さらなるN3マウスを繁殖させた。全ての値は所定の遺伝子型を有する試験した個々のマウスの値の平均であった。この方法で、本出願人らは、サイトカイン産生およびAHRの測定、ならびに本出願人らは生物学的システムで固有の変動性によるアッセイの変動を克服したことを確信する。

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【0127】

組換えハプロタイプを受け継いだN2マウスに関連するIL-4値は、二方式分布で分離したので（図3a）、高IL-4反応を調節する遺伝子座は、マーカーD11Mit271およびD11Mit22の間に位置することを示し得た（図3b）。さらに、高レベルのIL-4産生が、Kim1sscpに存在するBALB/c対立遺伝子を有する全てのマウスで観察され、そして低レベルのIL-4産生が、Kim1sscpにおいてホモ接合体HBA遺伝子型を有する全てのマウスで観察された。従って、Taprは、Kim1sscp、Rattus norvegicus Kidney Injury Molecule (Kim-1)のマウスホモログ中のイントロンマーカーと非組換え性である。対照的に、Taprは他の全てのマーカーから、少なくとも1つの組換えを有して分離される。TaprおよびKim1sscpが共に分離するという事実は、Tapr遺伝子座がKim1sscpの非常に近くに位置するか、または区別できないことを示した。D11Mit271とD11Mit22との間の組換えハプロタイプ頻度に基づいて、本出願人らは組換え頻度を0.0039と計算し、それは、Tapr遺伝子座は小さい0.3-0.5cM領域に位置することを示す。本出願人らはまた、TaprとIL-4との間の組換え頻度を0.08と計算した。従って、TaprはIL-4サイトカインクラスターから5-10cM離れた場所に位置するが、これは、ヒトアトピーおよび喘息に連鎖する5q23-35と高度に保存されたシンテニーを有するマウスゲノムの領域内にある。

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【0128】

相似アプローチを用いて、本出願人らは、情報を得られる組換えハプロタイプを有するマウスにおいて、アレルゲン誘発AHR表現型の分離を調査した。指数化AHR値を用いて、N2マウスは明らかに親の表現型を示し、それは感作N2マウスのグループにおけるAHR指数値の柱状グラフにおいて、二方式分布を生成した（図3c）。1,000を超えるN2マウスに関連するAHR表現型の分離を分析することによって、本出願人らは、AHR反応を調節する遺伝子座も、マーカーD11Mit271とD11Mit22との間に位置し（図3d）、そしてAHR表現型はKim1sscpと非組換えであることを示した。従って、本出願人らは、IL-4応答性およびAHRはどちらも、Tapr遺伝子座と同時分離することを示し、それは同じ遺伝子座がIL-4発現およびAHRをどちらも調節することを示唆する（図3）。

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【0129】

これらの発見はさらに、Tapr遺伝子座は、以前に「候補」アトピー感受性遺伝子または喘息感受性遺伝子と考えられていた、IL-4サイトカインクラスターおよびクラスター内のサイトカイン遺伝子に対して5cMを超えてcentromericであることを示す。本出願人らのマッピング結果はまた、Taprは、IL-12p40遺伝子および T_H1 -IL12調節遺伝子座、Tpmを含むマウス第11染色体の領域のどちらとも遺伝的に分離することを確立した。

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【0130】

マウスおよびヒトホモログは、T a p rをヒト5 q 3 3に固定する（a n c h o r）。T a p r遺伝子座の周囲に複合マップを構築するために、本出願人らは、M o u s e G e n o m e D a t a b a s e (M G D) 連鎖、戻し交配、および照射ハイブリッド地図から入手可能な情報を統合し、そしてヒトゲノムの地図において保存されたシンテニーの領域を同定した。現在の照射ハイブリッドマップは、公知の遺伝子またはu n i g e n e クラスターと広範囲の相同性を有する、いくつかの発現配列タグ（E S T）を含む、D 1 1 M i t 2 7 1およびD 1 1 M i t 2 2の近くに存在するマーカーを、マウスゲノムの物理的地図に配置する。本出願人らはさらに、これらのマーカーおよびその関連するE S Tを、公知の遺伝子クラスターに対する、以前には同定されていない類似性に関して調査した。本出願人らは、ヒトゲノムに対する比較のために、これらのマーカーを骨組みに集合させた。このアプローチを用いて、本出願人らは、特定の照射ハイブリッドマーカーと以下のヒト遺伝子との間に有意な類似性を発見した：K I A A 0 1 7 1、A d a m - 1 9、S o x - 3 0、P i r - 1 2 1、C r s p 9 (C r s p 3 3)、およびh H A V c r - 1 (h H A V c r - 1)。図4は、一旦本出願人らがこれらの遺伝子を本出願人らの側面に位置するマーカー間のマウスゲノムの物理的地図に固定したことを示す。本出願人らは、H u m a n G e n o m e B r o w s e rにおいてそれらの遺伝子の位置決定をし得る。

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【0131】

この領域におけるマウスおよびヒトゲノム間の高度の保存は、T a p r遺伝子座のヒト5 q 3 3. 2に対する連鎖を示す。図4に示すように、本出願人らは、ヒト地図のこの領域における全ての公知の遺伝子およびE S Tを同定した。ヒトh H A V - c rおよびK i m - 1のマウスホモログの近くにある特に関心のある遺伝子は、I L - 2誘導性T細胞キナーゼ（I t k）およびS P - 1転写因子（C r s p 9）の補調節因子を含み、これらはどちらもT細胞の分化に関与することが知られている。本出願人らは、これら候補遺伝子由来のコード領域を配列決定し、そしてI T KまたはC R S P - 9のどちらにも多型を発見しなかった。

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【0132】

新規T細胞表面タンパク質ファミリーのT a p r領域への位置決定。ラットK i m 1のマウスホモログは、0. 4 c M領域内に位置し、そしてT a p rと密接に連鎖するので、本出願人らは公共に利用可能なデータベースを調査し、そしていくらかの配列類似性を有するE S Tのクラスターを発見した。それらは部分的な適用範囲しか提供せず、そして大きな変異部分を含んでいた。K i m - 1の最も近いヒトホモログは、ヒトA型肝炎ウイルス細胞レセプター、h H A V - c rであり、そしてヒトゲノムのt B L A S T検索は、おそらく遺伝子ファミリーのメンバーである、K i m - 1の2つのさらなるホモログもまた、ヒト第5染色体およびマウス第11染色体に位置することを示唆した。

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【0133】

c o n A刺激脾臓細胞由来のc D N Aを使用して、本出願人らはK i m 1の2つのマウスオルソログを同定およびクローニングした。本出願人らはそれらをT i m 1およびT i m 2と名付け、図5 Aに示すようにT a p r領域に位置する。T I M - 3は、3番目の、より関連が遠い、K I M - 1のオルソログである。

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【0134】

この遺伝子ファミリーの3つのメンバーは全て、刺激T細胞によって発現され、そして3つの形式は全てマウス第11染色体／ヒト第5染色体のT a p r領域に位置し、ここでそれらは、免疫グロブリン（I g）ドメイン、ムチンドメイン、およびリン酸化部位を有する細胞内尾部を含む、共通の構造モチーフを有する細胞表面糖タンパク質をコードする。これらタンパク質の細胞機能は未知であるので、本出願人らはその遺伝子を、T細胞、免疫グロブリンドメイン、ムチンドメイン（T i m）遺伝子ファミリーのメンバーと呼ぶ。マウスT i m 1は、ラットK i m 1およびアフリカミドリザルおよびヒトで同定されたH A V c r - 1のマウスホモログである。T i m 2は、本研究以前にはどの有機体においても同定されたことのない、以前には未知の遺伝子である。

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【0135】

マウス *Tim1* 遺伝子は、305アミノ酸膜タンパク質をコードし、それはラット *KIM-1* と78%の全体同一性、そしてヒト *HAVcr-1* と35%の同一性を有する。図5Bに示した、マウス *TIM-1*、ラット *KIM-1*、ヒト *HAVcr-1* およびアフリカミドリザル *HAVcr-1* を用いたギャップ多重配列アライメント (*gapped multiple sequence alignment*) は、これらの種における *TIM-1* / *KIM-1* / *HAVcr-1* タンパク質間の相同性の程度を示す。*TIM-1* の細胞質領域は、2つのチロシン残基を含み、そして高度に保存されたチロシンキナーゼリン酸化モチーフ、*RAEDNIY*を含み、それは *TIM-1* の予測される *Itk* 部位および *EGFR* キナーゼ部位、*SRAEDNIYIVEDRP* と一体である。*TIM-1* のムチンドメインは、複数のO-結合糖鎖付加部位を有し、そして免疫グロブリンドメインに2つのN-結合糖鎖付加部位が見出される。

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【0136】

同様の305アミノ酸膜タンパク質である *TIM-2* は、マウス *TIM-1* と64%の同一性、ラット *KIM-1* と60%の同一性、および *hHAVcr-1* と32%の同一性を有する (図5A、B)。*TIM-1* と同様、*TIM-2* は2つの細胞外N-結合糖鎖付加部位、および多くのO-結合糖鎖付加部位を有するセリン、スレオニン-リッチなムチンドメインを有する。*TIM-2* もまた、細胞内チロシンキナーゼリン酸化モチーフ、*RTRCEDQVY* を有する。

【0137】

Tim3 は、複数の細胞外糖鎖付加部位および細胞内チロシンリン酸化モチーフを有する、同様の膜貫通糖タンパク質構造を有する281アミノ酸膜タンパク質をコードする。*TIM-3* においては、ムチンドメインは *TIM-1* および *TIM-2* におけるほど顕著ではないが (図5A)、T細胞に発現した *TIM-3* は、おそらくAPC上のリガンドと相互作用し、そしてAPC活性化を変化させる。*TIM-3* は、4つのN-結合糖鎖付加部位、および5つのO-結合糖鎖付加部位を有し、*TIM-3* は、*TIM-1* および *TIM-2* と同様、高度に糖鎖付加されており、そして抗原提示細胞のような他の細胞に存在するリガンドと相互作用し得ることを示唆する。

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【0138】

Tim4 は、マウスでは344アミノ酸のタンパク質、ヒトでは378アミノ酸のタンパク質をコードする。予測される *TIM-4* はまた、高度に保存されたシステイン残基を有する *IgV* 様ドメイン、スレオニン-リッチなムチン様ドメイン、および短い細胞内尾部を有して、他の *TIM* タンパク質の一般的な膜糖タンパク質構造モチーフを共有する。しかし、*TIM-4* は、他の *TIM* タンパク質に存在するリン酸化チロシンモチーフを欠き、従って他の *TIM* タンパク質の機能を調節し得る。

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【0139】

各 *TIM Ig* ドメインは、細胞接着、生存、および腫瘍形成、ならびにヘルパーT細胞の分化の調節に関与している *TIM* のような膜貫通タンパク質である、オステオポンチンで見出される *SVVYGLR* モチーフに似た、予測されるインテグリン結合モチーフを共有する。このインテグリン結合モチーフは、アルファ (9) 特異性およびアルファ (4) 特異性を示す。

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【0140】

3つの *Tim* 遺伝子に関する *BALB/c* および *HBA/DBA* コード領域の配列比較は、*TIM-1*、*TIM-3*、および *TIM-4* における主要な多型を明らかにしたが、*TIM-2* ではなかった。*TIM-1* において、これらの多型は3つのアミノ酸の違いおよび *HBA/DBA* における15のアミノ酸欠失をコードする。7つの予測されるアミノ酸の違いが *TIM-3* において同定された (図5c)。ゲノム配列が、欠失を含むこれらの多型は、スプライシング異型ではなく、真の多型であることを確認する。他のマウス株における *TIM-1* および *TIM-3* のゲノム部分のさらなる配列決定によって、本出願人らは、低減された *T_H2* および *AHR* 反応を発現する傾向がある点で *DBA/2* と類似の

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株であるC57／BL6もまた、Tim1およびTim3のHBA／DBA対立遺伝子を有することを発見した。TIM-1およびTIM-4の多型は、シグナルおよびムチン様ドメインに位置し、一方TIM-3において同定された多型は、Igドメインに集中している（図5c）。Igおよびムチンドメインを有する糖タンパク質において、どちらかのドメインにおける異型は、MAdCAM-1で示されたように、レセプターーリガンド相互作用に影響し得る。TIM-1およびTIM-4の予測される切断部位はシグナル配列における多型によって変化しないが、多型が切断の効率および／またはレセプターの細胞表面への往来に影響し得ることが可能である。これらのTim配列および多型は、免疫反応、およびヒトにおけるHAVウイルス病原性に重要である。

【0141】

本出願人らのN2戻し交配由来のゲノムDNAサンプルの分析は（図3）、TIM-1およびTIM-3多型はTaprと完全に同時分離することを示した。これらの観察はTIM-1、TIM-3、または両方における変化がAHRおよびTH2による炎症の変化をどの程度担うか識別しないが、本出願人らはヒトTIM-1（hHAVcr-1）および／またはTIM-3における多型は、喘息感受性とヒト染色体5qとの間の強力な関連の基礎となることを示唆する。この考えは、ヒトTim1のコード領域における主要な異型が、ヒトゲノムおよびESTデータベースの調査で明らかであるという事実によって支持される。これらヒトcDNA異型と、以前に記載されたサルHAVcr-1の異型およびここで同定されたマウス異型との比較は、TIM-1の予測されるタンパク質配列において広範囲の変異が存在することを示す（図5b、c）。この高い変異の程度が、TIM-1およびそのファミリーメンバーを、喘息感受性候補遺伝子として最も綿密に研究されたサイトカインおよびサイトカインレセプターのような、他の多くの候補遺伝子と区別する。それに加えて、Tim1と喘息感受性との間の関連は、ダニ感受性小児喘息の、Tim1およびTim3から約0.5メガベースであるマーカー、D5S820に対する有意な連鎖（平均LODスコア＝4.8）の報告によってさらに支持される（図4）。

【0142】

上記の遺伝的多型に加えて、選択的スプライシングによって生じる、TIM遺伝子におけるいくつかの発現多型が存在する。TIM-1、TIM-2、およびTIM-4 mRNAの選択的スプライシングは、いくつかのTIM異型を産生し、それらのいくつかは分泌される、可溶性形式のTIMレセプターである。これらのスプライシング異型は、代わりに（alternate）5'非翻訳領域を有するTIMスプライシング異型と共に、TIMタンパク質の細胞特異的、および状態特異的発現パターンに寄与し得る。

【0143】

T細胞はTaprに影響を与える。Tapr遺伝子座の機能をよりよく理解するために、本出願人らは、Taprの対立遺伝子異型が、T細胞または抗原提示細胞（APC）の機能に影響を与えるかどうか決定した。これらの実験のために、本出願人らは、HBAバックグラウンドを有する（HBA DO11.10）卵白アルブミン（OVA）-特異的T細胞レセプター（TCR）トランスジェニックマウス（Tg）を産生し、本出願人らはそれらをBALB／cバックグラウンドを有する（BALB／c DO11.10）TCR-Tgマウスと比較した。これら株のどちらか由来の精製CD4＋T細胞を、OVAおよびBALB／cまたはHBA骨髓のいずれか由来の樹状細胞（DC）と同時培養し、そして産生されたサイトカインを評価した。照射脾臓細胞および他の組織は、高レベルのTIM遺伝子を発現することが発見されたので、この実験のためにAPCとして照射脾臓細胞は使用しなかった。

【0144】

BALB／c DO11.10 T細胞は、抗原提示細胞の供給源とは独立した方式で、HBA DO11.10 T細胞より高レベルのIL-4およびIL-13を産生した（図6A）。それに加えて、一次または二次刺激いずれかの間のAPCの供給源にかかわらず、CD4 T細胞の供給源が、各抗原濃度において産生されたIL-4／IL-13の量を決定した。細胞型の各組み合わせの培養上清において、同等レベルのIL-12が検

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出され、さらにBALB/cおよびHBA DC機能は同様であることを示した。さらに、BALB DO11.10およびHBA DO11.10 T細胞は、同等レベルのIL-2を産生し、そして2次培養中にOVAに反応して同様のレベルの増殖を示し、HBAおよびBALB/c T細胞は同様に活性化されるが、それらが産生するTh2サイトカインのレベルがかなり異なることを示した。

【0145】

本出願人らは図6Bにおいて、本出願人らのDO11.10/DCシステムにおける1次培養の最初の12時間以内に、本出願人らはTIM-1のmRNAがBALB/cおよびHBA CD4+ T細胞の両方によって発現されることを発見したことを示す。1次刺激から4日以内に、本出願人らはBALB/c DO11.10の上清において有意なレベルのIL-13を発見し、そしてHBA DO11.10の上清においては検出しない。この違いは、36時間においてmRNAレベルで検出可能である(図6B)。12および36時間の間に、IL-13 mRNAの発現はHBA CD4 T細胞において低減され、一方IL-13の発現はBALB/c CD4 T細胞において維持される。従って、抗原に対する1次反応の間に、BALB/c CD4 T細胞は、HBA CD4 T細胞よりも強力なTh2反応を発現する。本出願人らの発見は、Taprは、1次抗原特異的反応の間にヘルパーT細胞の分化を調節することを示し、そして本出願人らはこれらの反応の最も初期段階の間に、CD4 T細胞においてTIM-1の発現を検出する。

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【0146】

成熟Th1およびTh2サブセットへの分化後に、ヘルパーT細胞は、Th1細胞はTIM-3を発現し、一方Th2細胞は選択的にTIM-1を発現するように、RT-PCRによって傾倒されたTIM発現を示す。全てのT細胞集団は弱いTIM-4発現を示す。TIM-1によるItkシグナルがTh2分化を促進するようであるが、TIMタンパク質によるEGFRシグナルが、エフェクターおよび特にメモリーT細胞集団における細胞生存を増強するようである。ItkはT細胞および肥満細胞にのみ発現されるので、TIM-1に対するItkキナーゼ活性は免疫細胞、特に喘息およびアレルギーに關与するものに制限される。しかし、EGFRのような他のタンパク質チロシンキナーゼが、虚血上皮細胞、照射脾臓細胞、および腫瘍細胞を含む、他の組織によって発現されるTIMタンパク質の機能に關与する。

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【0147】

これらの研究において、本出願人らは、喘息の基本的な特徴であるTh2サイトカイン産生および抗原誘発AHRの発現を調節する遺伝子座である、Taprをマッピングした。本出願人らは、繰り返しアトピーおよび喘息に連鎖されてきたヒトゲノムの領域である、ヒト染色体5qに相同的な染色体部分を有する、間隔特異的類遺伝子性マウス(HBA)を用いて、Taprを位置決定した。この領域はまた、脊髄形成異常および腫瘍性細胞発生異常と関連する5q症候群に繰り返し連鎖された。複雑な形質をより単純な、あるいは単一の遺伝子、形質に変換するこの類遺伝子性マウス戦略を用いて、本出願人らはTaprの間隔を0.4cM間隔まで狭め、この領域におけるいくつかの候補遺伝子を配列決定し、そしてTIM遺伝子ファミリーをポジショナルクローニングした。

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【0148】

TIM遺伝子ファミリーは、以前に記載されたことがない。本出願人らは、全長cDNA配列を同定およびクローニングし、そしてHBAマウスと比較してBALB/cのTIM-1タンパク質において有意な多型を発見した。本出願人らは、TIM-1およびTIM-3のBALB/c配列は、AHRおよびアレルギー性T細胞反応に対する感受性に關連し、一方HBA配列は、これらの反応に対する保護に關連することを見出した。TIM-3は、分化したTH1によって選択的に発現される。多型Tim3異型とTaprとの關連は、TIM-3がTH1およびTH2細胞機能を調節し得ることを示唆する。しかし、Tim3における変異をまた、Tim4またはTim1に緊密に連鎖したハプロタイプに歸し得る。

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【0149】

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本出願人らは、いくつかの理由により、T I M-1は免疫系の調節において（特に喘息およびアレルギー性疾患に関して）、およびストレス（低酸素、栄養素の欠損、照射、化学療法等）に対する反応における上皮および造血細胞の生存の調節において、非常に重要な役割を果たしていると考える。まず、T i m 1は、T i m 3と同様、T a p r効果が起こる可能性が最も高い時である、1次抗原刺激の間にC D 4 T細胞に発現される。T細胞は、A H Rの発現および喘息の病因に決定的な役割を果たし、本出願人らの結果は、T a p rは、I L-13の産生および続くT細胞分化を調節することによって、T h 2反応に対する初期のC D 4の傾倒を増強することによって、喘息に影響を与えることを示唆する。2番目に、乳児または小児期におけるヒトでのH A V感染は、喘息およびアレルギーの発現と逆比例して関連する。本出願人らは、H A VのT I M-1 / H A V c r-1との相互作用が、T細胞サイトカイン産生を変化させ得、そうでなければアトピーおよび喘息に罹患しやすい個人において、偏ったT h 1 / T h 2 バランスを逆行または予防でき得ることを示唆する。麻疹ウイルスレセプターであるS L A Mは、ウイルス相互作用によって変化し得る方式でT h 1 / T h 2 バランスを調節する、別のT細胞表面糖タンパク質の例である。麻疹ウイルスに関するS L A M、またはH I Vに関するC D 4、C C R 5、およびC X C R 4のような、いくつかのウイルスレセプターは、宿主自身の免疫系のレセプターであるので、感染が成功しない場合でも、ウイルスレセプターによるシグナル伝達は、サイトカインの放出および疾患の発現を引き起こし得る。

【0150】

3番目に、T I M-1における多型は、本出願人らが観察する、異なる型のヘルパーT細胞反応と関連する。従って、T I M-1の異型はそれ自身、免疫異常のいかなる公知の環境的原因も存在しない場合に起こる、遺伝的T h 1 / T h 2 素因に寄与し得る。霊長類におけるH A Vレセプターは、高度に変異することが公知であり、そして本出願人らは、ヒトT I M-1 / h H A V c r-1の多型対立遺伝子は、本出願人らがマウスで同定したものと同様、T h 2の偏りおよび喘息感受性における変異に関連し得ることを提案する。ウイルスレセプターとして作用し、そして感染に対する感受性を変化させる細胞表面分子の遺伝子における変異はまれなことではなく、従ってT I M-1およびT I M遺伝子ファミリーの他のメンバーにおける有意な遺伝的変異は、サイトカインのもののような他の遺伝子における変異よりも観察される可能性がより高い。なぜ喘息感受性対立遺伝子がヒト遺伝子プールにおいて優勢であり得るのかは明らかではないが、T a p rとH A V c rとの関連は、喘息感受性対立遺伝子の残存に関して興味深い説明を提供する。ヒトの進化の間に、T i m遺伝子ファミリーのある対立遺伝子が、アトピー性疾患および他の免疫異常に対する抵抗性を与え得たが、それらの抵抗性対立遺伝子の選択は、ウイルス病原体に対する抵抗性を与える代替対立遺伝子の選択と平衡に達したのかもしれない。

【0151】

まとめると、本出願人らの研究は、強力な候補喘息感受性遺伝子を位置決定し、そしてこの複雑な遺伝的形質の調査における内在する困難を克服する、類遺伝子性マウス戦略の最初の成功した利用を提示する。本出願人らは、ヒト染色体5 qに対して相同的な領域に存在する、以前には未知の遺伝子ファミリーを同定し、そしてそれはT h細胞の分化および喘息感受性において主要な役割を果たす。ヒトにおける以前の研究は、ヒト染色体5 qにおいていくつかの候補遺伝子を同定したが、本出願人らの研究で同定されたT i m 1遺伝子産物はまた、H A V感染と喘息感受性の低減との間の逆比例関係に関する説明を提供する。

【0152】

C D 4⁺ T細胞（T h）の部分集団は、区別できるパターンのサイトカインを産生し、そしてこれはT h細胞間の機能的不均一性の概念を導いた。1型T h細胞（T h 1）は、インターロイキン2（I L-2）および／またはインターフェロンγを産生し、遅延型過敏症（D T H）反応を誘発し、そしてマクロファージを活性化する。一方2型T h細胞（T h 2）は、I L-4、I L-5およびI L-10を産生し、そして特にI g E産生および好酸球性炎症に重要であり、そして細胞による免疫を抑制し得る。T h 2細胞は、アトピー

一の病因において中枢の役割を果たすと考えられる。いくつかの因子が、特定の免疫反応の間に、Tヘルパー細胞がTh2に対してTh1へ分化するかどうかを決定する。これらは、サイトカイン環境、TCRシグナルの強さおよび／または抗原密度、ならびに副刺激経路を含むが、必ずしもこれに限らない。CD4⁺Tヘルパー細胞のTh1またはTh2サブセットへの分化は、アトピー、自己免疫疾患、感染性疾患、および移植片拒絶の結果に深い影響を有する。

【0153】

非アトピー性の個人をアレルギー性疾患の発現から保護し、そしてアトピー性個人でアレルギー性反応を阻害し得る、免疫反応の特定の特徴はあまり理解されていない。いくつかのシステムにおいて、Th1細胞はTh2細胞を交差調節するので、アレルゲン特異的Th1細胞が、アレルギー性疾患および喘息を調節すると考えられた。Th1細胞はTh2細胞の発達および増殖を阻害し、そしてIgE産生はIL-4およびIFN- γ によって相互に調節される。これは、アレルギーからの保護は、阻害性アレルゲン特異的Th1細胞の発達によることを示唆する。非アレルギー性個人の末梢血由来のアレルゲン特異的T細胞クローンは、Th1サイトカインを産生することが示された。これらの観察はまた、喘息の衛生状態仮説を支持し、それは感染、特にTh1反応を誘発するものの罹患率が、公衆衛生基準の改善ならびにワクチンおよび抗生物質の使用によって、西洋化社会において減少したことを示唆する。結果として、Th2反応およびアトピーが、Th1による反応の非存在下で、より激しくそして迅速に発現する。

【0154】

本明細書中で同定されたTIM遺伝子はまた、候補癌遺伝子である。TIM遺伝子による細胞株のトランスフェクションは、細胞死への抵抗性を与え、そしてTIM-1において記載された予測されるEGFRキナーゼモチーフは、この細胞生存が調節される可能なメカニズムを提供する。さらに、TIM-1は、細胞をFasによるアポトーシスから保護するタンパク質である、TOSOと有意な程度の配列同一性（約20%）および構造的類似性（IgVドメイン、ムチン／シンデカンドメイン、膜貫通ドメイン、および同様のリン酸化チロシンモチーフを有する細胞内ドメインを有する膜貫通糖タンパク質）を示す。TIM遺伝子と同様、TOSOはおそらく癌遺伝子であり、造血系悪性腫瘍および固形腫瘍において頻繁な変化を有するゲノムの領域に位置する。

【0155】

（方法）

動物。C.D2 Es-HBAを含む類遺伝子性株を、DBA/2NをBALB/cAnPtバックグラウンドに、イントロGRESS的に（*introgressively*）戻し交配することによって産生した。BALB/cBy、DBA/2J、および（BALB/c $\times$ DBA/2）F1マウス（CByD2F1/J）を、Jackson Laboratory（Bar Harbor、ME）から得、一方BALB/cAnおよびDBA/2NをTaconic Labs. から得た。（BALB/c $\times$ HBA）F1マウスを、BALB/cByJとHBAとの間の交配によって産生した。N2マウスを、（BALB/c $\times$ HBA）F1をHBAに対して戻し交配することによって産生した。本出願人らの組換えN2動物の分析において、可能な場合にはいつも、組換えマウスを非組換え同胞と共に試験した。多数のアッセイにおいて個々のN2遺伝子型を調査するために、本出願人らは、選択されたN2マウスをHBAに対して戻し交配してN3マウスを産生することによって、選択された組換えハプロタイプを保存し、それを遺伝子型決定して組換えN2ハプロタイプを有するマウスを選択した。OVAペプチド323-339（pOVA³²³⁻³³⁹）を認識するTCRに関してトランスジェニックであり、そしてBALB/c（43）に対して戻し交配した、DO11.10マウスは、Dr. Dennis Lohによって親切に提供され、そして本出願人らの施設で繁殖させた。HBA DO11.10マウスを、DO11.10をHBAに対して戻し交配することによって産生した。DO11.10マウスを、TCR-Tgに関してFACS分析によって選択し、そして遺伝子型決定してD11Mit135とD11Mit168との間のHBA対立遺伝子に関して選

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択した。The Stanford University Committee on Animal Welfareが、全ての動物プロトコルを承認した。

【0156】

遺伝子型決定。D11Mit140とD11Mit269との間に存在する全ての利用可能な「D11Mit-」マーカーならびに、D11Mit271およびD11Mit22付近の全ての照射ハイブリッドマーカーを、DBA/2とBALB/cとの間のあらゆる多型に関して試験することによって、Tapr遺伝子座周囲のさらなるマーカーを同定した。MIT Map Pairプライマーを、Research Genetics (Huntsville, AL) から得、そして全ての他のプライマーを、Protein and Nucleic Acid Facility (Stanford, CA) において合成した。PCRを以前に記載したように行い、そしてSSLP多型を4-5%のMetaphorアガロース (BioWhittaker, Walkersville, MD) で分離した。SSCPに関して分析される産物を、³³P-dCTPを用いて増幅し、Sequie-Gen GT System (Bio-Rad, Hercules, CA) を用いて、40Wおよび4℃で変性アクリルアミドゲルにおいて分離した。

【0157】

免疫化プロトコル。サイトカイン産生アッセイにおいて研究されたマウスを、完全フロイントアジュバント (CFA) 中のKLH (Calbiochem, La Jolla, CA) を用いて初回抗原刺激した (Dekruffyら、J Immunol 149、3468-76 (1992))。気道過剰応答性の測定のために、マウスを0日目に硫酸アルミニウムカリウム (ミョウバン) と複合体化したOVAで腹腔内 (i.p.、50 μg) に、そして7、8、および9日目の軽い麻酔後、鼻腔内 (i.n.、50 μlのPBS中50 μgのOVA) に免疫化した。コントロールマウスには、ミョウバン単独のi.p. 注射および鼻腔内PBSを与えた。吸入メタコリンに対する気道過剰応答性を、最後のOVA鼻腔内投与の24時間後 (10日目) に測定した。

【0158】

気道応答性の測定。気道応答性を、以前に記載されたように (Hansenら、J Clin Invest 103、175-83 (1999))、身体全体の体積記録器 (Buxco Electronics Inc.、Troy, NY) においた意識のあるマウスからの、メタコリン誘発気流障害によって評価した。

【0159】

細胞培養。KLHで初回抗原刺激したマウス由来のリンパ節細胞を、以前に記載されたように調製した (Yeungら、J Immunol 161、4146-52 (1998))。トランスジェニックDO11.10 CD4 T細胞を、抗CD4磁気ビーズ (Miltentyi Biotech、ドイツ) とのインキュベーション後、MACSカラムを用いてポジティブに選択した。2×10⁴ 細胞/ウェルを、96穴丸底プレートにおいて250 μg/mlのOVAおよび1×10⁴ 細胞/ウェルの骨髓由来樹状細胞と共に培養した。7日後、DO11.10 T細胞を洗浄し、そして新しい抗原提示細胞および示した濃度の抗原で再刺激した。初代DO11.10培養の抗原濃度を、再刺激の間に滴定した。骨髓由来樹状細胞を、以前に記載されたように、いくらか改変して調製した；5×10⁶ の骨髓細胞を、直径9 cmの組織培養皿で、20-25 U/mlのGM-CSFを含む10 mlの培地と共に培養した。ゆるく接着した細胞を、培養6日目に2番目の皿に移動させた；4日以内に、移したこれらの細胞を樹状細胞の供給源として使用した。

【0160】

サイトカインELISA。ELISAを、Macaulayら、J Immunol 160、1694-700 (1998)；およびMacaulayら、J Immunol 158、4171-9 (1997) において以前に記載されたように行なった。

【0161】

モノクローナル抗体。ELISAおよびFACS分析のためのモノクローナル抗体を、硫酸アンモニウム沈殿およびイオン交換クロマトグラフィーによって、腹水液から精製した

。抗クローンタイプ (clonotype) 抗体 K J 1-26. 1 は、Dr. Philippa Marrack、National Jewish Medical Center によって寛大に提供され、そして抗体を、FACS の前に、標準的なプロトコールによって FITC と結合体化した。

【0162】

(実施例 2)

(ヒト Tim 配列の同定)

Th2 応答およびアレルゲン誘発気道過剰応答性の発達に対して保護を与える遺伝子座にある TIM 遺伝子ファミリーのポジショナルクローニングは、Th2 により駆動される応答およびアトピー性疾患の調節に関する我々の理解を非常に改善する機会を与える。それに加えて、TIM-3 はマウス Th1 細胞に特異的に発現し、そして抗 TIM-3 mAb は実験的自己免疫脳脊髄炎 (EAE) の重症度の増加を引き起こす。これは、T ヘルパーサブセット調節におけるその遺伝子ファミリーの重要性を強調する。

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【0163】

マウス Tim-3 および Tim-4 のオルソログであるヒト Tim cDNA を、PCR によってクローニングした。TIM-1 のヒトオルソログを、A 型肝炎ウイルスの細胞レセプターである、HAVcr-1 としてクローニングした。TIM ファミリー遺伝子は、ヒト第 5 染色体において、TIM-4、TIM-1、TIM-3 の順番で、介在する遺伝子無しにお互いにすぐ隣接している。第 12 および 19 染色体に TIM 偽遺伝子が存在する。その遺伝子ファミリーメンバーは中程度にしか関連していない。タンパク質配列および Tim 遺伝子ファミリー間の関係を、図 7 に示す。

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【0164】

TIM 遺伝子ファミリーメンバーの細胞質ドメインは、マウスオルソログとヒトオルソログとの間で最も保存されたドメインであり、例えばヒト TIM-3 細胞質ドメインとマウス TIM-3 細胞質ドメインとの間で 77% の同一性である。対照的に、TIM-3 全体はヒトおよびマウス間で 63% しか同一でない。各 TIM 遺伝子は、明らかな予測されるチロシンシグナル伝達モチーフを含む。TIM-1 の細胞質領域は、2 つのチロシン残基および高度に保存されたチロシンキナーゼリン酸化モチーフ、RAEDNIY を含む。SRAEDNIYIVEDRP の拡大した領域は、Itk および EGF レセプターリン酸化に対する推定部位を含む。Itk は、ホスホリパーゼ C- γ (PLC- γ) をリン酸化し、そしてそれによって T 細胞活性化およびヘルパー T 細胞分化に関与するシグナル伝達事象のカスケードを引き起こすことが知られている。さらに、Itk シグナル伝達は Th1/Th2 分化に影響を与え、そして Itk^{-/-} マウスは強い Th2 応答を発達しない。EGF レセプターキナーゼ活性は、細胞の生存および細胞死に対する耐性と関連する。同様に、TIM-3 は、細胞質ドメインに別個の保存されたチロシンリン酸化モチーフおよび SH2 結合モチーフを含む。これは、TIM のそのリガンドとの相互作用が、細胞内シグナル伝達経路に携わること、および各 TIM はこのシグナル伝達において別々であることを示唆する。

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【0165】

TIM タンパク質の細胞外 IgV ドメインはまた、 α (9) β (1) インテグリン、 α (4) β (1) インテグリン、および α (4) β (7) インテグリンによる接着に関与する、オステオポンチンの SVVYGLR モチーフと類似の推定インテグリン結合モチーフを含む。300.19 株の TIM-1 をトランスフェクトしたプレ B 細胞は、トランスフェクトされていない 300.19 細胞と比較して、細胞培養において高度の接着性および生存増加を示す。TIM-1 および TIM-2 をトランスフェクトした CHO 細胞もまた、トランスフェクトされていない CHO 細胞と比較して、生存の増強を示す。これらの結果は、TIM タンパク質が細胞接着および死を調節することを示す。

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【0166】

ヒト Tim1 遺伝子および Tim3 遺伝子における遺伝子多型。ヒト Tim1 遺伝子に存在する SNP またはヌクレオチド多型および欠失/挿入を同定する。SNP はゲノムにお

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いて非常にありふれており、300-600塩基対ごとに起こるので、Tim1のコード領域のみを分析した。さらに、よくある遺伝子変異も重要であるようである。最初に、30-40人の個人(60-80本の染色体)から取ったT細胞由来のcDNAを配列決定する。検出力の計算は、60染色体のコード領域において標的配列を調査することが、1%より大きい個体群頻度を有するSNPを容易に検出し、そして5%以上の個体群頻度を有する対立遺伝子を検出する機会を90%より多く有することを示す。従って、配列の異型に関して30-40人の個人をスクリーニングすることは、個体群中に存在する、ほとんどのよくある、機能的に関連する、非保存的なDNA異型を捕捉する。

【0167】

物理的にきわめて接近したDNA異型/SNPは、多くの場合強力な依存関係(すなわち連鎖不均衡)を示すので、DNA異型のグループ(SNPハプロタイプ)が一緒に受け継がれるかどうかを決定し、そしてこれらSNPの一部のみのスクリーニングがハプロタイプの同定に十分であるかどうかを決定した。様々な染色体の大きな領域の分析は、別個のハプロタイプブロック(数十から数百キロベース)が一般的に存在し、それぞれが見かけの組換え部位によって時々断続された、限られた多様性を有することを示す。ハプロタイプを発見するために、cDNAを配列決定し、そして複数の個人で繰り返し見られる配列変異の組み合わせを探す。

【0168】

末梢血単核細胞(PBMC)は38人のドナー由来であり、そしてインビトロでPHA(7.5 µg/ml)を用いて24および72時間、またはコンカナバリン(Concavalin A)(2 µg/ml)を用いて24時間刺激した。PMA(20 ng/ml)およびイオノマイシン(1 µM)を、刺激の最後の6時間に加えた。次いで細胞を回収し、そしてTrizol試薬(Invitrogen)を用いて全RNAを抽出した。配列決定のためのcDNA鋳型を得るために、Superscript II逆転写酵素(Invitrogen)を用いて、製造会社のプロトコルによって、RNAを逆転写した。このcDNAを用いて、Herculase Hot Starttm 高忠実度ポリメラーゼ(Stratagene)を用いて、Tim cDNAの全長をPCR増幅した。PCRプライマーは以下のものであった:(配列番号37)GTGTC TGACAGTGGCGTA(順向き)、(配列番号38)TTTGCC CAGGCAGAACCA(順向き)、CCACCCCAAGGTCACGACT(逆向き)、(配列番号39)ATGCCACGGACTAAGACC(逆向き)。PCR産物を、Qiagen QIAquickゲル抽出試薬を用いて精製し、そしてTim1に関して4つの内部配列決定プライマーを用いて、そしてTim3に関して2つの内部配列決定プライマーを用いて配列決定した。

【0169】

活性化T細胞から全RNAを取り、そしてSuperscript IIおよびオリゴdTを用いてそれを転写することによって、全長Tim1 RT-PCR産物を、これらの個人においてクローニングした。Tim1 cDNAを、Expand高忠実度ポリメラーゼ(Roche)を用いて増幅して、Tim1コード領域にまたがる1 kbの産物を産生し、それをPCR Purificationキット(Invitrogen)を用いて精製した。次いでこの精製産物をTOPO pEF6ベクター(Invitrogen)にクローニングし、続いてTOP10コンピテント細菌を形質転換した。細菌コロニーを、アンピシリン選択を用いてLBプレートで増殖させた。単一のコロニーを取り、そしてQiagenミニプレップキットを用いてプラスミドプレップを行った。HindIII消化を用いた制限マッピングを用いて、正しい方向で挿入を含むプラスミドを選択した。次いでこれらのプラスミドを、3つの異なるプライマー(順向き(T7)、内部および逆向き(BGH))を用いて配列決定し、そしてその配列をNCBIヒトTIM参照配列とSeqManプログラムにおいて整列させた。

【0170】

35人の個人由来の染色体(70個の染色体)からTim1を配列決定した後、Tim1

におけるいくつかの多型を同定し、それらを図8に示す。これらの多型に1-7の番号をつける（左カラム）。NCBIデータベースに記載されているヒトTIM-1の全配列（NM_012206）を、参照ポイントとして図8で提供する。この配列は、コード領域における複数の優勢な配列多型の存在のために、配列決定した染色体の20%以下に存在する。6個のさらなる配列異型が同定され、図8に示す、そして全ての多型は、マウスでもそうであるように、ムチンの細胞外ドメインで観察されたが、特定の変異はマウスで見られるものと異なっていた。重要なことに、これら異型の間に、様々な組み合わせで、限られた程度の関連が存在する。最も明らかな変異は、65%の染色体で観察された、多型1（157insMTTTP）と名付けられた挿入、および65%の染色体で観察された、多型5（187△Thr）の欠失である。多型4は40%の染色体で観察され、そして他の多型はそれぞれ≤5%の染色体で観察された。特に、これら異型（2-6）のほとんどは、最初のムチンをコードするエキソンであるエキソン3内に位置し、そして全ての異型はゲノムレベルで存在し、そしてスプライシング異型ではない。

【0171】

mRNAのこの配列分析に基づいて、より多数の患者／コントロールからのゲノムDNAを分析するより迅速な方法が開発された。図8に示した配列中に見られる変異に関して個人をスクリーニングするために、DNAをまず150PCR産物における単純配列長多型（SSLP）に関して試験し、それは主要な挿入（多型1）および欠失（多型5）を検出し得る。

【0172】

それに加えて、他の多型（2-4、6および7）を遺伝子型決定および新規多型を同定するために、PCR産物の一本鎖高次構造多型（SSCP）分析を用いる、比較的単純なアッセイを開発した。十分に最適化された条件において、SSCP分析は90%より多くの単一ヌクレオチド置換および全ての長さ多型を検出する。この分析のために、Tim遺伝子の各エキソンを増幅するPCRプライマーを同定し、そして標準的な非変性SSCPゲル電気泳動法を用いて異型を区別し得る（図9）。各エキソンの高分解能SSCP分析のために、ABI 377 DNA配列と共に非変性ポリアクリルアミドゲル電気泳動を使用する。蛍光末端標識プライマーを合成および精製する。ハイスループット遺伝子型決定処理の間に検出された新規SSCPパターンは、新規異型を同定する。この方法を用いて、患者およびコントロールの遺伝子型を迅速に分析する。

【0173】

Tim3遺伝子を、本質的に同じ方法論を用いて分析した。活性化T細胞由来のmRNAを配列決定して、Tim3多型、およびTim1とTim3との間の長い範囲のハプロタイプを同定する。Tim3 cDNAを示す60個の染色体を配列決定した後、Tim3は、これがマウスゲノムにおける場合と同様に、多型性であることが見出された。しかし、1つの多型のLeu140Argのみが優勢であり、示される染色体の約12%に見出される。

【0174】

（実施例3）

（Tim配列の発現）

マウスTIM-3タンパク質は、Th1クローンに発現するが、未処理（ナイーブな）T細胞またはTh2細胞には発現しない。TCRトランスジェニックT細胞を用いると、TIM-3タンパク質は、1回または2回のTh1指向性分化後のTh1細胞に発現しなかったが、3回およびそれ以上のTh1への刺激後に発現した。TIM-3 mRNA発現がいくらか早期に検出された。TIM-3遺伝子発現がヒトにおいて同じであるかどうかを決定するために、ヒトTh1細胞におけるTIM-3 mRNA発現およびTIM-1 mRNA発現を、IL-12および抗IL-4 mAbの存在下で抗原を用いた刺激によって作製した破傷風毒素特異的T細胞を用いて試験した。TIM-1と喘息の関連を考慮して、ヒトTh2細胞におけるTIM-1 mRNA発現およびTIM-3 mRNA発現を試験した。Th2細胞株を、アレルゲン、IL-4、および抗IL-12 mAb

によるインビトロ刺激によって、アレルギー性ドナーから産生した。RNAをPCRによってTIM遺伝子発現に関して分析した。

【0175】

TIM-3はTh1分化の後一般的に発現していたが、TIM-1は消失した。逆に、TIM-3はどのTh2にも発現しなかったが、TIM-1は全てのTh2細胞に発現した。おそらく、この混合した集団はTh1メモリー細胞およびTh2メモリー細胞をどちらも含むために、TIM-1およびTIM-3はどちらも、Th1細胞株およびTh2細胞株を誘導するために使用したドナー由来の、単球除去した未刺激末梢血単核細胞に発現する。これらの結果は、Th2に発現するTIM-1およびTh1に発現するTIM-3の相互関係を示唆する。このTIM-1およびTIM-3間の相互関係は、マウスにおいても観察された。

【0176】

ヒト組織において、4.4kbのTIM-1 mRNAは腎臓および精巣に非常に強力に発現した。4.4kb mRNAは、ほとんど全ての組織に存在したが、ほとんどにおいてかすかであった。5.5kbのバンドが結腸および肝臓で観察された。7.5kbのバンドが脾臓、胸腺、および末梢血白血球で観察され、そして4.4kbより小さいバンドがいくつかの臓器で観察された。これらの結果は、hTIM-1はほとんどのヒト組織においてあるレベルで発現しており、そして7.5kbのメッセージが、免疫学的目的のある組織においてhTIM-1をコードし得ることを示唆する。しかし、TIM-1のラットホモログであるKim-1 (Kidney Injury Molecule-1) の発現は、虚血性障害時に腎臓において増加する。発現分析において使用したMTNプロットは、死体から抽出したmRNAから調製したので、腎臓におけるTIM-1発現の増加を再分析した。正常腎臓生検から得た腎臓RNAにおいて、TIM-1の過剰発現は見られなかった。従って、腎臓および精巣で観察されたTIM-1の高レベルの発現は、組織障害により起こるTIM-1発現のアップレギュレーションによるものであった可能性がある。損傷した腎臓は、流入する炎症性細胞を、破壊的なTh1応答より、より保護的なTh2応答へ向けるタンパク質を発現し得る。

【0177】

(実施例4)

(TIMリガンドおよび抗体)

抗体の産生。マウスTIM-1に対するモノクローナル抗体の産生は、異なる組織、細胞株およびマウス株におけるTIM-1の細胞表面発現を調査することを可能にする。マウスTIM-1の対立遺伝子の両方を、高タンパク質発現のためにベクター (Invitrogen, pEF6-TOPO) にクローニングした。ラットを両方のTim1 cDNA構築物で免疫および追加免疫し、細胞表面分子に対する抗体を迅速に産生した。cDNAワクチン接種を用いるこの方法は、Tim1 cDNAはAPCによって取り込まれ、それがTIM-1を細胞表面分子として発現するので、細胞表面エピトープに対するmAbの産生に有利である。BALB/c TIM-1およびHBA TIM-1の両方と同等によく (TIM-1の免疫グロブリンドメインのような、TIM-1の保存されたドメインに結合することによって) 結合するmAbを産生するために、BALB/cおよびHBA Tim1 cDNAをどちらも (pEF6-mTIMbalbおよびpEF6-mTIMhba) を各ラットに注射した。

【0178】

Tim1 cDNA免疫ラットのさらなる追加免疫を、pEF6-mTIM-1-GFP発現構築物で安定にトランスフェクトしたCHO細胞で行なった。高レベルのマウスTIM-1を発現するCHOトランスフェクタントを、FACSによって分類し、そしてラットに注射した。別のmTIM-1を発現する細胞を、プレB細胞株300.19をpEF6-mTIM-1発現構築物で安定にトランスフェクトすることによって産生した。この株を使用して、ラット血清および抗TIM-1抗体のため融合後のハイブリドーマを、フローサイトメトリーによってスクリーニングする。非免疫ラット由来のコントロール血清

と比較して、ラット血清（および2次FITC-ヤギ抗ラットIg）の、安定なpEF6-mTIM1トランスフェクト300.19細胞への結合によって検出されるような、抗TIM-1に対して高いポリクローナル力価を有するラットを産生した。非トランスフェクト細胞またはTIM-2でトランスフェクトした細胞では応答性が存在しないので、この染色は、TIM-1に特異的である。

【0179】

ラット脾臓を取出し、そして脾臓細胞を骨髓腫細胞株（SP/2）と融合してハイブリドーマを産生した。ハイブリドーマ上清を、TIM-1トランスフェクト300.19細胞株を使用してスクリーニングし、モノクローナル抗TIM-1を産生するハイブリドーマクローンを同定する。TIM-1に対する（そして他のTIMタンパク質ではない）mAbの特異性を、TIM-2トランスフェクト細胞、およびmTIM-3トランスフェクト細胞またはTIM-3 Ig融合タンパク質を用いて確認する。

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【0180】

抗体染色。Th1細胞株およびTh2細胞株を、BALB/cおよびHBA DO11.10脾臓細胞両方から調製した。TIM-1 mRNA発現のRT-PCRは、標準的な偏向性条件下で2回の抗原による再刺激後、TIM-1はTh2株に発現するがTh1株には発現しないことを示した。Th2偏向性条件下で抗原/APCによる2回の刺激後のDO11.10T細胞は、ポリクローナルラット抗TIM-1抗血清で染色された。これらのTh2細胞は高レベルのTIM-1を発現した。

【0181】

Th2株におけるTIM-1の選択的な発現を示すこれらの実験を、抗Tim-1 mAbおよびノーザンブロットを用いて定量および確認する。BALBおよびHBA由来のDO11.10細胞を、抗原およびAPCと共に培養し、そして標準的な偏向条件（抗IL-12+IL-4または抗IL-4+IL-12）の下で1、2、および3週間再刺激する。刺激の各週後に、細胞を抗TIM-1 mAbで染色する。様々な時点において刺激細胞を回収することによって、Th1サブセットまたはTh2サブセットへの分化を受けたT細胞におけるTIM-1発現の動態を決定する。Tim-1表面発現がT細胞活性化後に変化するかどうかを決定するために、本発明者らはまた、いくつかの細胞をPMAおよびイオノマイシンで刺激することによって、抗原刺激の各回の1週間後に、休止T細胞および活性化T細胞におけるTIM-1発現を比較する。活性化細胞を、細胞内サイトカイン発現に関して染色し、T細胞のThサブセット分化を検証する。あるいは、刺激の各回の後に、PMA+イオノマイシンで活性化されたT細胞から回収したmRNAを用いた定量的RT-PCRまたはノーザンブロットを使用して、mRNA産生の相対的レベルを決定する。

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【0182】

TIM-1-Ig融合タンパク質。TIM-1ポリペプチドおよびマウス免疫グロブリンのFc領域との間の融合タンパク質である、BALB/c TIM-1-mIgG2aを調製した。ベクターを、マウスIgG2a Fcにおいて、Fcレセプターへの結合を最小にする変異を含むように、遺伝子操作した。TIM-1融合タンパク質を、TIM-1機能の特徴付けにおいて利用する。TIM-1 Ig融合タンパク質は、TIM-1リガンドに結合することによってTIM-1機能を阻害すること、およびTIM-1/TIM-1リガンド相互作用を妨害することが予測される。

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【0183】

ヒトIgG1のヒンジおよびFcフラグメントに融合したTIM-1のシステインリッチな免疫グロブリンドメインおよびムチン様領域の2/3を含む、精製したD1mu c-Fc融合タンパク質（IgVmu c-hIg）を、ゲルに流した。このタンパク質をCHO細胞に発現させ、そしてIgVmu c-hIgタンパク質を、CHO上清からプロテインAアガロースカラムを用いて精製した。精製IgVmu c-hIg融合タンパク質は、約2logのHAV伝染性を中和する。それに加えて、IgVmu c-hIgによるHAVの処理は、HAV粒子の沈降に大きなシフトを引き起こし、IgVmu c-hIgはウイ

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ルスゲノムの非外被化 (uncoating) を誘発したことを示す。一方、ムチンドメインを含まない Ig 様領域のみを含む融合タンパク質 (IgV-hIg) は、それを引き起こさなかった。この HAV 中和化システムおよび IgVmuc-hIg 融合タンパク質を使用して、TIM-1/HA V レセプター対立遺伝子の機能を分析する。

【0184】

マクロファージの拡大および活性化に対する抗 TIM-3 mAb のインビボ効果に基づいて、TIM-3 リガンドは、骨髓株の細胞に発現するという仮説をたてた。1000 U/ml の IL-4 および 800 U/ml の GM-CSF を用いて、確立されたプロトコルによって、血液単球から樹状細胞 (DC) を調製した。DC を、細胞を 2 日間、IL-1 β (10 ng/ml)、TNF- α (10 ng/ml)、IL-6 (1000 U/ml) および PGE₂ (1 μ g/ml) を補充した IL-4 (1000 U/ml) および GM-CSF (800 U/ml) 中に再プレートすることによって成熟させた。成熟 DC は、hTIM-3-Ig で陽性に染色されたが、ドナー間で変動性が存在し、成熟 DC は TIM-3 の IgV ドメインに対するリガンドを発現することを示唆する。骨髓由来内皮細胞は非常に弱く染色され、そして B 細胞株は TIM-3-Ig で染色されなかった。

【0185】

Tim1/huhavcr-1 の細胞質内尾部は比較的短い、それはマウス、ラット、ヒトおよびサル間で高度に保存された配列 (RAEDNIYI) を含み、そしてそれはリン酸化され、そして他のシグナル伝達分子との相互作用によってシグナル伝達し得る。T 細胞において RAEDNIYI モチーフに結合し得る、最も可能性ある候補分子は、チロシンキナーゼ Itk である。インターロイキン 2 誘導性チロシンキナーゼの Itk は、Tec ファミリーの非レセプタータンパク質チロシンキナーゼであり、それは T 細胞の活性化を引き起こす細胞内シグナル伝達事象に役割を果たす。Tec ファミリーメンバーは、保存された SH3、SH2、および多くのキナーゼファミリーに共通の触媒ドメインを含むが、それらはこの領域外の独特の配列によって区別される。Itk はホスホリパーゼ C- γ (PLC- γ) をリン酸化し、そして T 細胞活性化およびヘルパー T 細胞分化に関与するシグナル伝達事象のカスケードを引き起こすことが知られている。Itk シグナル伝達の非存在下で、Th2 細胞は発達しない。これらの結果は、TIM-1/huhavcr-1 が、Itk によってシグナル伝達し、それによって CD4⁺ T 細胞におけるサイトカインの発達を変化させ得ることを示唆する。

【0186】

(実施例 5)

(TIM ノックアウトマウス)

ノックアウト構築物を、Tim1 欠損マウスの作出において使用し、それは TIM-1 分子の非存在下で免疫反応の発達を分析することを可能にする。別のアプローチにおいて、TIM-1-Ig 融合タンパク質または抗 TIM-1 モノクローナル抗体を用いて、Tim1^{+/+} (野生型) マウスにおける TIM-1 の機能を阻害する。Tim1 ノックアウトマウスおよび抗 TIM-1 mAb アプローチは、T 細胞分化および喘息の病因における TIM-1 の役割の評価に関して相補的である。

【0187】

Tim1 の HBA マウスゲノム配列は、エキソン 4 における組み込まれた L1 レトロウイルスエレメントのために、BALB/c 配列と比較して、エキソン 4 の欠失を有する。1 つのエキソンの短縮が TIM-1 機能を低減する場合、Tim1 ノックアウトマウスの作出による TIM-1 機能の全ての欠失は、そのようなマウスの Th2 応答を生じる能力を著しく制限するはずである。TIM-1 KO マウスを、cre-lox 技術および BALB/c ES 細胞を用いて、TIM-1 のエキソン 1 およびエキソン 2 を欠失させることによって作出し、それは TIM-1 の細胞表面発現を排除するはずであり、それによって Th2 細胞および AHR の発達における TIM-1 機能の重要性を示す。

【0188】

適当な標的化構築物を作製するために、TIM 遺伝子ファミリーのメンバーを含む特異的

BACを、高密度フィルターセットを用いて、C57/B16 BACライブラリー (R
 PCI-23) をスクリーニングすることによって同定した。これらのBACクローンを
 使用して、Tim遺伝子ファミリーを含む約350 kbのゲノム領域をカバーする、50
 0 kbのコンテイングおよび物理的地図を作成した。完全なTim1遺伝子を含む1つの
 特異的BAC、RPCI-23-222F8を、TIM標的化構築物を作製するために選
 択した。標的構築物は、プロモーター領域、シグナルエキソン、およびIgVエキソン (1
 および2) を含む4 kbの領域を、この領域をはさむ5'および3'アームに
 よる相同的組換えによって、欠失させる。標的化構築物の相同的アームは、BALB/c
 およびHBA (C57B1/6) のDNAの両方と相同的であるので、この標的化ベクタ
 ーを、C57B1/6またはBALB/cのES細胞のいずれでも使用し得る。構築物を
 ES細胞に導入し、それをPCRおよびサザンブロットによって、標的化対立遺伝子に関
 してスクリーニングする。

【0189】

標的化ベクターのpLOXは、3つのloxP部位を含み、そして標的化ES細胞におい
 てcreリコンビナーゼを発現することによって、組換えは標的化領域の3つの形式を形
 成し、そのうち2つの対立遺伝子のAおよびBを、Tim1 KOマウスを作出するた
 めに使用する。選択されたES細胞クローンを、胚盤胞に導入してキメラマウスを産生し、
 そしてノックアウトの生殖細胞系列の遺伝のために繁殖させる。尾部DNAを、ノックア
 ウトの遺伝に関して分析する。まず対立遺伝子Aを使用し、そこでcre/lox組換え
 は一番外側のloxP部位を使用して、TIM-1のエキソン1およびエキソン2に加え
 てTKおよびネオマイシンの選択カセットを欠失させた。TIM-1ゲノム標的化領域か
 らneoおよびTKのカセット全体を除去することは、同じファミリーの他の遺伝子にき
 わめて近接するneoの転写に対して、2次的に生じ得る人工産物を混同するのを防ぐ。
 あるいは、もし対立遺伝子Aが致死的または混乱させる表現型を産生する場合、標的化対
 立遺伝子Bを用いてマウスを作出し、それにおいてneoおよびTKのカセットが欠失し
 、そしてTIM-1領域がlox-p部位によって挟まれる。この条件付き標的化アプロ
 ーチの使用は、T細胞特異的プロモーターの調節下にあるcreリコンビナーゼを発現す
 るマウスにおいて、Tim-1のT細胞特異的欠失を可能にするはずである。

【図面の簡単な説明】

【0190】

【図1】図1aは、HBAマウスはBALB/cマウスよりも有意に少ないIL-4を産
 生することを示す。150 µgのKLHで免疫したマウスのリンパ節細胞を回収し、B-
 細胞を涸渇し、そして10 µg/mlのKLHを含むCDMEで培養した。96時間後に
 上清を回収、そしてELISAによってIL-4レベルに関して評価した。データの全範
 囲を表すIL-4レベルのボックスプロット (各グループにおいてn=10) を示し、ボ
 ックスは上位および下位の四分位数 (quartiles) を含み、データセットの中央
 値を各ボックスの中に示す。BALB/c細胞および (BALB/c × HBA) F1細胞
 によって産生されたIL-4レベルは、HBAのIL-4レベルより有意に高い (P<0.
 0001 (スチューデントt検定))。図1bは、HBAマウスはBALB/cマウス
 より有意に少ないIL-13およびIL-10を産生することを示す。示したデータは、
 各実験グループにおける10匹の個々のマウス由来のリンパ節細胞培養によって産生され
 たサイトカインの平均値、±S.D.である。HBAのIL-13レベルおよびIL-1
 0レベルは、BALB/cまたは (BALB/c × HBA) F1のどちらの値より低かつ
 た (P<0.0001) (IL-5対BALB/c、P<0.05) (HBA IFN-
 γ対BALB/c、P<0.001)。図1cは、アレルギー誘発気道過剰応答性は、H
 BAマウスよりもBALB/cマウスで有意に大きいことを示す。肺気流の障害を測定し
 、そして示したデータは、様々なメタコリン濃度における各グループの感作マウス間で平
 均したpeak enhanced pause (Penh) 値、±S.E.M.を表す。
 (BALB × HBA) F1は、BALB/c表現型を示し、一方 (BALB × DBA)
 F1マウスはDBA/2様の表現型を示す。

【図2】図2は、HBA第11染色体の領域は、DBA/2から受け継がれたことを示す。HBA第11染色体は、SSLPマーカーによって表される、hba-a2およびes-3の間の、DBA由来の2つの領域を含む。DBA/2遺伝子型を有するHBA第11染色体の領域を、左の図において（青色で）強調する。マーカー（左）は、D11Mit230に対して遠位の2-5cMの分解能を提供する。マウス第11染色体が5q23-35と高度に保存されたシンテニーの領域を有する場合、さらなるマーカーがBALB/cおよびDBA/2間の情報を与える多型によって同定され、この領域において0.01-2cMの分解能を与えた（右カラムのマーカー）。一本鎖DNA高次構造多型（SSCP）マーカーを、SSLPマーカーと区別するために緑色で示し、そして赤色で表示された目的の特定の遺伝子の位置も示す。本発明者らのマーカー地図の配置がChromosome Committee ReportsおよびMIT連結地図と異なる場合、本発明者らの地図は以前の連結および物理的地図と一致する。 10

【図3】図3aは、N2マウスによるIL-4産生は、F1およびHBA表現型に対応するピークを有する双峰分布であることを示す。複数の実験において組換えN2マウスの相対的表現型を評価する方法として、本発明者らは複数の実験からのデータの整理統合を可能にする指数化関数（indexing function）を利用した。柱状グラフは、（BALB×HBA）F1、HBAホモ接合体、および示した組換えハプロタイプを有するN2マウスのIL-4指数値の、双峰分布を示す。F1およびHBAハプロタイプと関連する分布は独特である（ $P < 0.0001$ 、対応ある学生t検定）。図3bは、IL-4の調節はKim1sscpと共に分離することを示す。組換えN2ハプロタイプを、IL-4指数値によって、高IL-4表現型（指数 < 0.35 ）および低IL-4表現型（指数 > 0.65 ）と関連するグループへ分類した。ボックスの各カラムは、組換えハプロタイプを示す。D11Mit269からD11Mit154までの間の遺伝子座におけるこれらハプロタイプの対立遺伝子に、遺伝子型によって明暗をつけた（濃い=F1；薄い=HBA）。高IL-4産生は、4つのハプロタイプと分離し（左）、そして低IL-4産生は4つのハプロタイプと分離する（右）。IL-4表現型はKim1sscpと連結する。図3cは、N2マウスのAHRは、F1およびHBA表現型に対応するピークを持つ双峰分布であることを示す。Penh値から計算した指数値を示す。柱状グラフは、N2マウスのAHR指数値の双峰分布を示す。（BALB×HBA）F1、HBAホモ接合体、および組換えハプロタイプを示す。F1およびHBAハプロタイプと関連する分布は別個である（ $P < 0.0001$ 、対応ある学生t検定）。図3dは、AHR調節遺伝子座は、D11Mit22およびD11Mit271間のIL-4調節遺伝子座と同時分離することを示す。組換えN2ハプロタイプを、AHR指数値によって、高AHR表現型（指数 < 0.35 ）、および低AHR表現型（指数 > 0.65 ）と関連するグループへ分類した。ボックスの各カラムは、組換えハプロタイプを示す。D11Mit269からD11Mit154間の遺伝子座におけるこれらハプロタイプの対立遺伝子に、遺伝子型によって明暗をつける（濃い=F1；薄い=HBA）。高AHRは、4つのハプロタイプと分離し（左）、そして低AHR産生は3つのハプロタイプと分離する（右）。AHR調節遺伝子座は、D11Mit271およびD11Mit22間のKim1sscpと連結する。 40

【図4】図4は、Taprを含むマウス第11染色体の区間は、5q33と高度に相同的である。Tapr遺伝子座の周囲の複合地図を構築するために、本発明者らはマウス連結、戻し交配、および照射ハイブリッド地図から入手可能な情報を統合し、そしてヒトゲノムの現在の地図において、高度に保存されたシンテニーの領域を同定した（Human Genome Browser v3、UCSC、2001年3月）。マウス第11染色体（左）の物理的地図に位置するESTを、その登録番号で表示し、そしてヒト第5染色体（右）のESTに対応する、遺伝子に対する相同性によって整列させた（中央）。KIAA0171およびSgd間の公知の遺伝子を全て示す。

【図5a】図5aは、新規TIM遺伝子ファミリーおよびTIM-1およびTIM-3における主要な多型の同定、マウスTIM-1およびマウスTIM-2のクローニング、遺 50

伝子ファミリーのメンバー、マウスTIM遺伝子ファミリーメンバーの配列を示す。陰をつけたボックスは、2つのマウスTIM遺伝子間の同一性を示す。conA刺激脾臓細胞由来の全RNAを、Gibco Superscript IIを用いて逆転写した。全長Tim-3 cDNAのPCR産物を増幅し、Qiagen QIAquick PCR Purification試薬で精製し、そしてBiotech Core (Mountain View, CA) によって直接配列決定した。Tim-1およびTim-2 cDNAのPCR産物を、TOPO-XLクローニング試薬 (Invitrogen) を用いてelectrocompetent TOP10細胞にクローニングした。プラスミドを、標準的なアルカリ溶解プロトコルを用いて精製した。BALB/cおよびHBAプラスミドを、記載したように配列決定した。マウスTIM-1、ラットKIM-1、およびHAVcr-1の相同性。マウスTIM-1との同一性を、陰をつけたボックスによって表示する。おおよそのシグナル部位を白い反転した三角形によって表示し、そしてIgドメイン/ムチンドメイン境界を黒い菱形で示す。予測される膜貫通ドメインに下線を引く。BALB/cおよびHBA TIM-1およびTIM-3間の主要な多型を有するTIM-1およびTIM-3配列を示す。 10

【図5b】図5bは、新規TIM遺伝子ファミリーおよびTIM-1およびTIM-3における主要な多型の同定、マウスTIM-1およびマウスTIM-2のクローニング、遺伝子ファミリーのメンバー、マウスTIM遺伝子ファミリーメンバーの配列を示す。陰をつけたボックスは、2つのマウスTIM遺伝子間の同一性を示す。conA刺激脾臓細胞由来の全RNAを、Gibco Superscript IIを用いて逆転写した。全長Tim-3 cDNAのPCR産物を増幅し、Qiagen QIAquick PCR Purification試薬で精製し、そしてBiotech Core (Mountain View, CA) によって直接配列決定した。Tim-1およびTim-2 cDNAのPCR産物を、TOPO-XLクローニング試薬 (Invitrogen) を用いてelectrocompetent TOP10細胞にクローニングした。プラスミドを、標準的なアルカリ溶解プロトコルを用いて精製した。BALB/cおよびHBAプラスミドを、記載したように配列決定した。マウスTIM-1、ラットKIM-1、およびHAVcr-1の相同性。マウスTIM-1との同一性を、陰をつけたボックスによって表示する。おおよそのシグナル部位を白い反転した三角形によって表示し、そしてIgドメイン/ムチンドメイン境界を黒い菱形で示す。予測される膜貫通ドメインに下線を引く。BALB/cおよびHBA TIM-1およびTIM-3間の主要な多型を有するTIM-1およびTIM-3配列を示す。 20 30

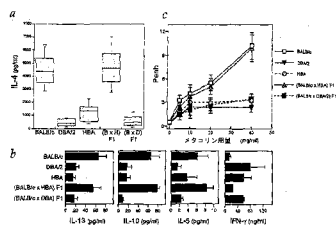
【図5c】図5cは、新規TIM遺伝子ファミリーおよびTIM-1およびTIM-3における主要な多型の同定、マウスTIM-1およびマウスTIM-2のクローニング、遺伝子ファミリーのメンバー、マウスTIM遺伝子ファミリーメンバーの配列を示す。陰をつけたボックスは、2つのマウスTIM遺伝子間の同一性を示す。conA刺激脾臓細胞由来の全RNAを、Gibco Superscript IIを用いて逆転写した。全長Tim-3 cDNAのPCR産物を増幅し、Qiagen QIAquick PCR Purification試薬で精製し、そしてBiotech Core (Mountain View, CA) によって直接配列決定した。Tim-1およびTim-2 cDNAのPCR産物を、TOPO-XLクローニング試薬 (Invitrogen) を用いてelectrocompetent TOP10細胞にクローニングした。プラスミドを、標準的なアルカリ溶解プロトコルを用いて精製した。BALB/cおよびHBAプラスミドを、記載したように配列決定した。マウスTIM-1、ラットKIM-1、およびHAVcr-1の相同性。マウスTIM-1との同一性を、陰をつけたボックスによって表示する。おおよそのシグナル部位を白い反転した三角形によって表示し、そしてIgドメイン/ムチンドメイン境界を黒い菱形で示す。予測される膜貫通ドメインに下線を引く。BALB/cおよびHBA TIM-1およびTIM-3間の主要な多型を有するTIM-1およびTIM-3配列を示す。 40 50

【図6】図6は、TaprはCD4 T細胞のIL-4およびIL-13応答を調節する

【図 9】 図 9 は、ヒト T I M-1 の S S C P 多型分析を示す。

10

【义 1】



【图 2】

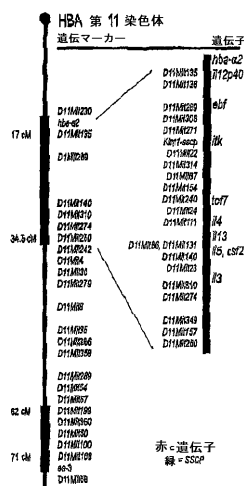


Figure 2

【図 3】

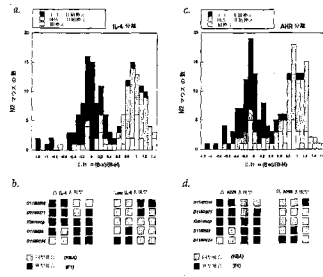


Figure 3

【図 4】

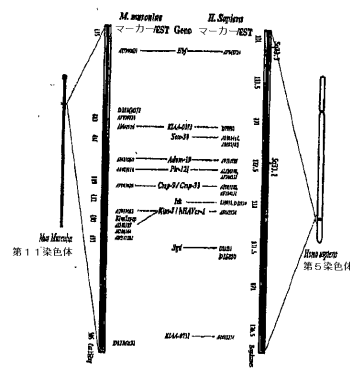


Figure 4

【図 5 a】

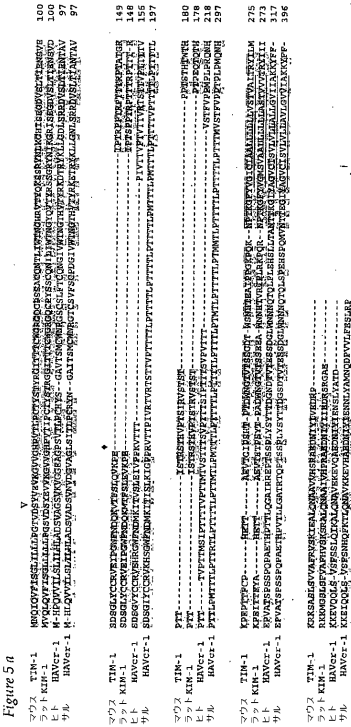


Figure 5 a

【図 5 b】

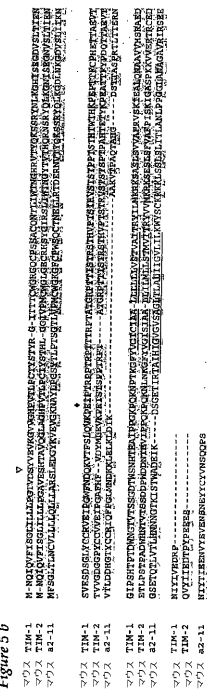


Figure 5 b

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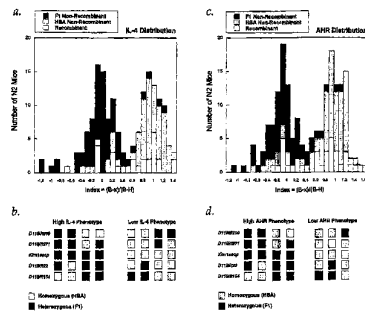
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- (71) Applicant: THE BOARD OF TRUSTEES OF THE LE- LAND STANFORD JUNIOR UNIVERSITY [US/US]; 900 Welch Road, Suite 350, Palo Alto, CA 94304 (US).
- (72) Inventors: MCINTIRE, Jennifer, Jones; 1106 Clark Way, Palo Alto, CA 94304 (US). DEKRUYFF, Rose- marie, H.; 825 Allardice Way, Stanford, CA 94305 (US). UMETSU, Dale, T.; 825 Allardice Way, Stanford, CA 94305 (US). FREEMAN, Gordon, J.; 44 Binney Street, Boston, MA 02115 (US). KUCHROO, Vijay; 1249 Boylston Street, 2nd Street, Boston, MA 02115 (US).
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[Continued on next page]

(54) Title: T CELL REGULATORY GENES AND METHODS OF USE THEREOF



(57) Abstract: A genetic locus and corresponding family of proteins associated with regulation of immune function and cell survival are provided. These genes encode cell surface molecules with conserved IgV and mucin domains. The locus comprising the TIM family is genetically associated with immune dysfunction, including asthma. Furthermore, the TIM gene family is located within a region of human chromosome 5 that is commonly deleted in malignancies and myelodysplastic syndrome. Polymorphisms in the gene sequences are associated with the development of airway hyperreactivity and allergic inflammation, and T cell production of IL-4 and IL-13 . The proteins include the human hepatitis A cellular receptor, *hHAVcr-1*.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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T CELL REGULATORY GENES AND METHODS OF USE THEREOF

BACKGROUND OF THE INVENTION

The astonishing complexity of the immune system is its greatest strength.

5 The 10^{12} - 10^{14} possible antibody specificities, the delicate interplay between the various regulatory and effector cells, the restriction of T cell responses according to MHC antigens; all these contribute to the ability of the host to effectively react against infectious agents and other antigens perceived as foreign. But this diversity has its drawbacks. Mistakes happen: the target of a response may turn out to be a normal self protein; inflammatory
10 responses are misregulated; and normal responses are undesirably directed against grafts and transplanted cells. Under these circumstances, the complexity of the system makes diagnosis and therapy extremely difficult.

The profile of cytokines produced by $CD4^+$ T cells during an immune response determines the nature of effector functions which develop and regulates the
15 outcome of an immune response. Production of IL-2 and IFN- γ during Th1-dominated responses is associated with vigorous cell-mediated immunity, the induction of IgG2a and inhibition of IgE synthesis, and with resistance to intracellular pathogens. In contrast, the production of IL-4, IL-5 and IL-10 during Th2-dominated responses is associated with humoral immunity and protection from autoimmune pathology. Overproduction of Th2-
20 cytokines by allergen-specific $CD4^+$ T cells can result in the development of allergic disease and asthma, while Th1 cells have been associated with a variety of pro-inflammatory diseases.

One approach to immune associated diseases is immunotherapy. Immunotherapy has proven to be effective when used properly, and it is hoped that
25 advances in immunologic intervention will further improve the efficacy. Alternative approaches have attempted to use cytokines to shift the immune response. IL-12, a heterodimeric cytokine produced by macrophages and dendritic cells, is potent in driving the development of Th1 cytokine synthesis in naive and memory $CD4^+$ T cells. Other cytokines, such as IL-13 and IL-4, have been associated with the differentiation of T cells to
30 a Th2 type.

Atopy, which includes asthma, allergic rhinitis, and atopic dermatitis, is a complex trait that arises as a result of environmentally induced immune responses in genetically susceptible individuals. The prevalence of all atopic diseases has dramatically increased in industrialized countries over the past two decades. Asthma is the most
35 common chronic disease of childhood and affects more than 15 million individuals in the United States, leading to direct treatment costs exceeding \$11 billion per annum. Epidemiological studies have suggested that the increase in asthma prevalence results

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from changes in hygiene and from reduced frequency of infections (e.g., tuberculosis or hepatitis A) within industrialized society. However, the specific molecular pathways that result in the increased asthma prevalence, and the genetic polymorphisms that confer asthma susceptibility are poorly understood.

5 Expression of asthma is influenced by multiple environmental and genetic factors that interact with each other in non-additive ways, complicating the identification of asthma susceptibility genes. Asthma susceptibility has been linked to several chromosomal regions, but with resolution no better than 5-10 cM, in which there are usually hundreds of candidate genes. Moreover, because the effects of genetic variation in any single gene are likely to have only modest effects in the overall pathogenesis of asthma, and because 10 gene-gene and gene-environment interactions confound the analysis, the location of putative susceptibility genes to regions amenable to positional cloning has proven difficult to refine. Nevertheless, asthma susceptibility has been linked to chromosomes 5, 6, 11, 14, and 12. Of these, chromosome 5q23-35 has received the greatest attention because it contains a large number of candidate genes (11, 12, 13, 14, 15, 16, 17, 18), including IL-9, 15 IL-12p40, the β -adrenergic receptor, and the IL-4 cytokine cluster, which contains the genes for IL-4, IL-5, and IL-13. However, the large size of the linked region of 5q complicates its analysis, and a gene for asthma from this site has not yet been conclusively identified.

20

Related Publications

The genetic sequence of the human hepatitis virus A cellular receptor may be found in Genbank, accession number XM_011327. A related sequence is provided in Genbank, accession number BAB55044. Monney *et al.* (2002) Nature 415:436 describe 25 cell surface molecules expressed on Th1 cells. U.S. Patent nos. 5721351, US 6204371, US 6288218 relate to sequences corresponding to a mouse TIM-3 allele.

SUMMARY OF THE INVENTION

Genetic sequences of a gene family encoding polypeptides associated with 30 immune function and cell survival are provided. These genes encode cell surface molecules with conserved IgV and mucin domains, herein referred to as T cell Immunoglobulin domain and Mucin domain (TIM) proteins. The locus comprising the TIM family is genetically associated with immune dysfunction, including asthma. Furthermore, the TIM gene family is located within a region of human chromosome 5 that is commonly 35 deleted in malignancies and myelodysplastic syndrome. Polymorphisms are identified in TIM-1, TIM-3 and TIM-4, which can be associated with Th1/Th2 differentiation and airway hyperresponsiveness (AHR).

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The nucleic acid compositions are used to produce the encoded proteins, which may be employed for functional studies, as a therapeutic, and in studying associated physiological pathways. TIM specific binding agents, including nucleic acids, antibodies, and the like, are useful as diagnostics for determining genetic susceptibility to atopy and asthma and as diagnostics for assessing tumor resistance to cancer therapy. TIM blocking agents find use as therapeutics in the treatment of immune dysfunction and disorders of cell survival, including malignancies.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1a, *HBA mice produce significantly less IL-4 than do BALB/c mice*. Lymph node cells of mice immunized with 150 μ g of KLH were harvested, B-cell depleted, and cultured in cDME with 10 μ g/ml KLH. Supernatants were harvested after 96 hours and assessed for IL-4 levels by ELISA. Shown is a box plot of IL-4 levels ($n=10$ in each group), representing the full range of data, with the boxes encompassing the upper and lower quartiles, with the median of the data set shown inside each box. IL-4 levels produced by BALB/c cells and by (BALB/c x HBA) F1 cells are significantly higher than HBA IL-4 levels ($P<0.0001$ (student's t-test)). *b*, *HBA mice produce significantly less IL-13 and IL-10 than do BALB/c mice*. Data shown are the average values of cytokines produced by lymph node cell cultures from ten individual mice in each experimental group, $\pm$ S.D. HBA IL-13 and IL-10 levels were lower than either the BALB/c or (BALB/c x HBA) F1 values, $P<0.0001$. HBA IL-5 versus BALB/c, $P<0.05$. HBA IFN- γ versus BALB/c, $P<0.001$. *c*, *Allergen-induced airway hyperreactivity is significantly greater in BALB/c than do HBA mice*. Pulmonary airflow obstruction was measured, and data shown represent peak enhanced pause (Penh) values averaged among sensitized mice in each group at various methacholine concentrations, $\pm$ S.E.M. (BALB x HBA) F1 demonstrate BALB/c phenotypes, while (BALB x DBA) F1 mice demonstrate DBA/2-like phenotypes.

Figure 2. Regions of HBA chromosome 11 were inherited from DBA/2. HBA chromosome 11 contains two regions derived from DBA between *hba-a2* and *as-3*, as delineated by SSLP markers. The regions of HBA chromosome 11 with DBA/2 genotypes are highlighted (in blue) in the diagram to the left. The markers (left) provide 2-5 cM resolution distal to *D11Mit230*. Where the mouse chromosome 11 has regions of highly conserved synteny with 5q23-35, additional markers were identified with informative polymorphisms between BALB/c and DBA/2, to provide 0.01-2 cM resolution in this region (right column markers). Single-stranded confirmation polymorphisms (SSCP) markers are shown in green to distinguish them from the SSLP markers, and the positions of particular genes of interest, denoted in red, are also shown. Where the arrangement of our marker map differs from the Chromosome Committee Reports and the MIT linkage map, our map

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concurs with previous linkage and physical maps.

Figure 3a. *IL-4 production by N2 mice is bimodal, with peaks corresponding to F1 and HBA phenotypes.* As a means of evaluating the relative phenotypes of recombinant N2 mice in multiple experiments, we utilized an indexing function that allowed us to consolidate data from multiple experiments. A histogram shows a bimodal distribution of IL-4 index values for N2 mice with (BALB x HBA) F1, HBA homozygous, and recombinant haplotypes indicated. Distributions associated with the F1 and HBA haplotypes are distinct ($P < 0.0001$, paired Student's t-test). *b. IL-4 regulation segregates with Kim1sscp.* Recombinant N2 haplotypes were sorted by IL-4 index values into groups associated with high IL-4 phenotypes (index < 0.35) and low IL-4 phenotypes (index > 0.65). Each column of boxes represents a recombinant haplotype. Alleles for these haplotypes at loci between D11Mit269 to D11Mit154 are shaded according to genotype (dark = F1; pale = HBA). High IL-4 production segregates with four haplotypes (left), and low IL-4 production segregates with four haplotypes (right). The IL-4 phenotype is linked to *Kim1sscp*. *c. AHR of N2 mice is bimodal, with peaks corresponding to F1 and HBA phenotypes.* Index values calculated from Penh values are shown. The histogram shows a bimodal distribution of AHR index values for N2 mice. (BALB x HBA) F1, HBA homozygous, and recombinant haplotypes are indicated. Distributions associated with the F1 and HBA haplotypes are distinct ($P < 0.0001$, paired student's t-test). *d. AHR Regulatory Locus cosegregates with the IL-4 Regulatory Locus between D11Mit22 and D11Mit271.* Recombinant N2 haplotypes were sorted by AHR index values into groups associated with high AHR phenotypes (index < 0.35) and low AHR phenotypes (index > 0.65). Each column of boxes represents a recombinant haplotype. Alleles for these haplotypes at loci between D11Mit269 to D11Mit154 are shaded according to genotype (dark = F1; pale = HBA). High AHR segregates with four haplotypes (left), and low IL-4 production segregates with three haplotypes (right). The AHR regulatory locus is linked to *Kim1sscp*, between D11Mit271 and D11Mit22.

Figure 4 *Mouse chromosome 11 interval containing Tapr is highly homologous to 5q33.* In order to construct a composite map around the *Tapr* locus, we integrated available information from the mouse linkage, backcross, and radiation hybrid maps and identified a region of highly conserved synteny in current maps of the human genome (Human Genome Browser v3, UCSC, March 2001). ESTs located on a physical map of mouse chromosome 11 (left) are denoted by their accession numbers and aligned by homology to genes (center), which correspond to ESTs on human chromosome 5 (right). All known genes between KIAA0171 and *Sgd* are shown.

Figure 5a, b, c. *Identification novel TIM gene family and major polymorphisms in TIM-1 and TIM-3.* Cloning of mouse TIM-1 and mouse TIM-2. Members of a gene family.

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Sequences of the mouse TIM gene family members are shown. Shaded boxes illustrate identity between two of the mouse TIM genes. Total RNA from conA-stimulated splenocytes was reverse transcribed using Gibco Superscript II. PCR products of full length *Tim-3* cDNA were amplified, purified with Qiagen QIAquick PCR Purification reagents, and sequenced directly by Biotech Core (Mountain View, CA). PCR products for *Tim-1* and *Tim-2* cDNA were cloned into electrocompetent TOP10 cells with TOPO-XL cloning reagents (Invitrogen). Plasmids were purified with a standard alkaline lysis protocol. BALB/c and HBA plasmids were sequenced, as described. Homology of mouse TIM-1, rat KIM-1, and HAVcr-1. Identity with mouse TIM-1 is denoted by the shaded boxes.

The approximate signal site is denoted by an open, inverted triangle and the Ig domain/mucin domain boundary is shown with a filled diamond. The predicted transmembrane domains are underlined. TIM-1 and TIM-3 sequences with major polymorphisms between BALB/c and HBA TIM-1 and TIM-3 shown.

Figure 6. *Tapr Regulates CD4 T cell IL-4 and IL-13 Responses.* T cells from BALB/c DO11.10 mice produce higher levels of IL-4 and IL-13 in response to antigen than do T cells from HBA DO11.10 mice. Splenic CD4+ cells were isolated by positive selection with anti-CD4 magnetic beads and then cocultured with bone marrow-derived DCs and OVA. After seven days, the cells were restimulated. Supernatants were harvested after 18-24 hours of the secondary culture. Data represent mean cytokine levels detected at increasing concentrations of OVA, $\pm$ S.D. Detection of expression of TIM-1 mRNA in purified CD 4 T cells during priming and differentiation.

Figure 7. Sequence alignment of Human and Mouse TIM protein sequences.

Figure 8. Polymorphisms in human TIM-1

Figure 9. SSCP polymorphism analysis of human TIM-1.

DETAILED DESCRIPTION OF THE EMBODIMENTS

Genetic sequences associated with immune function, including susceptibility to asthma, are provided. The sequences of murine Tim-1, Tim-2, Tim-3, and Tim-4, and the human counterpart sequences, are provided herein. The sequence of major polymorphisms are also provided. Genomic sequences demonstrate that these polymorphisms, including a deletion, are true polymorphisms, not splicing variants. Variants of the TIM-1 and TIM-3 sequences are associated with susceptibility to airway hyperreactivity and allergic T cell responses, and other variants associated with protection against these responses.

The extracellular domain of TIM gene family members contains two domains, an IgV domain and a mucin domain. This Ig/mucin structure is also found in

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MAdCAM (mucosal addressin cell adhesion molecule), which contains two Ig domains and a mucin domain; however, there is only a low level of homology between TIMs and MAdCAM. A similar degree of homology is also present between TIM-1 and TOSO (NP_005440.1 GI:4885641), a protein which, like TIM-1 is expressed on activated T cells, and which protects T cells from Fas-mediated apoptosis.

T cells express the TIM family of genes, which critically regulates CD4 T cell differentiation. Th1 cells preferentially express the TIM-3 protein, while Th2 cells preferentially express the TIM-1 protein. TIM-1 has been linked to airway hyperreactivity and TIM-3 to autoimmune disease, therefore the expression pattern on differentiating lymphoid cells and the kinetics of expression of TIM-1 on lymphoid cells reflect the function of these molecules.

In another aspect of the invention, methods are provided for determining susceptibility of an individual to developing asthma and atopic diseases, by determining the genotype at the Tim locus. Screening may analyze, for example, polymorphisms in any one of the TIM-1, TIM-3 or TIM-4 alleles provided herein, or otherwise determined. Methods are provided for screening such polymorphisms, *e.g.* SSCP analysis, size polymorphisms, and the like.

In another aspect of the invention, a method of screening for biologically active agents that modulate Tim gene or polypeptide function is provided, where the method comprises combining a candidate biologically active agent with any one of: (a) a Tim polypeptide; (b) a cell comprising a nucleic acid encoding a Tim polypeptide; or (c) a non-human transgenic animal model for Tim gene function comprising one of: (i) a knockout of an *Tim* gene; (ii) an exogenous and stably transmitted *Tim* gene sequence; or (iii) a *Tim* promoter sequence operably linked to a reporter gene; and determining the effect of said agent on Tim function.

The activity of TIM polypeptides may be modulated in order to direct immune function. TIM-1 is preferentially expressed in Th2 cells, and agents that modulate TIM-1 activity find use in the treatment of Th2 related disorders, including allergies, asthma, and the like. TIM-3 is preferentially expressed in Th1 cells, and agents that modulate TIM-3 activity find use in the treatment of pro-inflammatory immune diseases, including autoimmune diseases, graft rejection and the like.

CONDITIONS OF INTEREST

Atopic diseases are complex genetic traits that develop as a result of environmentally induced immune responses in genetically predisposed individuals. Both atopic and non-atopic individuals are exposed to the same environmental factors, but genetic differences that distinguish atopic from non-atopic individuals result in atopic

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disease in some individuals, manifested by allergic inflammation in the respiratory tract, skin or gastrointestinal tract, as well as by elevated serum IgE, eosinophilia and the symptoms of wheezing, sneezing or hives. In addition, allergic inflammatory responses are characterized by the presence of Th2 lymphocytes producing high levels of IL-4, IL-5, IL-9 and IL-13, which enhance the growth, differentiation and/or recruitment of eosinophils, mast cells, basophils and B cells producing IgE.

Allergens of interest include antigens found in food, such as strawberries, peanuts, milk proteins, egg whites, etc. Other allergens of interest include various airborne antigens, such as grass pollens, animal danders, house mite feces, etc. Molecularly cloned allergens include *Dermatophagoides pteronyssinus* (Der P1); Lol pi-V from rye grass pollen; a number of insect venoms, including venom from jumper ant *Myrmecia pilosula*; *Apis mellifera* bee venom phospholipase A2 (PLA₂ and antigen 5S; phospholipases from the yellow jacket *Vespula maculifrons* and white faced hornet *Dolichovespula maculata*; a large number of pollen proteins, including birch pollen, ragweed pollen, *Parol* (the major allergen of *Parietaria officinalis*) and the cross-reactive allergen *Parl* (from *Parietaria judaica*), and other atmospheric pollens including *Olea europaea*, *Artemisia* sp., *gramineae*, etc. Other allergens of interest are those responsible for allergic dermatitis caused by blood sucking arthropods, e.g. *Diptera*, including mosquitos (*Anopheles* sp., *Aedes* sp., *Culiseta* sp., *Culex* sp.); flies (*Phlebotomus* sp., *Culicoides* sp.) particularly black flies, deer flies and biting midges; ticks (*Dermacentor* sp., *Ornithodoros* sp., *Otobius* sp.); fleas, e.g. the order *Siphonaptera*, including the genera *Xenopsylla*, *Pulex* and *Ctenocephalides felis felis*. The specific allergen may be a polysaccharide, fatty acid moiety, protein, etc.

Polymorphisms in a number of interacting/epistatic atopy genes are thought to result in enhanced susceptibility to allergic disorders. Genome wide scans have sought to identify the responsible genes by linking various parameters of allergy and asthma with polymorphic DNA markers of specific genes, usually repeat sequences of DNA (microsatellites that contain di- tri- and tetra-nucleotide repeats). These studies have identified several chromosomal regions as likely to be involved in the pathogenesis of atopy, but with resolution no better than 5-10 cM in which there are typically hundreds of candidate genes. Nevertheless, asthma susceptibility has been linked to chromosomes 5q23-31, chromosome 6p21, chromosome 11q13, and chromosome 12q (9-13) by two or more of these genome wide scanning studies.

Asthma, as defined herein, is reversible airflow limitation in an individual over a period of time. Asthma is characterized by the presence of cells such as eosinophils, mast cells, basophils, and CD25⁺ T lymphocytes in the airway walls. There is a close interaction between these cells, because of the activity of cytokines which have a variety of communication and biological effector properties. Chemokines attract cells to the site of

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inflammation and cytokines activate them, resulting in inflammation and damage to the mucosa. With chronicity of the process, secondary changes occur, such as thickening of basement membranes and fibrosis. The disease is characterized by increased airway hyperresponsiveness to a variety of stimuli, and airway inflammation. A patient diagnosed as asthmatic will generally have multiple indications over time, including wheezing, asthmatic attacks, and a positive response to methacholine challenge, i.e., a PC20 on methacholine challenge of less than about 4 mg/ml. Guidelines for diagnosis may be found, for example, in the National Asthma Education Program Expert Panel Guidelines for Diagnosis and Management of Asthma, National Institutes of Health, 1991, Pub. No. 91-3042.

Asthma, allergic rhinitis (hay fever), atopic dermatitis (eczema) and food allergy are diseases that occur in the same families, implying common genetic mechanisms. These atopic diseases are exceedingly prevalent, affecting 20-40% of the general population and constitute a major public health problem. The economic costs for these disorders are enormous. For asthma alone, the estimated health care costs in 1996 were \$14 billion. In addition, the prevalence of all of the atopic diseases has increased dramatically in industrialized countries over the past two decades for reasons that are not yet clear. The prevalence of asthma in industrialized countries, for which the numbers are the most accurate, has doubled since 1982, and is projected to double again in prevalence by the year 2020.

Pro-inflammatory diseases associated with Th1 type T cells include autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, type I diabetes mellitus, *etc.* (RA) is a chronic autoimmune inflammatory synovitis affecting 0.8% of the world population. Current therapy for RA utilizes therapeutic agents that non-specifically suppress or modulate immune function. Such therapeutics, including the recently developed TNF α antagonists, are not fundamentally curative, and disease activity rapidly returns following discontinuation of therapy. Tremendous clinical need exists for fundamentally curative therapies that do not cause systemic immune suppression or modulation.

A quantitative increase in myelin-autoreactive T cells with the capacity to secrete IFN-gamma is associated with the pathogenesis of MS and EAE, suggesting that autoimmune inducer/helper T lymphocytes in the peripheral blood of MS patients may initiate and/or regulate the demyelination process in patients with MS. The overt disease is associated with muscle weakness, loss of abdominal reflexes, visual defects and paresthesias. During the presymptomatic period there is infiltration of leukocytes into the cerebrospinal fluid, inflammation and demyelination.

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10 IDDM is a cell-mediated autoimmune disorder leading to destruction of
insulin-secreting beta cells and overt hyperglycemia. T lymphocytes invade the islets of
Langerhans, and specifically destroy insulin-producing β -cells. The depletion of β cells
results in an inability to regulate levels of glucose in the blood. The disease progression
5 may be monitored in individuals diagnosed by family history and genetic analysis as being
susceptible. The most important genetic effect is seen with genes of the major
histocompatibility locus (*IDD1*), although other loci, including the insulin gene region
(*IDD2*) also show linkage to the disease (see Davies *et al, supra* and Kennedy *et al.*
(1995) *Nature Genetics* 9:293-298).

10

TIM GENE FAMILY

The provided TIM family genes and fragments thereof, encoded proteins,
genomic regulatory regions, and specific antibodies are useful in the identification of
individuals predisposed to development or resistance to asthma, and for the modulation of
15 gene activity in vivo for prophylactic and therapeutic purposes. The encoded proteins are
useful as an immunogen to raise specific antibodies, in drug screening for compositions
that mimic or modulate activity or expression, including altered forms of the proteins, and as
a therapeutic.

The TIM family genes are immediately adjacent to each other on human
20 chromosome 5, in the order TIM-4, TIM-1, TIM-3, with no intervening genes. This segment
of human chromosome 5 is commonly deleted in malignancies and dysplastic cell
populations, as in myelodysplastic syndrome (see Boultonwood, *et al*, (1997) *Genomics*
45:88-96). There are TIM pseudogenes on chromosomes 5, 12, and 19. Each TIM
protein, except TIM-4, contains a distinct predicted tyrosine signaling motif. The
25 cytoplasmic region of TIM-1 contains two tyrosine residues and includes a highly conserved
tyrosine kinase phosphorylation motif, RAEDNIY. The expanded region,
SRAEDNIYVEDRP, contains a predicted site for Itk phosphorylation and for EGF-receptor
phosphorylation.

The mouse *Tim1* gene encodes a 305 amino acid membrane protein. The
30 cytoplasmic region of TIM-1 contains two tyrosine residues and includes a highly conserved
tyrosine kinase phosphorylation motif, RAEDNIY. The mucin domain of TIM-1 has multiple
sites for O-linked glycosylation, and there two sites for N-linked glycosylation found in the
immunoglobulin domain.

Mouse TIM-2, a similar 305 amino acid membrane protein, has 64% identity
35 to mouse TIM-1, 60% identity to rat KIM-1, and 32% identity to hHAVcr-1. Like TIM-1, TIM-
2 has two extracellular N-linked glycosylation sites and a serine, threonine- rich mucin
domain with many O-linked glycosylation sites. TIM-2 also has an intracellular tyrosine

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kinase phosphorylation motif, RTRCEDQVY.

Tim3 encodes a 281 amino acid membrane protein in mice, and a 301 amino acid protein in humans, that has a similar, integral membrane glycoprotein structure with multiple extracellular glycosylation sites and an intracellular tyrosine phosphorylation motif.

5 Although the mucin domain is not as prominent in TIM-3 as it is in TIM-1 and TIM-2, TIM-3 expressed on T cells interacts with a ligand on APCs and alters APC activation. TIM-3 has four sites for N-linked and five sites for O-linked glycosylation, suggesting that TIM-3, like TIM-1 and TIM-2, is heavily glycosylated and might interact with a ligand present on other cells, such as antigen presenting cells.

10 *Tim4* encodes a 344 amino acid protein in mice, and a 378 amino acid protein in humans. The predicted TIM-4 also shares the general membrane glycoprotein structural motifs of the other TIM proteins, a with an IgV-like domain with highly conserved cysteine residues, a threonine-rich mucin-like domain, and a short intracellular tail.

Polymorphisms in the murine sequences are provided in the sequence listing 15 for the BALB/c and HBA/DBA strains. In TIM-1, these polymorphisms encode three amino acid differences and a fifteen amino acid deletion in HBA/DBA. Seven predicted amino acid differences were identified in TIM-3. The polymorphisms in TIM-1 and TIM-4 are located in the signal and mucin-like domains, while the polymorphisms identified in TIM-3 are clustered in the Ig domain.

20 Variants in coding regions of human *Tim1* are provided in the seqlist and Figure 8. Variations include an insertion (labeled polymorphism 1), 157insMTTTPV, observed in 65% of the chromosomes, and a deletion (polymorphism 5), 187ΔThr, observed in 65% of the chromosomes. Other polymorphisms are T140A (polymorphism 7); V161A; (polymorphism 2); V167I (polymorphism 3); T172A (polymorphism 4); N258D 25 (polymorphism 6). Polymorphism 4 was observed in 40% of the chromosomes, and the other polymorphisms were each observed in ≤5% of the chromosomes. Most of these variations (2-6) are located within exon 3, the first mucin-encoding exon, and all of the variants occur at the genomic level and are not splice variants. The association between *Tim1* and asthma susceptibility is further supported by reports of significant linkage of mite-sensitive childhood asthma to D5S820 (mean LOD score = 4.8), a marker which is 30 approximately 0.5 megabases from *Tim1*.

In human tissues, a 4.4 kb TIM-1 mRNA is present in almost all tissues, though it is faint in most. A 5.5-kb band was observed in colon and liver. A 7.5-kb band was observed in spleen, thymus, and peripheral blood leukocytes, and smaller than 4.4-kb 35 bands were observed in some organs. TIM-1 mRNA is expressed with alternate 5' untranslated regions, in different cell populations. Hypoxia and ischemia induces TIM-1 expression in epithelial cells, and radiation induces expression of TIM gene family mRNA.

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The TIM genes are expressed in tumor specimens. Human TIM-4 mRNA is expressed in glioblastoma tissue, and is also detected in mitogen stimulated or irradiated peripheral blood monocytes.

In one aspect, the invention provides for an isolated nucleic acid molecule other than a naturally occurring chromosome comprising a sequence encoding a TIM-1, TIM-2, TIM-3 or TIM-4 protein, or a homolog or variant thereof, which variant may be associated with susceptibility to airway hyperreactivity and allergic T cell responses. The nucleic acid may be operably linked to a vector and/or control sequences for expression in a homologous or heterologous host cell. Such a host cell can find use in the production of the encoded protein. In another aspect of the invention, a purified polypeptide is provided of TIM-1, TIM-2, TIM-3 or TIM-4 protein, or a homolog or variant thereof, which variant may be associated with susceptibility to airway hyperreactivity and allergic T cell responses. In another aspect, an antibody or other specific binding member that binds to the TIM-1, TIM-2, TIM-3 or TIM-4 polypeptide is provided.

Sequences of human and murine TIM sequences are provided in the sequence listing, as follows:

SEQ ID NO	Name	Length	Type
1	BALB/c TIM-1	305	Protein
2	BALB/c TIM-1	918	DNA
3	ES-HBA TIM-1	282	Protein
4	ES-HBA TIM-1	849	DNA
5	BALB/c TIM-2	305	Protein
6	BALB/c TIM-1	958	DNA
7	ES-HBA TIM-2	305	Protein
8	ES-HBA TIM-2	958	DNA
9	BALB/c TIM-3	281	Protein
10	BALB/c TIM-3	2725	DNA
11	ES-HBA TIM-3	281	Protein
12	ES-HBA TIM-3	862	DNA
13	BALB/c TIM-4	345	Protein
14	BALB/c TIM-4	1032	DNA
15	ES-HBA TIM-4	345	Protein
16	ES-HBA TIM-4	1032	DNA
17	hTIM-1 allele 1	359	Protein
18	hTIM-1 allele 1	1080	DNA
19	hTIM-1 allele 2	359	Protein
20	hTIM-1 allele 2	1080	DNA
21	hTIM-1 allele 3	365	Protein
22	hTIM-1 allele 3	1098	DNA
23	hTIM-1 allele 4	359	Protein
24	hTIM-1 allele 4	1079	DNA
25	hTIM-1 allele 5	364	Protein
26	hTIM-1 allele 5	1095	DNA
27	hTIM-1 allele 6	364	Protein
28	hTIM-1 allele 6	1099	DNA
29	hTIM-3 allele 1	301	Protein
30	hTIM-3 allele 1	1116	DNA

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31	hTIM-3 allele 2	301	Protein
32	hTIM-3 allele 2	1116	DNA
33	hTIM-4 allele 1	378	Protein
34	hTIM-4 allele 1	1156	DNA
35	hTIM-4 allele 2	378	Protein
36	hTIM-4 allele 2	1156	DNA

The DNA sequence encoding Tim polypeptides may be cDNA or genomic DNA or a fragment thereof. Fragments of interest for probes, producing polypeptides, *etc.* may comprise one or more polymorphic residues. The term Tim gene shall be intended to mean the open reading frame encoding any one of the specific Tim polypeptides, introns, as well as adjacent 5' and 3' non-coding nucleotide sequences involved in the regulation of expression, up to about 1 kb beyond the coding region, but possibly further in either direction. The gene may be introduced into an appropriate vector for extrachromosomal maintenance or for integration into the host.

In some embodiments, the Tim gene sequence is other than human TIM-1 allele 1, as set forth in the sequence listing; and/or other than mouse TIM-3 DBA allele.

The term "cDNA" as used herein is intended to include all nucleic acids that share the arrangement of sequence elements found in native mature mRNA species, where sequence elements are exons and 3' and 5' non-coding regions. Normally mRNA species have contiguous exons, with the intervening introns removed by nuclear RNA splicing, to create a continuous open reading frame encoding a Tim protein.

A genomic sequence of interest comprises the nucleic acid present between the initiation codon and the stop codon, as defined in the listed sequences, including all of the introns that are normally present in a native chromosome. It may further include the 3' and 5' untranslated regions found in the mature mRNA. It may further include specific transcriptional and translational regulatory sequences, such as promoters, enhancers, *etc.*, including about 1 kb, but possibly more, of flanking genomic DNA at either the 5' or 3' end of the transcribed region. The genomic DNA may be isolated as a fragment of 100 kbp or smaller; and substantially free of flanking chromosomal sequence.

The sequence of the 5' region, and further 5' upstream sequences and 3' downstream sequences, may be utilized for promoter elements, including enhancer binding sites, that provide for expression in tissues where *Tim* genes are expressed. The tissue specific expression is useful for determining the pattern of expression, and for providing promoters that mimic the native pattern of expression. Naturally occurring polymorphisms in the promoter region are useful for determining natural variations in expression, particularly those that may be associated with disease. Alternatively, mutations may be introduced into the promoter region to determine the effect of altering expression in experimentally defined systems. Methods for the identification of specific DNA motifs

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involved in the binding of transcriptional factors are known in the art, *e.g.* sequence similarity to known binding motifs, gel retardation studies, *etc.* For examples, see Blackwell *et al.* (1995) *Mol Med* **1**: 194-205; Mortlock *et al.* (1996) *Genome Res.* **6**: 327-33; and Joulin and Richard-Foy (1995) *Eur J Biochem* **232**: 620-626.

5 The regulatory sequences may be used to identify *cis* acting sequences required for transcriptional or translational regulation of TIM expression, especially in different tissues or stages of development, and to identify *cis* acting sequences and *trans* acting factors that regulate or mediate TIM expression. Such transcription or translational control regions may be operably linked to a TIM gene in order to promote expression of wild
10 type or altered TIM or other proteins of interest in cultured cells, or in embryonic, fetal or adult tissues, and for gene therapy.

The nucleic acid compositions of the subject invention may encode all or a part of the subject polypeptides. Fragments may be obtained of the DNA sequence by chemically synthesizing oligonucleotides in accordance with conventional methods, by
15 restriction enzyme digestion, by PCR amplification, *etc.* For the most part, DNA fragments will be of at least 15 nt, usually at least 18 nt, more usually at least about 50 nt. Such small DNA fragments are useful as primers for PCR, hybridization screening, *etc.* Larger DNA fragments, *i.e.* greater than 100 nt are useful for production of the encoded polypeptide. For use in amplification reactions, such as PCR, a pair of primers will be used. The exact
20 composition of the primer sequences is not critical to the invention, but for most applications the primers will hybridize to the subject sequence under stringent conditions, as known in the art. It is preferable to choose a pair of primers that will generate an amplification product of at least about 50 nt, preferably at least about 100 nt. Algorithms for the selection of primer sequences are generally known, and are available in commercial
25 software packages. Amplification primers hybridize to complementary strands of DNA, and will prime towards each other.

The TIM genes are isolated and obtained in substantial purity, generally as other than an intact mammalian chromosome. Usually, the DNA will be obtained substantially free of other nucleic acid sequences that do not include an TIM sequence or
30 fragment thereof, generally being at least about 50%, usually at least about 90% pure and are typically "recombinant", *i.e.* flanked by one or more nucleotides with which it is not normally associated on a naturally occurring chromosome.

The DNA sequences are used in a variety of ways. They may be used as probes for identifying TIM related genes. Mammalian homologs have substantial sequence
35 similarity to the subject sequences, *i.e.* at least 75%, usually at least 90%, more usually at least 95% sequence identity with the nucleotide sequence of the subject DNA sequence. Sequence similarity is calculated based on a reference sequence, which may be a subset

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of a larger sequence, such as a conserved motif, coding region, flanking region, etc. A reference sequence will usually be at least about 18 nt long, more usually at least about 30 nt long, and may extend to the complete sequence that is being compared. Algorithms for sequence analysis are known in the art, such as BLAST, described in Altschul *et al.* (1990)

5 J Mol Biol **215**:403-10.

Nucleic acids having sequence similarity are detected by hybridization under low stringency conditions, for example, at 50°C and 10XSSC (0.9 M saline/0.09 M sodium citrate) and remain bound when subjected to washing at 55°C in 1XSSC. Sequence identity may be determined by hybridization under stringent conditions, for example, at 10 50°C or higher and 0.1XSSC (9 mM saline/0.9 mM sodium citrate). By using probes, particularly labeled probes of DNA sequences, one can isolate homologous or related genes. The source of homologous genes may be any species, *e.g.* primate species, particularly human; rodents, such as rats and mice, canines, felines, bovine, ovine, equine, yeast, *Drosophila*, *Caenorhabditis*, etc.

15 The DNA may also be used to identify expression of the gene in a biological specimen. The manner in which one probes cells for the presence of particular nucleotide sequences, as genomic DNA or RNA, is well established in the literature and does not require elaboration here. mRNA is isolated from a cell sample. mRNA may be amplified by RT-PCR, using reverse transcriptase to form a complementary DNA strand, followed by 20 polymerase chain reaction amplification using primers specific for the subject DNA sequences. Alternatively, mRNA sample is separated by gel electrophoresis, transferred to a suitable support, *e.g.* nitrocellulose, nylon, etc., and then probed with a fragment of the subject DNA as a probe. Other techniques, such as oligonucleotide ligation assays, *in situ* hybridizations, and hybridization to DNA probes arrayed on a solid chip may also find use. 25 Detection of mRNA hybridizing to the subject sequence is indicative of *TIM* gene expression in the sample.

The subject nucleic acid sequences may be modified for a number of purposes, particularly where they will be used intracellularly, for example, by being joined to a nucleic acid cleaving agent, *e.g.* a chelated metal ion, such as iron or chromium for 30 cleavage of the gene; or the like.

The sequence of the *TIM* locus, including flanking promoter regions and coding regions, may be mutated in various ways known in the art to generate targeted changes in promoter strength, sequence of the encoded protein, etc. The DNA sequence or product of such a mutation will be substantially similar to the sequences provided herein, 35 *i.e.* will differ by at least one nucleotide or amino acid, respectively, and may differ by at least two but not more than about ten nucleotides or amino acids. The sequence changes may be substitutions, insertions or deletions. Deletions may further include larger changes,

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such as deletions of a domain or exon. Other modifications of interest include epitope tagging, e.g. with the FLAG system, HA, etc. For studies of subcellular localization, fusion proteins with green fluorescent proteins (GFP) may be used. Such mutated genes may be used to study structure-function relationships of TIM polypeptides, or to alter properties of the protein that affect its function or regulation. For example, constitutively active transcription factors, or a dominant negatively active protein that binds to the TIM DNA target site without activating transcription, may be created in this manner.

Techniques for *in vitro* mutagenesis of cloned genes are known. Examples of protocols for scanning mutations may be found in Gustin *et al.*, *Biotechniques* 14:22 (1993); Barany, *Gene* 37:111-23 (1985); Colicelli *et al.*, *Mol Gen Genet* 199:537-9 (1985); and Prentki *et al.*, *Gene* 29:303-13 (1984). Methods for site specific mutagenesis can be found in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, CSH Press 1989, pp. 15.3-15.108; Weiner *et al.*, *Gene* 126:35-41 (1993); Sayers *et al.*, *Biotechniques* 13:592-6 (1992); Jones and Winistorfer, *Biotechniques* 12:528-30 (1992); Barton *et al.*, *Nucleic Acids Res* 18:7349-55 (1990); Marotti and Tomich, *Gene Anal Tech* 6:67-70 (1989); and Zhu *Anal Biochem* 177:120-4 (1989).

Arrays provide a high throughput technique that can assay a large number of polynucleotides in a sample. In one aspect of the invention, an array is constructed comprising one or more of the TIM genes, proteins or antibodies, preferably comprising all of these sequences, which array may further comprise other sequences known to be up- or down-regulated in T cells, monocytes, and the like. This technology can be used as a tool to test for differential expression, or for genotyping. Arrays can be created by spotting polynucleotide probes onto a substrate (e.g., glass, nitrocellulose, etc.) in a two-dimensional matrix or array having bound probes. The probes can be bound to the substrate by either covalent bonds or by non-specific interactions, such as hydrophobic interactions. Techniques for constructing arrays and methods of using these arrays are described in, for example, Schena *et al.* (1996) *Proc Natl Acad Sci U S A*, 93(20):10614-9; Schena *et al.* (1995) *Science* 270(5235):467-70; Shalon *et al.* (1996) *Genome Res.* 6(7):639-45; USPN 5,807,522, EP 799 897; WO 97/29212; WO 97/27317; EP 785 280; WO 97/02357; USPN 5,593,839; USPN 5,578,832; EP 728 520; USPN 5,599,695; EP 721 016; USPN 5,556,752; WO 95/22058; and USPN 5,631,734.

The probes utilized in the arrays can be of varying types and can include, for example, synthesized probes of relatively short length (e.g., a 20-mer or a 25-mer), cDNA (full length or fragments of gene), amplified DNA, fragments of DNA (generated by restriction enzymes, for example) and reverse transcribed DNA. Both custom and generic arrays can be utilized in detecting differential expression levels. Custom arrays can be prepared using probes that hybridize to particular preselected subsequences of mRNA

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gene sequences or amplification products prepared from them.

Arrays can be used to, for example, examine differential expression of genes and can be used to determine gene function. For example, arrays can be used to detect differential expression, or expression of polymorphic sequences, in TIM genes. Exemplary uses of arrays are further described in, for example, Pappalarado *et al.* (1998) Sem. Radiation Oncol. 8:217; and Ramsay. (1998) Nature Biotechnol. 16:40. Furthermore, many variations on methods of detection using arrays are well within the skill in the art and within the scope of the present invention. For example, rather than immobilizing the probe to a solid support, the test sample can be immobilized on a solid support which is then contacted with the probe. Additional discussion regarding the use of microarrays in expression analysis can be found, for example, in Duggan, et al., Nature Genetics Supplement 21:10-14 (1999); Bowtell, Nature Genetics Supplement 21:25-32 (1999); Brown and Botstein, Nature Genetics Supplement 21:33-37 (1999); Cole et al., Nature Genetics Supplement 21:38-41 (1999); Debouck and Goodfellow, Nature Genetics Supplement 21:48-50 (1999); Bassett, Jr., et al., Nature Genetics Supplement 21:51-55 (1999); and Chakravarti, Nature Genetics Supplement 21:56-60 (1999).

Pharmacogenetics is the linkage between an individual's genotype and that individual's ability to metabolize or react to a therapeutic agent. Differences in metabolism or target sensitivity can lead to severe toxicity or therapeutic failure by altering the relation between bioactive dose and blood concentration of the drug. In the past few years, numerous studies have established good relationships between polymorphisms in metabolic enzymes or drug targets, and both response and toxicity. These relationships can be used to individualize therapeutic dose administration.

Genotyping of polymorphic alleles is used to evaluate whether an individual will respond well to a particular therapeutic regimen. The polymorphic sequences are also used in drug screening assays, to determine the dose and specificity of a candidate therapeutic agent. A candidate TIM polymorphism is screened with a target therapy to determine whether there is an influence on the effectiveness in treating asthma. Drug screening assays are performed as described above. Typically two or more different sequence polymorphisms are tested for response to a therapy.

The subject gene may be employed for synthesis of a complete TIM protein, or polypeptide fragments thereof, particularly fragments corresponding to functional domains; binding sites; *etc.*; and including fusions of the subject polypeptides to other proteins or parts thereof. For expression, an expression cassette may be employed, providing for a transcriptional and translational initiation region, which may be inducible or constitutive, where the coding region is operably linked under the transcriptional control of the transcriptional initiation region, and a transcriptional and translational termination

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region. Various transcriptional initiation regions may be employed that are functional in the expression host.

Polypeptides of particular interest that are fragments of the TIM polypeptides include specific domains of the TIM polypeptides, where a domain may comprise, for example, the extracellular domain, or the domains within the extracellular domain: the mucin domain and/or the Ig domain. Domains may also comprise the cytoplasmic domain, e.g. a fragment encompassing the tyrosine kinase phosphorylation motif, RAEDNIY, or the expanded region, SRAEDNIYVEDRP. Polypeptides encoded by the soluble splice variants are also of interest. The sequence of the Ig domains are as follows: human TIM-1 Ig domain, SEQ ID NO: 17, 19, 21, 23, 25, 27, residues 21-126; human TIM-3 Ig domain, SEQ ID NO: 29 and 31, residues 22-131; human TIM-4 Ig domain, SEQ ID NO: 33 and 35, residues 25-133; mouse TIM-1 Ig domain, SEQ ID NO: 1 and 3, residues 21-129; mouse TIM-2 Ig domain, SEQ ID NO: 7, residues 22-128; mouse TIM-3 Ig domain, BALB/c allele, SEQ ID NO: 9, residues 22-132; mouse TIM-3 Ig domain, DBA/2 allele, SEQ ID No: 11, residues 22-132; mouse TIM-4 Ig domain, SEQ ID NO: 13 and 15, residues 25-135.

Functionally equivalent polypeptides may find use, where the equivalent polypeptide may contain deletions, additions or substitutions of amino acid residues that result in a silent change, thus producing a functionally equivalent differentially expressed on pathway gene product. Amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. "Functionally equivalent", as used herein, refers to a protein capable of exhibiting a substantially similar *in vivo* activity as the polypeptide encoded by a TIM gene.

The polypeptides may be expressed in prokaryotes or eukaryotes in accordance with conventional ways, depending upon the purpose for expression. For large scale production of the protein, a unicellular organism, such as *E. coli*, *B. subtilis*, *S. cerevisiae*, or cells of a higher organism such as vertebrates, particularly mammals, e.g. COS 7 cells, may be used as the expression host cells. In many situations, it may be desirable to express the TIM gene in mammalian cells, where the TIM gene will benefit from native folding and post-translational modifications. Small peptides can also be synthesized in the laboratory, including specific peptide epitopes, domains, and the like, where peptides will usually be at least about 8 amino acids in length, more usually at least about 20 amino acids in length, up to complete domains, and the full length protein. Peptides may comprise polymorphic regions of the protein. Also included are fusion proteins, where all or a fragment of the TIM protein is fused to a heterologous polypeptide, e.g. green fluorescent protein, antibody Fc regions, poly-histidine, and the like.

In mammalian host cells, a number of viral-based expression systems may

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be used, including retrovirus, lentivirus, adenovirus, adeno-associated virus, and the like. In cases where an adenovirus is used as an expression vector, the coding sequence of interest can be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in
5 the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing differentially expressed or pathway gene protein in infected hosts.

Specific initiation signals may also be required for efficient translation of the
10 genes. These signals include the ATG initiation codon and adjacent sequences. In cases where a complete gene, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only a portion of the gene coding sequence is inserted, exogenous translational control signals must be provided. These exogenous
15 translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc.

With the availability of the polypeptides in large amounts, by employing an expression host, the polypeptides may be isolated and purified in accordance with
20 conventional ways. A lysate may be prepared of the expression host and the lysate purified using HPLC, exclusion chromatography, gel electrophoresis, affinity chromatography, or other purification technique. The purified polypeptide will generally be at least about 80% pure, preferably at least about 90% pure, and may be up to and including 100% pure. Pure is intended to mean free of other proteins, as well as cellular debris.

The polypeptide may be labeled, either directly or indirectly. Any of a variety of suitable labeling systems may be used, including but not limited to, radioisotopes such as ¹²⁵I; enzyme labeling systems that generate a detectable colorimetric signal or light when exposed to substrate; and fluorescent labels. Indirect labeling involves the use of a protein, such as a labeled antibody, that specifically binds to the polypeptide of interest. Such
30 antibodies include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments and fragments produced by a Fab expression library.

SPECIFIC BINDING MEMBERS

The term "specific binding member" or "binding member" as used herein
35 refers to a member of a specific binding pair, i.e. two molecules, usually two different molecules, where one of the molecules (i.e., first specific binding member) through chemical or physical means specifically binds to the other molecule (i.e., second specific

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binding member). The complementary members of a specific binding pair are sometimes referred to as a ligand and receptor; or receptor and counter-receptor. For the purposes of the present invention, the two binding members may be known to associate with each other, for example where an assay is directed at detecting compounds that interfere with the association of a known binding pair. Alternatively, candidate compounds suspected of being a binding partner to a compound of interest may be used.

Specific binding pairs of interest include carbohydrates and lectins; complementary nucleotide sequences; peptide ligands and receptor; effector and receptor molecules; hormones and hormone binding protein; enzyme cofactors and enzymes; enzyme inhibitors and enzymes; lipid and lipid-binding protein; etc. The specific binding pairs may include analogs, derivatives and fragments of the original specific binding member. For example, a receptor and ligand pair may include peptide fragments, chemically synthesized peptidomimetics, labeled protein, derivatized protein, etc.

In a preferred embodiment, the specific binding member is an antibody. The term "antibody" or "antibody moiety" is intended to include any polypeptide chain-containing molecular structure with a specific shape that fits to and recognizes an epitope, where one or more non-covalent binding interactions stabilize the complex between the molecular structure and the epitope. Antibodies that bind specifically to one of the TIM proteins are referred to as anti-TIM. The archetypal antibody molecule is the immunoglobulin, and all types of immunoglobulins, IgG, IgM, IgA, IgE, IgD, etc., from all sources, e.g. human, rodent, rabbit, cow, sheep, pig, dog, other mammal, chicken, other avians, etc., are considered to be "antibodies." Antibodies utilized in the present invention may be polyclonal antibodies, although monoclonal antibodies are preferred because they may be reproduced by cell culture or recombinantly, and can be modified to reduce their antigenicity.

Polyclonal antibodies can be raised by a standard protocol by injecting a production animal with an antigenic composition, which may be a polypeptide or a cDNA expressed *in vivo*. See, e.g., Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. When utilizing an entire protein, or a larger section of the protein, antibodies may be raised by immunizing the production animal with the protein and a suitable adjuvant (e.g., Freund's, Freund's complete, oil-in-water emulsions, etc.) When a smaller peptide is utilized, it is advantageous to conjugate the peptide with a larger molecule to make an immunostimulatory conjugate. Commonly utilized conjugate proteins that are commercially available for such use include bovine serum albumin (BSA) and keyhole limpet hemocyanin (KLH). In order to raise antibodies to particular epitopes, such as polymorphic residues, peptides derived from the full sequence may be utilized. The immunogen is injected into the animal host, preferably according to a predetermined

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schedule incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Alternatively, for monoclonal antibodies, hybridomas may be formed by
5 isolating the stimulated immune cells, such as those from the spleen of the inoculated animal. These cells are then fused to immortalized cells, such as myeloma cells or transformed cells, which are capable of replicating indefinitely in cell culture, thereby producing an immortal, immunoglobulin-secreting cell line. The immortal cell line utilized is preferably selected to be deficient in enzymes necessary for the utilization of certain
10 nutrients. Many such cell lines (such as myelomas) are known to those skilled in the art, and include, for example: thymidine kinase (TK) or hypoxanthine-guanine phosphoribosyl transferase (HGPRT). These deficiencies allow selection for fused cells according to their ability to grow on, for example, hypoxanthine aminopterinthymidine medium (HAT).

Preferably, the immortal fusion partners utilized are derived from a line that
15 does not secrete immunoglobulin. The resulting fused cells, or hybridomas, are cultured under conditions that allow for the survival of fused, but not unfused, cells and the resulting colonies screened for the production of the desired monoclonal antibodies. Colonies producing such antibodies are cloned, expanded, and grown so as to produce large quantities of antibody, see Kohler and Milstein, 1975 Nature 256:495 (the disclosures of
20 which are hereby incorporated by reference).

Large quantities of monoclonal antibodies from the secreting hybridomas may then be produced by injecting the clones into the peritoneal cavity of mice and harvesting the ascites fluid therefrom. The mice, preferably primed with pristane, or some other tumor-promoter, and immunosuppressed chemically or by irradiation, may be any of
25 various suitable strains known to those in the art. The ascites fluid is harvested from the mice and the monoclonal antibody purified therefrom, for example, by CM Sepharose column or other chromatographic means. Alternatively, the hybridomas may be cultured *in vitro* or as suspension cultures. Batch, continuous culture, or other suitable culture processes may be utilized. Monoclonal antibodies are then recovered from the culture
30 medium or supernatant.

In addition, the antibodies or antigen binding fragments may be produced by genetic engineering. In this technique, as with the standard hybridoma procedure, antibody-producing cells are sensitized to the desired antigen or immunogen. The messenger RNA isolated from the immune spleen cells or hybridomas is used as a
35 template to make cDNA using PCR amplification. A library of vectors, each containing one heavy chain gene and one light chain gene retaining the initial antigen specificity, is produced by insertion of appropriate sections of the amplified immunoglobulin cDNA into

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the expression vectors. A combinatorial library is constructed by combining the heavy chain gene library with the light chain gene library. This results in a library of clones which co-express a heavy and light chain (resembling the Fab fragment or antigen binding fragment of an antibody molecule). The vectors that carry these genes are co-transfected into a host (e.g. bacteria, insect cells, mammalian cells, or other suitable protein production host cell.). When antibody gene synthesis is induced in the transfected host, the heavy and light chain proteins self-assemble to produce active antibodies that can be detected by screening with the antigen or immunogen.

Chimeric antibodies may be made by recombinant means by combining the murine variable light and heavy chain regions (VK and VH), obtained from a murine (or other animal-derived) hybridoma clone, with the human constant light and heavy chain regions, in order to produce an antibody with predominantly human domains. The production of such chimeric antibodies is well known in the art, and may be achieved by standard means (as described, e.g., in U.S. Patent No. 5,624,659, incorporated fully herein by reference). Humanized antibodies are engineered to contain even more human-like immunoglobulin domains, and incorporate only the complementarity-determining regions of the animal-derived antibody. This is accomplished by carefully examining the sequence of the hyper-variable loops of the variable regions of the monoclonal antibody, and fitting them to the structure of the human antibody chains. Although facially complex, the process is straightforward in practice. See, e.g., U.S. Patent No. 6,187,287, incorporated fully herein by reference.

Alternatively, polyclonal or monoclonal antibodies may be produced from animals that have been genetically altered to produce human immunoglobulins. Techniques for generating such animals, and deriving antibodies therefrom, are described in U.S. Patents No. 6,162,963 and 6,150,584, incorporated fully herein by reference.

Alternatively, single chain antibodies (Fv, as described below) can be produced from phage libraries containing human variable regions. See U.S. Patent No. 6,174,708. Intrathecal administration of single-chain immunotoxin, LMB-7 [B3(Fv)- PE38], has been shown to cure of carcinomatous meningitis in a rat model. *Proc Natl. Acad. Sci U S A* 92, 2765-9, all of which are incorporated by reference fully herein.

In addition to entire immunoglobulins (or their recombinant counterparts), immunoglobulin fragments comprising the epitope binding site (e.g., Fab', F(ab')₂, or other fragments) are useful as antibody moieties in the present invention. Such antibody fragments may be generated from whole immunoglobulins by pepsin, papain, or other protease cleavage. "Fragment," or minimal immunoglobulins may be designed utilizing recombinant immunoglobulin techniques. For instance "Fv" immunoglobulins for use in the present invention may be produced by linking a variable light chain region to a variable

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heavy chain region via a peptide linker (e.g., poly-glycine or another sequence which does not form an alpha helix or beta sheet motif).

Fv fragments are heterodimers of the variable heavy chain domain (V_H) and the variable light chain domain (V_L). The heterodimers of heavy and light chain domains that occur in whole IgG, for example, are connected by a disulfide bond. Recombinant Fvs in which V_H and V_L are connected by a peptide linker are typically stable. These are single chain Fvs which have been found to retain specificity and affinity and have been shown to be useful for imaging tumors and to make recombinant immunotoxins for tumor therapy. However, researchers have found that some of the single chain Fvs have a reduced affinity for antigen and the peptide linker can interfere with binding. Improved Fv's have been also been made which comprise stabilizing disulfide bonds between the V_H and V_L regions, as described in U.S. Patent No. 6,147,203, incorporated fully herein by reference. Any of these minimal antibodies may be utilized in the present invention, and those which are humanized to avoid HAMA reactions are preferred for use in embodiments of the invention.

In addition, derivatized immunoglobulins with added chemical linkers, detectable moieties, such as fluorescent dyes, enzymes, substrates, chemiluminescent moieties and the like, or specific binding moieties, such as streptavidin, avidin, or biotin, and the like may be utilized in the methods and compositions of the present invention. For convenience, the term "antibody" or "antibody moiety" will be used throughout to generally refer to molecules which specifically bind to an epitope of the brain tumor protein targets, although the term will encompass all immunoglobulins, derivatives, fragments, recombinant or engineered immunoglobulins, and modified immunoglobulins, as described above.

Candidate antibodies can be tested for activity by any suitable standard means. As a first screen, the antibodies may be tested for binding against the immunogen. As a second screen, antibodies may be screened for cross-reactivity between alleles and between TIM family members, and tested for activity in inhibition of TIM function. For these screens, the candidate antibody may be labeled for detection. Antibodies that alter the biological activity of a TIM protein may be assayed in functional formats.

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DIAGNOSIS

Diagnosis of asthma or atopy associated with Tim polymorphisms is performed by protein, DNA or RNA sequence and/or hybridization analysis of any convenient sample from a patient, e.g. biopsy material, blood sample, scrapings from cheek, etc. A nucleic acid sample from a patient having asthma that may be associated with TIM, is analyzed for the presence of a predisposing polymorphism in TIM. A typical patient genotype would have at least one predisposing mutation on at least one chromosome. The presence of a polymorphic TIM sequence that affects the activity or

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- expression of the gene product, and confers an increased susceptibility to asthma is considered a predisposing polymorphism. Individuals are screened by analyzing their DNA or mRNA for the presence of a predisposing polymorphism, as compared to an asthma neutral sequence. Specific sequences of interest include any polymorphism that leads to clinical bronchial hyperreactivity or is otherwise associated with asthma, including, but not limited to, insertions, substitutions and deletions in the coding region sequence, intron sequences that affect splicing, or promoter or enhancer sequences that affect the activity and expression of the protein. Examples of specific *TIM* polymorphisms are provided in the Examples.
- Screening may also be based on the functional or antigenic characteristics of the protein. Immunoassays designed to detect predisposing polymorphisms in *TIM* proteins may be used in screening. Where many diverse mutations lead to a particular disease phenotype, functional protein assays have proven to be effective screening tools.
- Biochemical studies may be performed to determine whether a candidate sequence polymorphism in the *TIM* coding region or control regions is associated with disease. For example, a change in the promoter or enhancer sequence that affects expression of *TIM* may result in predisposition to asthma. Expression levels of a candidate variant allele are compared to expression levels of the normal allele by various methods known in the art. Methods for determining promoter or enhancer strength include quantitation of the expressed natural protein; insertion of the variant control element into a vector with a reporter gene such as β -galactosidase, luciferase, chloramphenicol acetyltransferase, etc. that provides for convenient quantitation; and the like. The activity of the encoded *TIM* protein may be determined by comparison with the wild-type protein.
- A number of methods are available for analyzing nucleic acids for the presence of a specific sequence. Where large amounts of DNA are available, genomic DNA is used directly. Alternatively, the region of interest is cloned into a suitable vector and grown in sufficient quantity for analysis. Cells that express *TIM* genes, such as trachea cells, may be used as a source of mRNA, which may be assayed directly or reverse transcribed into cDNA for analysis. The nucleic acid may be amplified by conventional techniques, such as the polymerase chain reaction (PCR), to provide sufficient amounts for analysis. The use of the polymerase chain reaction is described in Saiki, *et al.* (1985) *Science* 239:487, and a review of current techniques may be found in Sambrook, *et al. Molecular Cloning: A Laboratory Manual*, CSH Press 1989, pp.14.2-14.33. Amplification may also be used to determine whether a polymorphism is present, by using a primer that is specific for the polymorphism. Alternatively, various methods are known in the art that utilize oligonucleotide ligation as a means of detecting polymorphisms, for examples see Riley *et al.* (1990) *N.A.R.* 18:2887-2890; and Delahunty *et al.* (1996) *Am. J. Hum. Genet.*

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58:1239-1246.

A detectable label may be included in an amplification reaction. Suitable labels include fluorochromes, *e.g.* fluorescein isothiocyanate (FITC), rhodamine, Texas Red, phycoerythrin, allophycocyanin, 6-carboxyfluorescein (6-FAM), 2',7'-dimethoxy-4',5'-dichloro-6-carboxyfluorescein (JOE), 6-carboxy-X-rhodamine (ROX), 6-carboxy-2',4',7',4,7-hexachlorofluorescein (HEX), 5-carboxyfluorescein (5-FAM) or N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA), radioactive labels, *e.g.* ^{32}P , ^{35}S , ^3H ; *etc.* The label may be a two stage system, where the amplified DNA is conjugated to biotin, haptens, *etc.* having a high affinity binding partner, *e.g.* avidin, specific antibodies, *etc.*, where the binding partner is conjugated to a detectable label. The label may be conjugated to one or both of the primers. Alternatively, the pool of nucleotides used in the amplification is labeled, so as to incorporate the label into the amplification product.

The sample nucleic acid, *e.g.* amplified or cloned fragment, is analyzed by one of a number of methods known in the art. The nucleic acid may be sequenced by dideoxy or other methods, and the sequence of bases compared to a neutral *TIM* sequence. Hybridization with the variant sequence may also be used to determine its presence, by Southern blots, dot blots, *etc.* The hybridization pattern of a control and variant sequence to an array of oligonucleotide probes immobilised on a solid support, as described in US 5,445,934, or in WO95/35505, may also be used as a means of detecting the presence of variant sequences. Single strand conformational polymorphism (SSCP) analysis, denaturing gradient gel electrophoresis (DGGE), mismatch cleavage detection, and heteroduplex analysis in gel matrices are used to detect conformational changes created by DNA sequence variation as alterations in electrophoretic mobility. Alternatively, where a polymorphism creates or destroys a recognition site for a restriction endonuclease (restriction fragment length polymorphism, RFLP), the sample is digested with that endonuclease, and the products size fractionated to determine whether the fragment was digested. Fractionation is performed by gel or capillary electrophoresis, particularly acrylamide or agarose gels.

The hybridization pattern of a control and variant sequence to an array of oligonucleotide probes immobilised on a solid support, as described in US 5,445,934, or in WO95/35505, may be used as a means of detecting the presence of variant sequences. In one embodiment of the invention, an array of oligonucleotides are provided, where discrete positions on the array are complementary to at least a portion of mRNA or genomic DNA of the *TIM* locus. Such an array may comprise a series of oligonucleotides, each of which can specifically hybridize to a nucleic acid, *e.g.* mRNA, cDNA, genomic DNA, *etc.* from the *TIM* locus.

Antibodies specific for *TIM* polymorphisms may be used in screening

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immunoassays. A reduction or increase in neutral TIM and/or presence of asthma associated polymorphisms is indicative that asthma is TIM-associated. A sample is taken from a patient suspected of having TIM-associated asthma. Samples, as used herein, include biological fluids such as tracheal lavage, blood, cerebrospinal fluid, tears, saliva, lymph, dialysis fluid and the like; organ or tissue culture derived fluids; and fluids extracted from physiological tissues. Also included in the term are derivatives and fractions of such fluids. Biopsy samples are of particular interest, *e.g.* trachea scrapings, *etc.* The number of cells in a sample will generally be at least about 10^5 , usually at least 10^4 more usually at least about 10^6 . The cells may be dissociated, in the case of solid tissues, or tissue sections may be analyzed. Alternatively a lysate of the cells may be prepared.

Diagnosis may be performed by a number of methods. The different methods all determine the absence or presence or altered amounts of normal or abnormal TIM in patient cells suspected of having a predisposing polymorphism in TIM. For example, detection may utilize staining of cells or histological sections, performed in accordance with conventional methods. The antibodies of interest are added to the cell sample, and incubated for a period of time sufficient to allow binding to the epitope, usually at least about 10 minutes. The antibody may be labeled with radioisotopes, enzymes, fluorescers, chemilumescers, or other labels for direct detection. Alternatively, a second stage antibody or reagent is used to amplify the signal. Such reagents are well known in the art. For example, the primary antibody may be conjugated to biotin, with horseradish peroxidase-conjugated avidin added as a second stage reagent. Final detection uses a substrate that undergoes a color change in the presence of the peroxidase. The absence or presence of antibody binding may be determined by various methods, including flow cytometry of dissociated cells, microscopy, radiography, scintillation counting, *etc.*

An alternative method for diagnosis depends on the *in vitro* detection of binding between antibodies and TIM in a lysate. Measuring the concentration of TIM binding in a sample or fraction thereof may be accomplished by a variety of specific assays. A conventional sandwich type assay may be used. For example, a sandwich assay may first attach TIM-specific antibodies to an insoluble surface or support. The particular manner of binding is not crucial so long as it is compatible with the reagents and overall methods of the invention. They may be bound to the plates covalently or non-covalently, preferably non-covalently.

Other immunoassays are known in the art and may find use as diagnostics. Ouchterlony plates provide a simple determination of antibody binding. Western blots may be performed on protein gels or protein spots on filters, using a detection system specific for TIM as desired, conveniently using a labeling method as described for the sandwich assay.

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The *TIM* genes are useful for analysis of TIM expression, *e.g.* in determining developmental and tissue specific patterns of expression, and for modulating expression *in vitro* and *in vivo*. Vectors useful for introduction of the gene include plasmids and viral vectors. Of particular interest are retroviral-based vectors, *e.g.* Moloney murine leukemia virus and modified human immunodeficiency virus; adenovirus vectors, *etc.* that are maintained transiently or stably in mammalian cells. A wide variety of vectors can be employed for transfection and/or integration of the gene into the genome of the cells. Alternatively, micro-injection may be employed, fusion, or the like for introduction of genes into a suitable host cell. See, for example, Dhawan *et al.* (1991) *Science* **254**:1509-1512 and Smith *et al.* (1990) *Molecular and Cellular Biology* 3268-3271.

The expression vector will have a transcriptional initiation region oriented to produce functional mRNA. The native transcriptional initiation region or an exogenous transcriptional initiation region may be employed. The promoter may be introduced by recombinant methods *in vitro*, or as the result of homologous integration of the sequence into a chromosome. Many strong promoters are known in the art, including the β -actin promoter, SV40 early and late promoters, human cytomegalovirus promoter, retroviral LTRs, metallothionein responsive element (MRE), tetracycline-inducible promoter constructs, *etc.*

Expression vectors generally have convenient restriction sites located near the promoter sequence to provide for the insertion of nucleic acid sequences. Transcription cassettes may be prepared comprising a transcription initiation region, the target gene or fragment thereof, and a transcriptional termination region. The transcription cassettes may be introduced into a variety of vectors, *e.g.* plasmid; retrovirus, *e.g.* lentivirus; adenovirus; and the like, where the vectors are able to transiently or stably be maintained in the cells, usually for a period of at least about one day, more usually for a period of at least about several days to several weeks.

Antisense molecules are used to down-regulate expression of *TIM* in cells. The anti-sense reagent may be antisense oligonucleotides (ODN), particularly synthetic ODN having chemical modifications from native nucleic acids, or nucleic acid constructs that express such anti-sense molecules as RNA. The antisense sequence is complementary to the mRNA of the targeted gene, and inhibits expression of the targeted gene products. Antisense molecules inhibit gene expression through various mechanisms, *e.g.* by reducing the amount of mRNA available for translation, through activation of RNase H, or steric hindrance. One or a combination of antisense molecules may be administered, where a combination may comprise multiple different sequences.

Antisense molecules may be produced by expression of all or a part of the

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target gene sequence in an appropriate vector, where the transcriptional initiation is oriented such that an antisense strand is produced as an RNA molecule. Alternatively, the antisense molecule is a synthetic oligonucleotide. Antisense oligonucleotides will generally be at least about 7, usually at least about 12, more usually at least about 20 nucleotides in length, and not more than about 500, usually not more than about 50, more usually not more than about 35 nucleotides in length, where the length is governed by efficiency of inhibition, specificity, including absence of cross-reactivity, and the like. It has been found that short oligonucleotides, of from 7 to 8 bases in length, can be strong and selective inhibitors of gene expression (see Wagner *et al.* (1996) Nature Biotechnology 14:840-844).

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TRANSGENIC ANIMALS

The subject nucleic acids can be used to generate genetically modified non-human animals or site specific gene modifications in cell lines. The term "transgenic" is intended to encompass genetically modified animals having a deletion or other knock-out of *TIM* gene activity, having an exogenous *TIM* gene that is stably transmitted in the host cells, or having an exogenous *TIM* promoter operably linked to a reporter gene. Transgenic animals may be made through homologous recombination, where the *TIM* locus is altered. Alternatively, a nucleic acid construct is randomly integrated into the genome. Vectors for stable integration include plasmids, retroviruses and other animal viruses, YACs, and the like. Of interest are transgenic mammals, *e.g.* cows, pigs, goats, horses, *etc.*, and particularly rodents, *e.g.* rats, mice, *etc.*

A "knock-out" animal is genetically manipulated to substantially reduce, or eliminate endogenous *TIM* function. Different approaches may be used to achieve the "knock-out". A chromosomal deletion of all or part of the native *TIM* homolog may be induced. Deletions of the non-coding regions, particularly the promoter region, 3' regulatory sequences, enhancers, or deletions of gene that activate expression of *TIM* genes. A functional knock-out may also be achieved by the introduction of an anti-sense construct that blocks expression of the native *TIM* genes (for example, see Li and Cohen (1996) *Cell* 85:319-329).

Transgenic animals may be made having exogenous *TIM* genes. The exogenous gene is usually either from a different species than the animal host, or is otherwise altered in its coding or non-coding sequence. The introduced gene may be a wild-type gene, naturally occurring polymorphism, or a genetically manipulated sequence, for example those previously described with deletions, substitutions or insertions in the coding or non-coding regions. The introduced sequence may encode an *TIM* polypeptide, or may utilize the *TIM* promoter operably linked to a reporter gene. Where the introduced gene is a coding sequence, it usually operably linked to a promoter, which may be

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constitutive or inducible, and other regulatory sequences required for expression in the host animal.

Specific constructs of interest, but are not limited to, include anti-sense *TIM*, which will block *TIM* expression, expression of dominant negative *TIM* mutations, and over-expression of a *TIM* gene. A detectable marker, such as *lac Z* may be introduced into the *TIM* locus, where upregulation of *TIM* expression will result in an easily detected change in phenotype.

The modified cells or animals are useful in the study of *TIM* function and regulation. Animals may be used in functional studies, drug screening, etc., e.g. to determine the effect of a candidate drug on asthma. A series of small deletions and/or substitutions may be made in the *TIM* gene to determine the role of different exons in DNA binding, transcriptional regulation, etc. By providing expression of *TIM* protein in cells in which it is otherwise not normally produced, one can induce changes in cell behavior. These animals are also useful for exploring models of inheritance of asthma, e.g. dominant v. recessive; relative effects of different alleles and synergistic effects between *TIM* and other asthma genes elsewhere in the genome.

DNA constructs for homologous recombination will comprise at least a portion of the *TIM* gene with the desired genetic modification, and will include regions of homology to the target locus. DNA constructs for random integration need not include regions of homology to mediate recombination. Conveniently, markers for positive and negative selection are included. Methods for generating cells having targeted gene modifications through homologous recombination are known in the art. For various techniques for transfecting mammalian cells, see Keown *et al.* (1990) Methods In Enzymology 185:527-537.

Drug screening may be performed in combination with the subject animal models. Many mammalian genes have homologs in yeast and lower animals. The study of such homologs' physiological role and interactions with other proteins can facilitate understanding of biological function. In addition to model systems based on genetic complementation, yeast has been shown to be a powerful tool for studying protein-protein interactions through the two hybrid system described in Chien *et al.* (1991) P.N.A.S. 88:9578-9582. Two-hybrid system analysis is of particular interest for exploring transcriptional activation by *TIM* proteins.

COMPOUND SCREENING

One can identify ligands or substrates that bind to, modulate or mimic the action of *TIM*. Areas of investigation are the development of treatments for immune disorders, asthma, cancer, ischemia-reperfusion injury, and other diseases that are

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associated with cellular responses to stress. Drug screening identifies agents that provide an inhibition, replacement, or enhancement for TIM function in affected cells. For example, agents that reverse or inhibit TIM function may reduce bronchial reactivity in asthma by reducing levels of Th2 cytokines, and TIM inhibitors may enhance tumor sensitivity to cancer therapy, by potentiating the effects of radiation and chemotherapeutic treatments that induce apoptosis. Of particular interest are screening assays for agents that have a low toxicity for human cells. A wide variety of assays may be used for this purpose, including labeled *in vitro* protein-protein binding assays, protein-DNA binding assays, electrophoretic mobility shift assays, immunoassays for protein binding, and the like. The purified protein may also be used for determination of three-dimensional crystal structure, which can be used for modeling intermolecular interactions, transcriptional regulation, etc.

The term "agent" as used herein describes any molecule, e.g. protein or pharmaceutical, with the capability of altering or mimicking the physiological function of TIM, such as a signal tyrosine kinase inhibitor, or a peptide inhibitor of an integrin binding site. Generally a plurality of assay mixtures are run in parallel with different agent concentrations to obtain a differential response to the various concentrations. Typically, one of these concentrations serves as a negative control, i.e. at zero concentration or below the level of detection.

Candidate agents encompass numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of more than 50 and less than about 2,500 daltons. Candidate agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The candidate agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules including, but not limited to: peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof.

Candidate agents are obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides and oligopeptides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means, and may be used to produce combinatorial libraries. Known pharmacological agents may be subjected to directed or random chemical

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modifications, such as acylation, alkylation, esterification, amidification, *etc.* to produce structural analogs.

Where the screening assay is a binding assay, one or more of the molecules may be joined to a label, where the label can directly or indirectly provide a detectable signal. Various labels include radioisotopes, fluorescers, chemiluminescers, enzymes, specific binding molecules, particles, *e.g.* magnetic particles, and the like. Specific binding molecules include pairs, such as biotin and streptavidin, digoxin and antidigoxin *etc.* For the specific binding members, the complementary member would normally be labeled with a molecule that provides for detection, in accordance with known procedures.

A variety of other reagents may be included in the screening assay. These include reagents like salts, neutral proteins, *e.g.* albumin, detergents, *etc.* that are used to facilitate optimal protein-protein binding and/or reduce non-specific or background interactions. Reagents that improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, *etc.* may be used. The mixture of components are added in any order that provides for the requisite binding. Incubations are performed at any suitable temperature, typically between 4 and 40°C. Incubation periods are selected for optimum activity, but may also be optimized to facilitate rapid high-throughput screening. Typically between 0.1 and 1 hours will be sufficient.

Other assays of interest detect agents that mimic TIM function. For example, candidate agents are added to a cell that lacks functional TIM, and screened for the ability to reproduce TIM in a functional assay.

THERAPEUTIC METHODS

Agents that modulate activity of TIM genes or proteins provide a point of therapeutic or prophylactic intervention, particularly agents that inhibit or upregulate activity of the polypeptide, or expression of the gene. Numerous agents are useful in modulating this activity, including agents that directly modulate expression, *e.g.* expression vectors, antisense specific for the targeted polypeptide; and agents that act on the protein, *e.g.* specific antibodies and analogs thereof, small organic molecules that block catalytic activity, *etc.*

Methods can be designed to selectively deliver nucleic acids to certain cells. Examples of such cells include T cells, *etc.* Certain treatment methods are designed to selectively express an expression vector to cells of interest. One technique for achieving selective expression in nerve cells is to operably link the coding sequence to a promoter that is primarily active in immune system cells, *e.g.* IL-2 promoter, T cell antigen receptor promoter, and the like. Alternatively, or in addition, the nucleic acid can be administered with an agent that targets the nucleic acid to the cells of interest. For instance, the nucleic

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acid can be administered with an antibody that specifically binds to a cell-surface antigen. When liposomes are utilized, substrates that bind to a cell-surface membrane protein associated with endocytosis can be attached to the liposome to target the liposome to nerve cells and to facilitate uptake.

- 5 Antisense molecules can be used to down-regulate expression in cells. The antisense reagent may be antisense oligonucleotides (ODN), particularly synthetic ODN having chemical modifications from native nucleic acids, or nucleic acid constructs that express such antisense molecules as RNA. The antisense sequence is complementary to the mRNA of the targeted gene, and inhibits expression of the targeted gene products.
- 10 Antisense molecules inhibit gene expression through various mechanisms, e.g. by reducing the amount of mRNA available for translation, through activation of RNase H, or steric hindrance. One or a combination of antisense molecules may be administered, where a combination may comprise multiple different sequences.

- Antisense molecules may be produced by expression of all or a part of the target gene sequence in an appropriate vector, where the transcriptional initiation is oriented such that an antisense strand is produced as an RNA molecule. Alternatively, the antisense molecule is a synthetic oligonucleotide. Antisense oligonucleotides will generally be at least about 7, usually at least about 12, more usually at least about 20 nucleotides in length, and not more than about 500, usually not more than about 50, more usually not
- 20 more than about 35 nucleotides in length, where the length is governed by efficiency of inhibition, specificity, including absence of cross-reactivity, and the like. It has been found that short oligonucleotides, of from 7 to 8 bases in length, can be strong and selective inhibitors of gene expression (see Wagner *et al.* (1996) Nature Biotechnology 14:840-844).

- A specific region or regions of the endogenous sense strand mRNA sequence is chosen to be complemented by the antisense sequence. Selection of a specific sequence for the oligonucleotide may use an empirical method, where several candidate sequences are assayed for inhibition of expression of the target gene *in vitro* or in an animal model. A combination of sequences may also be used, where several regions of the mRNA sequence are selected for antisense complementation.
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- Antisense oligonucleotides may be chemically synthesized by methods known in the art (see Wagner *et al.* (1993) *supra.* and Milligan *et al.*, *supra.*) Preferred oligonucleotides are chemically modified from the native phosphodiester structure, in order to increase their intracellular stability and binding affinity. A number of such modifications have been described in the literature, which alter the chemistry of the backbone, sugars or
- 30 heterocyclic bases.

Among useful changes in the backbone chemistry are phosphorothioates; phosphorodithioates, where both of the non-bridging oxygens are substituted with sulfur;

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phosphoroamidites; alkyl phosphotriesters and boranophosphates. Achiral phosphate derivatives include 3'-O'-5'-S-phosphorothioate, 3'-S-5'-O-phosphorothioate, 3'-CH₂-5'-O-phosphonate and 3'-NH-5'-O-phosphoroamidate. Peptide nucleic acids replace the entire ribose phosphodiester backbone with a peptide linkage. Sugar modifications are also used to enhance stability and affinity. The alpha.-anomer of deoxyribose may be used, where the base is inverted with respect to the natural .beta.-anomer. The 2'-OH of the ribose sugar may be altered to form 2'-O-methyl or 2'-O-allyl sugars, which provides resistance to degradation without comprising affinity. Modification of the heterocyclic bases must maintain proper base pairing. Some useful substitutions include deoxyuridine for deoxythymidine; 5-methyl-2'-deoxycytidine and 5-bromo-2'-deoxycytidine for deoxycytidine. 5-propynyl-2'-deoxyuridine and 5-propynyl-2'-deoxycytidine have been shown to increase affinity and biological activity when substituted for deoxythymidine and deoxycytidine, respectively.

The compounds having the desired pharmacological activity may be administered in a physiologically acceptable carrier to a host for treatment of asthma attributable to a defect in TIM function. The compounds may also be used to enhance TIM function. The therapeutic agents may be administered in a variety of ways, orally, topically, parenterally *e.g.* subcutaneously, intraperitoneally, by viral infection, intravascularly, *etc.* Inhaled treatments are of particular interest. Depending upon the manner of introduction, the compounds may be formulated in a variety of ways. The concentration of therapeutically active compound in the formulation may vary from about 0.1-100 wt.%.

The pharmaceutical compositions can be prepared in various forms, such as granules, tablets, pills, suppositories, capsules, suspensions, salves, lotions and the like. Pharmaceutical grade organic or inorganic carriers and/or diluents suitable for oral and topical use can be used to make up compositions containing the therapeutically-active compounds. Diluents known to the art include aqueous media, vegetable and animal oils and fats. Stabilizing agents, wetting and emulsifying agents, salts for varying the osmotic pressure or buffers for securing an adequate pH value, and skin penetration enhancers can be used as auxiliary agents.

It is to be understood that this invention is not limited to the particular methodology, protocols, cell lines, animal species or genera, and reagents described, as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

As used herein the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a

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cell" includes a plurality of such cells and reference to "the array" includes reference to one or more arrays and equivalents thereof known to those skilled in the art, and so forth. All technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs unless clearly indicated otherwise.

All publications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing, for example, the cell lines, constructs, and methodologies that are described in the publications which might be used in connection with the presently described invention. The publications discussed above and throughout the text are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention.

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the subject invention, and are not intended to limit the scope of what is regarded as the invention. Efforts have been made to ensure accuracy with respect to the numbers used (*e.g.* amounts, temperature, concentrations, *etc.*) but some experimental errors and deviations should be allowed for. Unless otherwise indicated, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees centigrade; and pressure is at or near atmospheric.

EXPERIMENTAL

To analyze the human 5q23-35 region for asthma susceptibility genes, we utilized a mouse model, which offers several potential advantages. Environmental variation can be controlled, multiple phenotypes can be tested simultaneously, and inbred strains can be sensitized with allergen to develop airway hyperreactivity (AHR), a cardinal feature of human asthma. We utilized congenic inbred mouse strains that differed only by a small chromosomal region homologous to human chromosome 5q, thereby allowing this region to be studied in the absence of genetic variation outside the region. Positional cloning revealed a novel gene family encoding T cell membrane proteins (*Tim*), TIM-1, TIM-2, TIM-3, TIM-4, TIM-5, TIM-6, and TIM-7, in which major sequence variants of TIM-1, TIM-3, and TIM-4, cosegregate completely with *Tapr*.

IL-4 production and airway hyperreactivity are reduced in HBA mice. We examined congenic mice produced on a BALB/c genomic background with discrete genomic intervals inherited from individual DBA/2 chromosomes. BALB/c mice develop Th2 biased, atopy-resembling immune responses with enhanced AHR, while DBA/2 mice

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develop reduced IL-4 responses that protect against the development of AHR. By screening several of these congenic strains for reduced Th2 responsiveness, we identified one congenic line, C.D2 Es-Hba (HBA), which contained a segment of chromosome 11 inherited from DBA/2 mice, homologous to human 5q23-35. Fig. 1a shows that lymph node cells from immunized control BALB/c mice, as expected, produced high levels of IL-4, confirming the proclivity of BALB/c mice to develop Th2-biased immune responses. In contrast, lymph node cells from HBA mice produced significantly lower levels of IL-4, similar to that observed in DBA/2 mice. In addition, HBA mice produced significantly less IL-13 and IL-10, and somewhat lower levels of IL-5 compared to BALB/c mice, whereas production of IFN- γ was increased, as shown in Fig. 1b. These results indicated that the DBA/2-derived region of HBA chromosome 11, which has large regions of conserved synteny with human 5q23-35, contains a gene that reduces antigen-specific IL-4, IL-13, and IL-10 production, enhances IFN- γ production, and converts the BALB/c cytokine phenotype into a DBA/2 cytokine phenotype.

The HBA mice were examined for the capacity to develop antigen-induced airway hyperreactivity (AHR), which is associated with Th2-biased immune responses. Upon sensitization and challenge with allergen, control BALB/c mice developed high AHR, whereas similarly immunized HBA congenic mice, like DBA/2 mice, expressed normal airway reactivity in response to methacholine (Fig. 1c). Collectively, these results strongly suggested that genetic variation in a single locus on chromosome 11 regulated both Th2 cytokine production and AHR; therefore, we tentatively refer to the relevant genetic determinant(s) in HBA mice as a single locus, *T cell and Airway Phenotype Regulator (Tapr)*.

We also examined (BALB/c x HBA) F1 mice, which like the BALB/c mice, produced high levels of IL-4, IL-13, and IL-10 (Fig. 1a and 1b) and developed elevated antigen-induced AHR (Fig. 1c). These results indicate that a DBA/2 allele on chromosome 11, in isolation of other genes that regulate IL-4 synthesis, reduced IL-4 production and AHR in a recessive manner. In contrast, (BALB/c x DBA/2) F1 mice produced low levels of IL-4 and had normal airway responsiveness on immunization (Fig. 1), indicating that loci from other regions of the DBA genome also modulated IL-4 production and antigen-induced AHR, and that the DBA/2 alleles, in aggregate, functioned in a dominant manner to limit IL-4 production and AHR. These results underscore the multigenic, complex nature of atopic traits and demonstrate the potential advantages of using a congenic strain to isolate and characterize a single locus without interference from multiple epistatic genes that also influence the asthmatic phenotype.

Genetic Mapping of Tapr, the locus which controls AHR and IL-4 responsiveness. Previously, the congenic region in the HBA mice was delineated with 36

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genome-wide markers, including two chromosome 11 markers, hemoglobin- $\alpha 2$ (*hba- $\alpha 2$*) and esterase-3 (*es-3*) loci. The HBA genome, outside of chromosome 11, was inherited from BALB/c. A more precise analysis with 25 simple sequence length polymorphism (SSLP) markers known to be polymorphic between DBA/2 and BALB/c mice showed that HBA mice inherited two segments of chromosome 11 from DBA/2 (Fig. 2, left column). The proximal region contained a 20 cM segment with homology to chromosome 5q23-35, which afforded the possibility that a genetic locus implicated in human asthma linkage studies could be identified in a mouse model of asthma.

To map at higher resolution the T_H2 -AHR controlling locus, *Tapr*, (BALB/c x HBA) F1 mice were backcrossed to HBA mice to produce N2 animals. With this backcross approach, the set of alleles contributed by the HBA parent is pre-determined, and the set of alleles contributed by the F1 parent can be assessed by genotyping. Thus, recombination events that produce informative haplotypes within the congenic region can be detected in the N2 mice and used to assess the linkage of *Tapr* to loci in the congenic interval. Because of the recessive nature of *Tapr*, we tested N2 mice from these backcrosses to identify the minimum homozygous region of HBA-derived genes sufficient to confer the HBA *Tapr* phenotype. More than 2,000 N2 animals were generated and genotyped. Using SSLP markers, we selected those N2 mice with informative recombination events, and the N2 mice were phenotyped for the capacity to produce IL-4 in response to immunization with keyhole limpet hemocyanin (KLH). In this primary analysis, we determined that the relevant locus resided within the proximal congenic region, between *D11Mit135* and *D11Mit260*. In order to map *Tapr* at higher resolution, 22 additional markers were identified and utilized to provide 0.1-1 cM resolution in the area of interest.

To accurately compare the results of IL-4 cytokine analyses performed over several months time, an IL-4 index for each experiment was generated for each N2 mouse, $\left(\frac{B-x}{B-H} \right)$, where B=IL-4 production by cells from BALB/c mice, H=IL-4 production by cells from HBA mice, and x=IL-4 production by cells from the N2 mouse being assessed. High concentrations of IL-4 (BALB/c-like) are represented by index values near 0, and low concentrations of IL-4 (HBA-like) are represented by index values near 1.0. The "B and "H" values were established with 3-5 control mice for each group of 3-6 N2 mice carrying informative recombinations that we tested. The index values fall within a bimodal distribution (Fig. 3a), in which the phenotype index associated with N2 mice that had nonrecombinant HBA genotypes was significantly higher ($P<0.0001$, in a paired Student's t-test) than the phenotype index associated with N2 mice that had nonrecombinant (BALB/c x HBA)F1 genotypes.

For the mice with unique genotypes, we used several methods to ensure the

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adequacy of single measurements of cytokine production and AHR, since this is critical in linkage analysis. First, at the same time that we tested each of the N2 mice carrying recombinations of interest, we also tested "non-recombinant" siblings of each "recombinant N2" that were strictly HBA or F1 (BALB x HBA) in genotype. Furthermore, we bred
 5 additional N3 mice by crossing some of the N2 mice carrying recombinations of interest back to HBA mice, in order to have more individual mice with that particular N2 genotype. All values were the average of the values for the individual mice tested with a given genotype. In this way, we are confident of the measures of cytokine production and AHR, and that we have overcome assay variations due to variables inherent in biological
 10 systems.

Because the IL-4 values associated with the N2 mice that inherited recombinant haplotypes segregated in a bimodal distribution (Fig. 3a), were able to demonstrate that the genetic locus that controls high IL-4 responses is located between markers *D11Mit271* and *D11Mit22* (Fig. 3b). Moreover, high levels of IL-4 production were
 15 observed in all mice with a BALB/c allele present at *Kim1sscp*, and low levels of IL-4 production were observed in all mice with homozygous HBA genotypes at *Kim1sscp*. Thus, *Tapr* was nonrecombinant with *Kim1sscp*, an intronic marker within a mouse homologue of *Rattus norvegicus* Kidney Injury Molecule (*Kim-1*). In contrast, *Tapr* segregated from all
 20 other markers with at least one recombination. The fact that *Tapr* and *Kim1sscp* segregated together, indicated that the *Tapr* locus is located very close to or is indistinguishable from *Kim1sscp*. Based on the frequency recombinant haplotypes between *D11Mit271* and *D11Mit22*, we calculate a recombination frequency, 0.0039, which indicates that the *Tapr* locus maps to a small, 0.3-0.5 cM, region. We also calculated a recombination frequency of 0.08 between *Tapr* and IL-4. Therefore, *Tapr* is located 5-10
 25 cM away from the IL-4 cytokine cluster but is within the a region of the mouse genome that has highly conserved synteny with the 5q23-35 region that has been linked to human atopy and asthma.

Using an analogous approach, we examined the segregation of allergen-induced AHR phenotypes in mice with informative recombinant haplotypes. With indexed
 30 AHR values, N2 mice clearly exhibit parental phenotypes, which produced a bimodal distribution in a histogram of AHR index values in a group of sensitized N2 mice (Fig. 3c). By analyzing the segregation of AHR phenotypes associated with more than 1,000 N2 mice, we demonstrated that the genetic locus which controls AHR responses is also located between markers *D11Mit271* and *D11Mit22* (Fig. 3d) and that the AHR phenotype was
 35 nonrecombinant with *Kim1sscp*. Thus, we demonstrate that both IL-4 responsiveness and AHR cosegregate with the *Tapr* locus, which suggests that the same locus regulates both IL-4 expression and AHR (Fig. 3).

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These findings further demonstrate that the *Tapr* locus is more than 5 cM centromeric to the IL-4 cytokine cluster and the cytokine genes in the cluster previously thought to be 'candidate' atopy or asthma susceptibility genes. Our mapping results also establish that *Tapr* is genetically separable from both the IL-12p40 gene and the region of mouse chromosome 11 that includes the T_H1-IL12 regulatory locus, *Tpm*.

Mouse and human homologues anchor Tapr to human 5q33. In order to construct a composite map around the *Tapr* locus, we integrated available information from the Mouse Genome Database (MGD) linkage, backcross, and radiation hybrid maps and identified a region of conserved synteny in maps of the human genome. Current radiation hybrid maps place the markers that are near *D11Mit271* and *D11Mit22*, including several expressed sequence tags (ESTs) that have extensive homology to known genes or unigene clusters, onto a physical map of the mouse genome. We further examined these markers and their associated ESTs for previously unidentified similarity to known gene clusters. We assembled these markers onto a scaffold for comparison to the human genome. Using this approach, we found significant similarity between particular radiation hybrid markers and the following human genes: *KIAA0171*, *Adam-19*, *Sox-30*, *Pir-121*, *Crsp9* (*Crsp33*), and *hHAVcr-1* (*hHAVcr-1*). Fig. 4 demonstrates that once we anchored these genes to a physical map of the mouse genome between our flanking markers, we were able to locate those genes in the Human Genome Browser.

The high degree of conservation between the mouse and human genomes in this region indicates linkage of the *Tapr* locus to human 5q33.2. As shown Fig. 4, we identified all known genes and ESTs in this region of the human map. Genes of particular interest near human *hHAV-cr* and the mouse homologue of *Kim-1*, include IL-2 Inducible T cell kinase (*Itk*) and a coregulator of the SP-1 transcription factor (*Crsp9*), both known to be involved in T cell differentiation. We sequenced coding regions from these candidate genes and found no polymorphisms in either ITK or CRSP-9.

Localization of a Family of Novel T cell Surface Proteins to the Tapr Region. Because the mouse homolog of rat *Kim1* is located within the 0.4 cM region and is tightly linked with *Tapr*, we examined publicly available databases and found clusters of ESTs with some sequence similarity that provided only partial coverage and contained large segments of variation. The closest human homolog of *Kim-1* is the human hepatitis A virus cellular receptor, *hHAV-cr*, and tBLAST searches of the human genome suggested that two additional homologs of *Kim-1*, perhaps members of a gene family, also are located on human chromosome 5 and mouse chromosome 11.

Using cDNA from conA-stimulated splenocytes, we identified and cloned two

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mouse orthologues of *Kim1*, which we term *Tim1* and *Tim2*, that map to the *Tapr* region, as shown in Fig. 5A. *TIM-3* is a third, more distantly related, orthologue of *KIM-1*.

All three members of this gene family are expressed by stimulated T cells, and all three forms map to the *Tapr* region of mouse chromosome 11/ human chromosome 5 where they encode cell surface glycoproteins with common structural motifs, including an immunoglobulin (Ig) domain, mucin domain, and intracellular tail with phosphorylation sites. Because the cellular functions of these proteins is unknown, we refer to the genes as members of a *T cell, Immunoglobulin domain, Mucin domain (Tim)* gene family. Mouse *Tim1* is the mouse homologue of rat *Kim1* and the *HAVcr-1* identified in African green monkeys and humans. *Tim2* is a previously unknown gene that had not been identified in any organism prior to this study.

The mouse *Tim1* gene encodes a 305 amino acid membrane protein, that has 78% overall identity with rat *KIM-1* and 35% identity with human *HAVcr-1*. A gapped multiple sequence alignment with mouse *TIM-1*, rat *KIM-1*, human *HAVcr-1* and African green monkey *HAVcr-1*, shown in Fig. 5B, demonstrates the degree of homology between the *TIM-1/KIM-1/HAVcr-1* proteins in these species. The cytoplasmic region of *TIM-1* contains two tyrosine residues and includes a highly conserved tyrosine kinase phosphorylation motif, RAEDNIY, which is integral to the predicted *Itk* and *EGFR* kinase site of *TIM-1*, SRAEDNIYVEDRP. The mucin domain of *TIM-1* has multiple sites for O-linked glycosylation, and there two sites for N-linked glycosylation found in the immunoglobulin domain.

TIM-2, a similar 305 amino acid membrane protein, has 64% identity to mouse *TIM-1*, 60% identity to rat *KIM-1*, and 32% identity to h*HAVcr-1* (Figure 5A, B). Like *TIM-1*, *TIM-2* has two extracellular N-linked glycosylation sites and a serine, threonine-rich mucin domain with many O-linked glycosylation sites. *TIM-2* also has an intracellular tyrosine kinase phosphorylation motif, RTRCEDQVY.

Tim3 encodes a 281 amino acid membrane protein that has a similar, integral membrane glycoprotein structure with multiple extracellular glycosylation sites and an intracellular tyrosine phosphorylation motif. Although the mucin domain is not as prominent in *TIM-3* as it is in *TIM-1* and *TIM-2* (Figure 5A), *TIM-3* expressed on T cells likely interacts with a ligand on APCs and alters APC activation. *TIM-3* does have four sites for N-linked and five sites for O-linked glycosylation, suggesting that *TIM-3*, like *TIM-1* and *TIM-2*, is heavily glycosylated and might interact with a ligand present on other cells, such as antigen presenting cells.

Tim4 encodes a 344 amino acid protein in mice, and a 378 amino acid protein in humans. The predicted *TIM-4* also shares the general membrane glycoprotein structural motifs of the other *TIM* proteins, a with an IgV-like domain with highly conserved

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cysteine residues, a threonine-rich mucin-like domain, and a short intracellular tail. However, TIM-4 lacks the phosphotyrosine motif present in the other TIM proteins, and therefore may modulate the function of the other TIM proteins.

Each of the TIM Ig domain shares an predicted integrin-binding motif that is similar to the SVVYGLR motif found in osteopontin, an transmembrane protein like the TIMs that is implicated in the regulation of cell adhesion, survival, and oncogenesis, as well as in the regulation of helper T cell differentiation. This integrin binding motif demonstrates alpha(9) and alpha(4) specificity.

Comparison of the sequences of the BALB/c and HBA/DBA coding regions for the three *Tim* genes revealed major polymorphisms in TIM-1, TIM-3, and TIM-4, but not TIM-2. In TIM-1, these polymorphisms encode three amino acid differences and a fifteen amino acid deletion in HBA/DBA. Seven predicted amino acid differences were identified in TIM-3 (Fig. 5c). Genomic sequences confirm that these polymorphisms, including the deletion, are true polymorphisms, not splicing variants. By further sequencing genomic segments of TIM-1 and TIM-3 in other mouse strains, we found that C57/BL6, a strain similar to DBA/2 with respect to its tendency to develop reduced T_H2 and AHR responses, also has the HBA/DBA allele of *Tim1* and *Tim3*. The polymorphisms in TIM-1 and TIM-4 are located in the signal and mucin-like domains, while the polymorphisms identified in TIM-3 are clustered in the Ig domain (Fig. 5c). In glycoproteins with Ig and mucin domains, variants in either domain may affect receptor-ligand interactions, as shown for MadCAM-1. Although the predicted cleavage sites of TIM-1 and TIM-4 are unaltered by the polymorphism in the signal sequence, it is possible that the polymorphism may affect the efficiency of cleavage and/or trafficking of the receptor to the cell surface. These *Tim* sequences and polymorphisms are important for immune responses, and for HAV viral pathogenesis in humans.

Analysis of genomic DNA samples from our N2 backcross (Fig. 3) demonstrated that the TIM-1 and TIM-3 polymorphisms cosegregate completely with *Tapr*. While these observations do not distinguish the extent to which changes in TIM-1, TIM-3, or both, are responsible for changes in AHR and T_H2 -mediated inflammation, we suggest that polymorphisms in human TIM-1(hHAVcr-1) and/or TIM-3 underlie the strong association between asthma susceptibility and human chromosome 5q. This idea is supported by the fact that major variants in coding regions of human *Tim1* are evident on examination of human genome and EST databases. Comparison of these human cDNA variants with the previously described variants of monkey HAVcr-1 and the mouse variants identified here demonstrates that there is extensive variation in the predicted protein sequences of TIM-1 (Fig 5b,c). This high degree of variation distinguishes TIM-1 and its family members from

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many other candidate genes, such as the cytokines and the cytokine receptors that have been most closely studied as asthma susceptibility candidate genes. In addition, the association between *Tim1* and asthma susceptibility is further supported by reports of significant linkage of mite-sensitive childhood asthma to D5S820 (mean LOD score = 4.8),
 5 a marker which is approximately 0.5 megabases from *Tim1* and *Tim3* (Fig. 4).

In addition to the above genetic polymorphisms, there are several expression polymorphisms in the TIM genes that arise due to alternate splicing. Alternate splicing of TIM-1, TIM-2 and TIM-4 mRNA produces several TIM variants, some of which are secreted, soluble forms of the TIM receptors. These splice variants, along with TIM splice
 10 variants that have alternate 5' untranslated regions, may contribute to the cell-specific and condition-specific expression patterns of the TIM proteins.

T cells confer the Tapr effect. To better understand the function of the *Tapr* locus we determined whether allelic variation of *Tapr* affected the function of T cells or of antigen presenting cells (APC). For these experiments, we generated ovalbumin (OVA)-specific T cell receptor (TCR) transgenic mice (Tg) with the HBA background (HBA DO11.10), which we compared to TCR-Tg mice with the BALB/c background (BALB/c DO11.10). Purified CD4+ T cells from either of these strains were cocultured with OVA and dendritic cells (DCs) derived from either BALB/c or HBA bone marrow, and the cytokines
 20 produced were evaluated. Irradiated spleen cells were not used as APCs for this experiment, because it was found that irradiated spleen cells and other tissues express high levels of the TIM genes.

BALB/c DO11.10 T cells produced higher levels of IL-4 and IL-13 than did HBA DO11.10 T cells, in a manner that was independent of the source of the antigen presenting cells (Fig. 6A). In addition, the source of the CD4 T cells determined the amount of IL-4/IL-13 produced at each antigen concentration, regardless of the source of the APC during either the primary or secondary stimulation. Equivalent levels of IL-12 were detected in culture supernatants for each combination of cell types, further demonstrating that BALB/c and HBA DC function were comparable. Furthermore, BALB DO11.10 and
 30 HBA DO11.10 T cells produced equivalent levels of IL-2 and demonstrated comparable levels of proliferation in response to OVA during the secondary cultures, indicating that HBA and BALB/c T cells are similarly activated, although the levels of Th2 cytokines they produce are quite distinct.

We show in Figure 6B that within the first twelve hours of primary culture in
 35 our DO11.10/DC system, we find that mRNA for TIM-1 is expressed by both BALB/c and HBA CD4+ T cells. Within four days of primary stimulation, we find significant levels of IL-13 in supernatants of the BALB/c DO11.10 and detect none in the HBA DO11.10

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supernatants. This differentiation is detectable in mRNA levels at 36 hours (Fig. 6B). Between twelve and thirty six hours, expression of IL-13 mRNA is reduced in HBA CD4 T cells, while IL-13 expression is maintained in the BALB/c CD4 T cells. Thus, during the primary response to antigen, BALB/c CD4 T cells develop a stronger Th2 response than do HBA CD4 T cells. Our findings demonstrate that *Tapr* regulates helper T cell differentiation during primary antigen specific responses, and we detect TIM-1 expression in CD4 T cells during the earliest stages of these responses.

Following differentiation into mature Th1 and Th2 subsets, helper T cells demonstrate committed TIM expression by RT-PCR, such that Th1 cells express TIM-3, while Th2 cells preferentially express TIM-1. All T cell populations demonstrate weak TIM-4 expression. While the Itk signal through TIM-1 is likely to promote Th2 differentiation, the EGFR signal through the TIM proteins is likely to enhance cell survival in effector and especially memory T cell populations. Since Itk is expressed only in T cell and mast cells, the Itk kinase activity on TIM-1 is restricted to immune cells, particularly those involved in asthma and allergy. However, other protein tyrosine kinases, such as EGFR, are involved in the function of TIM proteins expressed by other tissues, including ischemic epithelial cells, irradiated spleen cells, and tumor cells.

In these studies, we mapped *Tapr*, a locus that regulates the development of Th2 cytokine production and antigen-induced AHR, a cardinal feature of asthma. We localized *Tapr* using an interval specific congenic mouse (HBA) that carried a chromosomal segment homologous to human chromosome 5q, a region of the human genome that has been repeatedly linked to atopy and asthma. This region has also been repeatedly linked to 5q- syndrome associated with myelodysplasia and neoplastic cytogenic abnormalities. Using this congenic mouse strategy that converted a complex trait into a simpler, possibly single gene, trait, we narrowed the interval of *Tapr* to 0.4 cM interval, sequenced several candidate genes in this region, and positionally cloned the TIM gene family.

The TIM gene family has not been previously described. We identified and cloned the full cDNA sequence and discovered significant polymorphisms in the TIM-1 proteins of BALB/c compared to HBA mice. We found that the BALB/c sequences for TIM-1 and TIM-3 are associated with susceptibility to AHR and allergic T cell responses, whereas the HBA sequences are associated with protection against these responses. TIM-3 is preferentially expressed by differentiated T_H1 . The association of polymorphic *Tim3* variants with *Tapr* suggests that TIM-3 might regulate T_H1 and T_H2 cell function. However, the variations in *Tim3* might also be attributed to a haplotype tightly linked to *Tim4* or *Tim1*.

We believe that TIM-1 plays a very important role in the regulation of the immune system (particularly with respect to asthma and allergic disease) and in the the

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regulation of epithelial and hematopoietic cell survival in response to stress (hypoxia, nutritional deficiency, irradiation, chemotherapy, etc.) for several reasons. First, *Tim1*, like *Tim3*, is expressed in CD4 T cells during primary antigen stimulation, when it is most likely that the *Tapr* effect occurs. T cells play a critical role in the development of AHR and in the pathogenesis of asthma, our results suggest that *Tapr* affects asthma by enhancing early CD4 commitment to Th2 responses by controlling the production of IL-13 and subsequent T cell differentiation. Second, HAV infection in humans during infancy or childhood is inversely associated with the development of asthma and allergy. We suggest that the HAV interaction with TIM-1/HAVER-1 may alter the T cell cytokine production may able to reverse or prevent the biased Th1/Th2 balance in individuals otherwise prone to atopy and asthma. SLAM, a measles virus receptor, is an example of another T cell surface glycoprotein that regulates the Th1/Th2 balance in a manner that may be altered by viral interaction. Because some viral receptors, such as SLAM for the measles virus or CD4, CCR5, and CXCR4 for HIV, are receptors of the host's own immune system, even when an infection does not succeed, virus-receptor mediated signal transduction can lead to the release of cytokines and the development of disease.

Third, the polymorphisms in TIM-1 are associated with the different types of helper T cell responses that we observe. Therefore, the variants of TIM-1 may themselves contribute to the genetic Th1/Th2 predisposition that occurs in the absence of any known environmental cause of immune deviation. The HAV receptor in primates is known to be highly variable, and we propose that polymorphic alleles of human TIM-1/hHAVER-1, like those we have identified in mice, may be associated with variations in Th2 bias and asthma susceptibility. Mutations in the genes for cell surface molecules that serve as viral receptors and that alter susceptibility to infection are not uncommon, and therefore significant genetic variation in TIM-1 and other members of the TIM gene family is far more likely to be observed than variation in other genes such as those for cytokines. It is unclear why asthma susceptibility alleles might be prevalent in the human gene pool, but the association of *Tapr* with HAVER-1 provides an interesting explanation for the persistence of asthma susceptibility alleles. During human evolution certain alleles of the *Tim* gene family may have conferred resistance to atopic diseases and other immune disorders, but selection of those resistance alleles may have been counterbalanced by selection of alternate alleles that confer resistance to viral pathogenesis.

In summary, our studies represent the first successful utilization of a congenic mouse strategy to locate a strong candidate asthma susceptibility gene and overcome the inherent difficulties in the examination of this complex genetic trait. We identified a previously unknown gene family that exists in a region homologous to human

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chromosome 5q, and which plays a major role in Th cell development and in asthma susceptibility. While prior studies in humans identified several candidate genes on human chromosome 5q, the *Tim1* gene product identified in our study also provides an explanation for the inverse relationship between HAV infection and reduced asthma susceptibility.

5 Subpopulations of CD4⁺ T cells (Th) produce distinct patterns of cytokines, and this has led to the concept of functional heterogeneity among Th cells. Type 1 Th cells (Th1) produce interleukin 2 (IL-2) and/or interferon γ , elicit delayed type hypersensitivity (DTH) responses and activate macrophages. Type 2 Th cells (Th2), on the other hand, produce IL-4, IL-5 and IL-10 and are especially important for IgE production and eosinophilic inflammation, and may suppress cell mediated immunity. Th2 cells are
10 believed to play a pivotal role in the pathogenesis of atopy. Several factors determine whether a T helper cell will differentiate into Th1 versus Th2 during a particular immune response. These include, but are not necessarily restricted to, the cytokine milieu, the strength of the TCR signal and/or antigen density, and the costimulatory pathways. CD4⁺ T
15 helper cell differentiation into Th1 or Th2 subsets has profound effects on the outcome of atopy, autoimmune diseases, infectious diseases, and graft rejection.

The specific features of immune responses that protect nonatopic individuals from the development of allergic diseases and which could inhibit allergic responses in atopic individuals are poorly understood. Because Th1 cells cross regulate Th2 cells in
20 some systems, allergen-specific Th1 cells have been assumed to regulate allergic disease and asthma. Th1 cells inhibit the development and proliferation of Th2 cells, and IgE production is reciprocally regulated by IL-4 and IFN- γ . This suggests that protection from allergy is due to the development of inhibitory allergen-specific Th1 cells. Allergen-specific T cell clones derived from the peripheral blood of nonallergic individuals have been shown
25 to produce Th1 cytokines. These observations have also supported the hygiene hypothesis of asthma, which suggests that the prevalence of infections, particularly those that induce Th1 responses, are reduced in westernized societies by improved public health measures and the use of vaccines and antibiotics. As a result, Th2 responses and atopy develop more intensely and rapidly in the absence of Th1 mediated responses.

30 The TIM genes identified herein are also candidate oncogenes. Transfection of cell lines with TIM genes confers resistance to cell death, and the predicted EGFR kinase motif described in TIM-1 provides a probable mechanism by which this cell survival is controlled. Furthermore, TIM-1 demonstrates a significant degree of sequence identity (approximately 20%) and structural similarity (a transmembrane glycoprotein with
35 an IgV domain, mucin/syndecan domain, transmembrane domain, and intracellular domain with similar phosphotyrosine motifs) with TOSO, a protein that protects cells from Fas-mediated apoptosis. Like the TIM genes, TOSO is a likely oncogene, which maps to a

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region of the genome with frequent changes in hematologic malignancies and solid tumors.

METHODS

- Animals. Congenic lines, including C.D2 Es-HBA were generated by introgressively backcrossing DBA/2N onto a BALB/cAnPt background. BALB/cBy, DBA/2J, and (BALB/c x DBA/2) F1 mice (CByD2F1/J) were obtained from the Jackson Laboratory (Bar Harbor, ME), while BALB/cAn and DBA/2N were obtained from Taconic Labs. (BALB/c x HBA) F1 mice were produced with a cross between BALB/cByJ and HBA. N2 mice were generated by backcrossing (BALB/c x HBA) F1 to HBA. In our analysis of recombinant N2 animals, recombinant mice were tested along with non-recombinant siblings, whenever possible. In order to examine individual N2 genotypes in multiple assays, we preserved selected recombinant haplotypes by backcrossing selected N2 mice to HBA to generate N3 mice, which were genotyped to chose mice carrying the recombinant N2 haplotype. DO11.10 mice, which are transgenic for TCR recognizing OVA peptide 323-339 (pOVA³²³⁻³³⁹) and backcrossed to BALB/c(43), were kindly provided by Dr. Dennis Loh and were bred in our facilities. HBA DO11.10 mice were produced by backcrossing DO11.10 to HBA. DO11.10 mice were selected by FACS analysis for the TCR-Tg and genotyped to select for HBA alleles between *D11Mit135* and *D11Mit168*. The Stanford University Committee on Animal Welfare approved all animal protocols.
- Genotyping. Additional markers around the *Tapr* locus were identified by testing all available "D11Mit-" markers present between *D11Mit140* and *D11Mit269* and all radiation hybrid markers near *D11Mit271* and *D11Mit22* for any polymorphisms between DBA/2 and BALB/c. MIT MapPair primers were obtained from Research Genetics (Huntsville, AL), and all other primers were synthesized in the Protein and Nucleic Acid Facility (Stanford, CA). PCR was performed as previously described, and SSLP polymorphisms were resolved with 4-5% Metaphor agarose (BioWhittaker, Walkersville, MD). Products analyzed for SSCP were amplified with ³³P-dCTP and separated on denaturing acrylamide gels at 40W and 4°C, with a Sequi-Gen GT System (Bio-Rad, Hercules, CA).
- Immunization protocols. Mice studied in cytokine production assays were primed with KLH (Calbiochem, La Jolla, CA) in complete Freund's adjuvant (CFA) (DeKruyff et al. *J Immunol* **149**, 3468-76 (1992)). For measurement of airway hyperreactivity, mice were immunized with OVA intraperitoneally (i.p., 50 µg) complexed with aluminum potassium sulfate (alum) on day 0, and intranasally (i.n. 50 µg OVA in 50 µl of PBS) after light anesthesia on days 7, 8 and 9. Control mice received i.p. injections of alum alone and intranasal PBS. Airway hyperreactivity to inhaled methacholine was measured 24 hours after the last intranasal dose of OVA (day 10).

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Measurement of Airway Responsiveness. Airway responses were assessed by methacholine-induced airflow obstruction from conscious mice placed in a whole body plethysmograph (Buxco Electronics Inc., Troy, NY), as described previously (Hansen et al. *J Clin Invest* **103**, 175-83 (1999)).

5 *Cell Culture.* Lymph node cells from mice primed with KLH were prepared as described previously (Yeung et al. *J Immunol* **161**, 4146-52 (1998)). Transgenic DO11.10 CD4 T cells were positively selected using MACS columns following incubation with anti-CD4 magnetic beads (Miltenyi Biotech, Germany). 2×10^4 cells/well were cocultured in 96-well round bottom plates with 250 μ g/ml OVA and 1×10^4 bone marrow-derived dendritic cells/well. After seven days, the DO11.10 T cells were washed and restimulated with fresh antigen presenting cells and antigen at the concentration indicated. Antigen concentration for the primary DO11.10 cultures was titrated during the restimulation. Bone marrow-derived dendritic cells were generated as previously described with some modifications; 5×10^6 bone marrow cells were cultured in 9-cm diameter tissue culture dishes with 10 ml culture medium containing 20-25 U/ml GM-CSF. Loosely adherent cells were transferred onto a second dish on the sixth day of culture; within four days, these transferred cells were used as a source of dendritic cells.

15 *Cytokine ELISA.* ELISAs were performed as previously described in Macaulay et al. *J Immunol* **160**, 1694-700 (1998); and Macaulay et al. *J Immunol* **158**, 4171-9 (1997).

20 *Monoclonal Antibodies.* Monoclonal antibodies for ELISA and FACS analysis were purified from ascites fluid by ammonium sulfate precipitation and ion-exchange chromatography. Anti-clonotypic antibody KJ1-26.1, was generously provided by Dr. Philippa Marrack, National Jewish Medical Center, and the antibody was FITC-conjugated according to standard protocols prior to FACS.

Example 2

Identification of Human Tim Sequences

30 The positional cloning of the TIM gene family within a locus that confers protection against the development of Th2 responses and allergen-induced airway hyperreactivity provides an opportunity to greatly improve our understanding of the regulation of Th2 driven responses and atopic diseases. In addition, TIM-3 is specifically expressed on murine Th1 cells and anti-TIM-3 mAb leads to increased severity of experimental autoimmune encephalomyelitis (EAE). This emphasizes the importance of the gene family in T helper subset regulation.

35 The human Tim cDNAs, which are the orthologues of murine Tim-3 and Tim-4 were cloned by PCR. The human orthologue of TIM-1 was cloned as HAVcr-1, the

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cellular receptor for hepatitis A virus. The TIM family genes are immediately adjacent to each other on human chromosome 5, in the order TIM-4, TIM-1, TIM-3, with no intervening genes. There are TIM pseudogenes on chromosomes 12 and 19. The gene family members are only moderately related. The protein sequences and relationship among the

5 Tim gene family are shown in Figure 7.

The cytoplasmic domains of TIM gene family members are the most conserved domain between mouse and human orthologues, e.g., 77% identity between the human and mouse TIM-3 cytoplasmic domains. In contrast, the whole TIM-3 is only 63% identical between human and mouse. Each TIM gene contains a distinct predicted tyrosine

10 signaling motif. The cytoplasmic region of TIM-1 contains two tyrosine residues and includes a highly conserved tyrosine kinase phosphorylation motif, RAEDNIY. The expanded region, SRAEDNIYIVEDRP, contains a predicted site for Itk and EGF receptor phosphorylation. Itk is known to phosphorylate phospholipase C- γ (PLC- γ), and thereby trigger a cascade of signaling events that are involved in T cell activation and helper T cell

15 differentiation. Furthermore, Itk signaling affects Th1/Th2 differentiation, and Itk^{-/-} mice do not develop strong Th2 responses. EGF receptor kinase activity is associated with cell survival and resistance to cell death. Similarly, TIM-3 contains distinct, conserved tyrosine phosphorylation and SH2 binding motifs in the cytoplasmic domain. This suggests that the interaction of a TIM with its ligand will engage an intracellular signaling pathway and that

20 each TIM will be distinct in this signaling.

The extracellular IgV domain of the TIM proteins also contains a predicted integrin-binding motif that is similar to the SVVYGLR motif of osteopontin that is involved in adhesion via α 9 β 1, α 4 β 1, and α 4 β 7 integrins. TIM-1 transfected pre-B cells of the 300.19 line demonstrate a high degree of adhesion an

25 increased survival in cell culture, as compared to non-transfected 300.19 cells. TIM-1 and TIM-2 transfected CHO cells also demonstrate enhanced survival compared to untransfected CHO cells. These results demonstrate that the TIM proteins regulate cell adhesion and death

Genetic polymorphisms in the human Tim1 and Tim3 genes. SNPs or

30 nucleotide polymorphisms and deletions/insertions present in the human *Tim1* gene are identified. Because SNPs are extremely common in the genome, occurring every 300-600 base pairs, only the coding region of *Tim1* was analyzed. Moreover, genetic variations that are common are also likely to be important. Initially cDNA is sequenced from T cells taken from 30-40 individuals (60-80 chromosomes). Power calculations show that surveying

35 target sequences in coding regions of 60 chromosomes will easily detect SNPs with a population frequency of greater than 1%, and having a more than 90% chance of detecting alleles with a population frequency of 5% or greater. Therefore, screening 30-40

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individuals for sequence variations captures most of the common, functionally relevant, non-conservative, DNA variation present in a population.

Since DNA variants/SNPs in close physical proximity often show strong dependency relationships (i.e., linkage disequilibrium), it is determined if a group of DNA variants (SNP haplotypes) are inherited together, and determined if screening for only a portion of these SNPs will be sufficient for identifying the haplotype. Analysis of large regions of various chromosomes indicate that discrete haplotype blocks (of tens to hundreds of kilobases) are generally present, each with limited diversity punctuated by apparent sites of recombination. To find haplotypes, cDNA is sequenced and searched for combinations of sequence variations that are seen repeatedly in multiple individuals.

Peripheral blood mononuclear cells (PBMC) were from 38 donors, and were stimulated *in vitro* with PHA (7.5 μ g/ml) for 24 and 72 hours, or with Concanavalin A (2 μ g/ml) for 24 hours. PMA (20ng/ml) and Ionomycin (1 μ M) were added during the last six hours of stimulation. The cells were then harvested and the total RNA was extracted using Trizol reagent (Invitrogen). To obtain cDNA templates for sequencing, RNA was reverse transcribed using Superscript II reverse transcriptase (Invitrogen), according to the manufacturer's protocol. The cDNA were used to PCR amplify the full length of *Tim* cDNA using Herculase Hot Start[™] high fidelity polymerase (Stratagene). The PCR primers were: (SEQ ID NO:37) GTGTCTGACAGTGGCGTA (forward), (SEQ ID NO:38) TTTGCCCAGGCAGAACCA (forward), CCACCCAAGGTCACGACT (reverse), (SEQ ID NO:39) ATGCCACGACTAAGACC (reverse). The PCR products were purified with Qiagen QIAquick gel extraction reagents, and sequenced using four internal sequencing primers for *Tim1* and two internal sequencing primers for *Tim3*.

The full length *Tim1* RT-PCR product was cloned in these individuals by taking total RNA from activated T cells and transcribing it with Superscript II and oligo dT. *Tim1* cDNA was amplified with Expand high fidelity polymerase (Roche) to generate a 1 kb product spanning the *Tim1* coding region, which was purified with a PCR Purification kit (Invitrogen). This purified product was then cloned into the TOPO pEF6 vector (Invitrogen), followed by transformation of TOP10 competent bacteria. Bacterial colonies were grown on LB plates with ampicillin selection. Single colonies were picked and plasmid preps generated using Qiagen mini prep kits. Restriction mapping using Hind III digestion was used to select plasmids containing inserts in the correct orientation. These plasmids were then sequenced with three different primers, forward (T7), internal and reverse (BGH), and the sequences aligned in SeqMan program with NCBI human TIM reference sequence.

After sequencing *Tim1* from the chromosomes from 35 individuals (70 chromosomes) several polymorphisms in *Tim1* were identified, which are shown in Figure 8. These polymorphisms are numbered 1-7 (left column). The full sequence of human

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TIM-1, which is listed in the NCBI database (NM_012206), is provided in Figure 8 as a reference point. This sequence is present in less than 20% of the chromosomes that were sequenced, due to the existence of multiple, prevalent sequence polymorphisms in the coding region. 6 additional sequence variations were identified, shown in Figure 8, and all of the polymorphisms were observed in the mucin, extracellular domain, as was true for mice, although the specific variations were distinct from those seen in mice. Importantly, there is a limited degree of association between these variants, in various combinations. The most pronounced variations are the insertion labeled polymorphism 1, 157insMTTTP, which was observed in 65% of the chromosomes, and the deletion in polymorphism 5, 187ΔThr, was observed in 65% of the chromosomes. Polymorphism 4 was observed in 40% of the chromosomes, and the other polymorphisms were each observed in ≤5% of the chromosomes. Notably, most of these variations (2-6) are located within exon 3, the first mucin-encoding exon, and all of the variants occur at the genomic level and are not splice variants.

Based on this sequence analysis of mRNA, a more rapid method for analyzing the genomic DNA from the larger number of patients/controls has been developed. To screen individuals for the variations seen in sequences shown in Figure 8, the DNA is initially tested for simple sequence length polymorphisms (SSLP) in a 150 PCR product, which can detect the major insertion, polymorphism 1, and the deletion, polymorphism 5.

In addition, to genotype the other polymorphisms (2-4, 6, and 7) and identify novel polymorphisms, a relatively simple assay using single strand conformational polymorphism (SSCP) analysis of PCR products has been developed. Under well-optimized conditions, SSCP analysis detects more than 90% of single nucleotide substitutions and all length polymorphisms. For this analysis, PCR primers have been identified that amplify each exon of the *Tim* genes, and variants can be distinguished using standard non-denaturing SSCP gel electrophoresis methods (Figure 9). Non-denaturing polyacrylamide gel electrophoresis is used with an ABI 377 DNA sequence for high resolution SSCP analysis of each exon. Fluorescent end-labeled primers are synthesized and purified. Novel SSCP patterns that are detected during the high-throughput genotyping process will identify novel variants. Using this method, the genotype of patients and controls is rapidly analyzed.

The *Tim3* gene was analyzed using essentially the same methodologies. mRNA from activated T cells is sequenced to identify *Tim3* polymorphisms, as well as long range haplotypes between the *Tim1* and *Tim3*. After sequencing *Tim3* cDNA representing 60 chromosomes, it has been found that *Tim3* is polymorphic, as it is in the mouse genome. However, only one polymorphism, Leu140Arg, is prevalent, found in

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approximately 12% of the chromosomes represented.

Example 3

Expression of Tim Sequences

5 Murine TIM-3 protein is expressed on Th1 clones but not on naive T cells or Th2 cells. Using TCR transgenic T cells, TIM-3 protein was not expressed on Th1 cells after one or two rounds of Th1-directed differentiation but was expressed after the third and further rounds of Th1 stimulation. TIM-3 mRNA expression was detected somewhat earlier. In order to determine if TIM-3 gene expression was the same in human, TIM-3 and TIM-1
10 mRNA expression in human Th1 cells was examined using tetanus toxoid specific T cells generated by stimulation with antigen in the presence of IL-12 and anti IL-4 mAb. Given the association of TIM-1 with asthma, TIM-1 and TIM-3 mRNA expression in human Th2 cells was examined. Th2 cell lines were generated from allergic donors by *in vitro* stimulation with allergen, IL-4, and anti IL-12 mAb. RNA was analyzed by PCR for TIM
15 gene expression.

TIM-3 was generally expressed after Th1 differentiation whereas TIM-1 was lost. Conversely, TIM-3 was not expressed in any of the Th2 but TIM-1 was expressed in all Th2 cells. Both TIM-1 and TIM-3 are expressed in monocyte-depleted, unstimulated peripheral blood mononuclear cells from the donors used to derive the Th1 and Th2 cell
20 lines, presumably because this mixed population contains both Th1 and Th2 memory cells. These results suggest a reciprocal relationship with TIM-1 being expressed in Th2 and TIM-3 in Th1. This reciprocal relationship between TIM-1 and TIM-3 has also been observed in the mouse.

In human tissues, a 4.4 kb TIM-1 mRNA was very strongly expressed in
25 kidney and testis. The 4.4-kb mRNA was present in almost all tissues, though it was faint in most. A 5.5-kb band was observed in colon and liver. A 7.5-kb band was observed in spleen, thymus, and peripheral blood leukocytes, and smaller than 4.4-kb bands were observed in some organs. These results suggest that hTIM-1 is expressed at some level in most human tissues and that a message of 7.5-kb may code for hTIM-1 in tissues of
30 immunological interest. However, expression of Kim-1 (Kidney Injury Molecule-1), the rat homologue of TIM-1, increases in kidney upon ischemic injury. Since the MTN blots used in the expression analysis were prepared from mRNA extracted from cadavers, the increased expression of TIM-1 in kidney was re-analyzed. TIM-1 was not found to be overexpressed in kidney RNA obtained from normal kidney biopsies. Therefore, it is likely
35 that the high levels of expression of TIM-1 observed in kidney and testis were due to an up-regulation in the expression of TIM-1 resulting from tissue injury. The injured kidney may express proteins that direct incoming inflammatory cells towards a more protective Th2

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response rather than a destructive Th1 response.

Example 4

TIM Ligands and Antibodies

5 *Generation of Antibodies.* Generation of monoclonal antibodies against mouse TIM-1 allows examination of the cell surface expression of TIM-1 in different tissues, cell lines and mouse strains. Both alleles of mouse TIM-1 have been cloned into a vector for high protein expression (Invitrogen, pEF6-TOPO). Rats have been immunized and boosted with both Tim1 cDNA constructs to rapidly generate antibodies against cell surface
10 molecules. This method with cDNA vaccination favors the production of mAb against cell surface epitopes since the Tim1 cDNA will be taken up by APC, which will express the TIM-1 as a cell surface molecule. In order to generate mAb that would bind equally well to both the BALB/c and the HBA TIM-1 (by binding to conserved domains of TIM-1 such as the Immunoglobulin domain of TIM-1), both the BALB/c and HBA Tim1 cDNA (pEF6-mTIMbalb and pEF6-mTIMhba) were injected into each rat.
15

Further boosting of the Tim1 cDNA-immunized rats was done with CHO cells stably transfected with the pEF6-mTIM-1-GFP expression constructs. CHO transfectants expressing high levels of mouse TIM-1 were sorted by FACS, and injected into the rats. Another mTIM-1 expressing cell was generated by stably transfecting the pre-B cell line
20 300.19 with the pEF6-mTIM-1 expression constructs. This line is used to screen the rat serum and the hybridomas following fusion for anti-TIM-1 antibody by flow cytometry. Rats have been generated which have high polyclonal titers against anti-TIM-1, as detected by the binding of rat serum (and a secondary FITC-goat anti-rat Ig) to stable pEF6-mTIM1-transfected 300.19 cells, as compared with control serum from unimmunized rats. This staining is specific for TIM-1 since there is no reactivity with nontransfected cells or cells
25 transfected with TIM-2.

The rat spleen is removed and the splenocytes fused with a myeloma cell line (SP/2) to produce hybridomas. Hybridoma supernatants are screened using the TIM-1 transfected 300.19 cell lines to identify hybridoma clones that produce monoclonal anti-
30 TIM-1. Specificity of the mAb for TIM-1 (and not other TIM proteins) is confirmed using TIM-2 transfected cells and mTIM-3 transfected cells or TIM-3 Ig fusion protein.

Antibody Staining. Th1 and Th2 cell lines were generated from both BALB/c and HBA DO11.10 spleen cells. RT-PCR for TIM-1 mRNA expression demonstrated that
35 TIM-1 is expressed in Th2 lines, but not in Th1 lines, following two rounds of restimulation with antigen under standard polarizing conditions. DO11.10 T cells following two rounds of stimulation with antigen/APC under Th2 polarizing conditions were stained with the

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polyclonal rat anti-TIM-1 antiserum. These Th2 cells expressed high levels of TIM-1.

These experiments showing preferential expression of TIM-1 in Th2 lines are quantified and confirmed using anti-Tim-1 mAbs and Northern blots. DO11.10 cells from BALB and HBA are cultured with antigen and APC, and restimulated for 1, 2, and 3 weeks under standard polarizing conditions (anti-IL-12 plus IL-4 or anti-IL-4 plus IL-12). After each week of stimulation, cells are stained with anti-TIM-1 mAb. By harvesting stimulated cells at various time points the kinetics of TIM-1 expression on T cells undergoing differentiation to Th1 or Th2 subset is determined. To determine if Tim-1 surface expression changes following T cell activation, we will also compare TIM-1 expression on resting and activated T cells one week after each round of antigen stimulation, by stimulating some cells with PMA and ionomycin. Activated cells are stained for intracellular cytokine expression to verify the Th subset differentiation of the T cells. Alternatively, quantitative RT-PCR or northern blots using mRNA harvested from T cells activated with PMA plus ionomycin, following each round of stimulation, are used to determine relative levels of mRNA production.

TIM-1-Ig fusion proteins BALB/c TIM-1-mIgG2a has been prepared, which is a fusion protein between the TIM-1 polypeptide and the Fc region of mouse immunoglobulin. The vector has been engineered to contain a mutation in murine IgG2a Fc that minimizes binding to Fc receptors. The TIM-1 fusion protein is utilized in characterization of TIM-1 function. The TIM-1 Ig fusion protein is expected to block TIM-1 function by binding to the TIM-1 ligand and interrupt TIM-1/TIM-1-ligand interactions.

Purified D1muc-Fc fusion protein containing the cys-rich immunoglobulin domain and 2/3 of the mucin-like region of TIM-1 fused to the hinge and Fc fragment of human IgG1 (IgVmuc-hlg) was run on a gel. This protein was expressed in CHO cells, and the IgVmuc-hlg protein was purified from CHO supernatants with protein-A agarose columns. Purified IgVmuc-hlg fusion protein neutralizes about 2 logs of HAV infectivity. In addition, treatment of HAV with IgVmuc-hlg produced a major shift in the sedimentation of the HAV particles, indicating that IgVmuc-hlg induced uncoating of the viral genome, whereas a fusion protein containing only the Ig-like region without the mucin domain (IgV-hlg) did not. This HAV neutralization system and the IgVmuc-hlg fusion protein will be used to analyze the function of TIM-1/HAV receptor alleles.

Based upon the *in vivo* effect of anti-TIM-3 mAb on macrophage expansion and activation, it was hypothesized that the TIM-3 ligand would be expressed on cells of the myeloid lineage. Dendritic cells (DC) were prepared from blood monocytes according to established protocols with 1000 U/ml IL-4 and 800 U/ml GM-CSF. DC were matured by replating the cells for 2 days in IL-4 (1000 U/ml) and GM-CSF (800 U/ml) supplemented

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with IL-1 β (10 ng/ml), TNF- α (10 ng/ml), IL-6 (1000 U/ml), and PGE $_2$ (1 μ g/ml). Mature DCs stained positively with hTIM-3-Ig, though there was variability among donors, suggesting that mature DC express a ligand for the IgV domain of TIM-3. Bone marrow derived endothelial cells stained very weakly and B cell lines did not stain with TIM-3-Ig.

5

Although the intracytoplasmic tail of Tim1/huhavcr-1 is relatively short, it contains a sequence that is highly conserved between mouse, rat, human and monkey (RAEDNIYI), and which may be phosphorylated, and signal through interaction with other signal transduction molecules. The most likely candidate molecule that can bind the RAEDNIYI motif in T cells is the tyrosine kinase Itk. The interleukin-2 inducible tyrosine kinase, Itk is a nonreceptor protein tyrosine kinase of the Tec family that participates in the intracellular signaling events leading to T cell activation. Tec family members contain the conserved SH3, SH2, and catalytic domains common to many kinase families, but they are distinguished by unique sequences outside of this region. It is known that Itk phosphorylate phospholipase C- γ (PLC- γ), and triggers a cascade of signaling events that are involved in T cell activation and helper T cell differentiation. In the absence of Itk signaling, Th2 cells do not develop. These results suggest that TIM-1/huhavcr-1 may signal through Itk, thereby altering the cytokine development in CD4 T cells.

20

Example 5

TIM Knockout Mice

A knockout construct is used in the generation of a *Tim1* deficient mouse, which enables analysis of development of immune responses in the absence of TIM-1 molecules. In another approach, TIM-1-Ig fusion proteins or anti-TIM-1 monoclonal antibody are used to block the function of TIM-1 in *Tim1*^{+/+} (wild type) mice. The Tim1 knockout mice and the anti-TIM-1 mAb approaches are complementary for the evaluation of the role of TIM-1 in T cell differentiation and in asthma pathogenesis.

The HBA mouse genomic sequence of *Tim1* has a deletion of exon 4, compared to the BALB/c sequence, due to an integrated L1 retroviral element at exon 4. If truncation of one exon reduces TIM-1 function, then total deletion of TIM-1 function by generation of Tim1 knockout mice, should severely limit the capacity of such mice to generate Th2 responses. TIM-1 KO mice are generated by deleting the TIM-1 exons 1 and 2, using cre-lox technology and BALB/c ES cells, which should eliminate cell surface expression of TIM-1, thereby demonstrating the importance of TIM-1 function in the development of Th2 cells and AHR.

To create an appropriate targeting construct, specific BACs containing members of the TIM gene family were identified by screening a C57/Bl6 BAC library (RPCI-

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23) using high density filter sets. These BAC clones were used to build a 500 kB contig and physical map that covers the approximately 350 kb genomic region encompassing the *Tim* gene family. One specific BAC, RPCI-23-222F8, that contains the complete *Tim1* gene was chosen to generate a TIM targeting construct. The targeting construct deletes a 4 kb region encompassing the promoter region, signal exon, and IgV exon (exons 1 and 2), via homologous recombination with 5' and 3' arms flanking this region. This targeting vector can be used in either C57Bl/6 or BALB/c ES cells, since the homologous arms of the targeting construct are homologous to both BALB/c and HBA (C57Bl/6) DNA. The construct is introduced into ES cells, which are screened for the targeted allele by PCR and by Southern blots.

The targeting vector, pLOX, contains three loxP sites, and by expressing cre recombinase in the targeted ES cells, recombination will generate three forms of the targeted region, of which two alleles, A and B, are used to create *Tim1* KO mice. Selected ES cell clones are introduced into blastocysts to generate chimeric mice and bred for germline transmission of the knockout. Tail DNAs are analyzed for transmission of the knockout. Allele A is used initially, in which cre/lox recombination has used the outermost loxP sites to delete the TK and neomycin selection cassettes in addition to TIM-1 exons 1 and 2. The removal of the entire neo and TK cassette from the TIM-1 genomic targeting region prevents confounding artifacts that can arise secondary to the transcription of neo in close proximity to other genes of the same family. Alternatively, if allele A produces a lethal or confounding phenotype, mice are generated with targeted allele B, in which the neo and TK cassette has been deleted and the TIM-1 region is flanked by lox-p sites. Use of this conditional targeting approach should allow T cell-specific deletion of Tim-1 in mice expressing cre-recombinase under the control of a T cell specific promoter.

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WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising a mammalian TIM gene sequence.
- 5 2. An isolated nucleic acid molecule according to Claim 1, wherein said mammalian TIM gene sequence is a human sequence selected from the group consisting of alleles 2, 3, 4, 5, and 6 of TIM-1, TIM-3, and TIM-4.
- 10 3. An isolated nucleic acid according to Claim 1, wherein said mammalian TIM gene sequence is a mouse sequence selected from the group consisting of TIM-1, TIM-2, TIM-3 and TIM-4.
- 15 4. An isolated nucleic acid comprising at least 20 nucleotides of contiguous sequence as set forth in any one of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34 or 36.
- 20 5. An isolated nucleic acid comprising a sequence as set forth in any one of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34 or 36.
- 25 6. An isolated nucleic acid molecule according to Claim 1, comprising a probe for detection of a TIM gene polymorphism.
7. An array of oligonucleotides comprising:
two or more probes according to Claim 6.
- 25 8. A cell comprising a nucleic acid composition according to claim 1.
9. A purified polypeptide composition comprising at least 50 weight % of the protein present as the product of the nucleic acid of Claim 1 or a fragment thereof.
- 30 10. The purified polypeptide according to Claim 9, wherein said polypeptide comprises a sequence as set forth in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33 and 35.
- 35 11. An antibody specific for a mammalian TIM polypeptide.
12. A method for detecting a predisposition to asthma in an individual,

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the method comprising:

analyzing said individual for the presence of at least one predisposing TIM polymorphism; wherein the presence of said predisposing polymorphism is indicative of an increased susceptibility to asthma.

5

13. The method according to Claim 12, wherein said analyzing step comprises detection of specific binding between genomic DNA or mRNA of said individual with a probe or probes according to Claim 6.

10

14. The method according to Claim 12, wherein said analyzing step comprises detection of specific binding between cells or protein from said individual and the antibody according to Claim 11.

15. A non-human transgenic animal model for TIM gene function comprising a knockout of a TIM gene.

16. A method of screening for biologically active agents that modulate TIM function, the method comprising:

combining a candidate biologically active agent with any one of:

20

(a) a mammalian TIM polypeptide;

(b) a cell comprising a nucleic acid encoding a mammalian TIM polypeptide;

or

(c) a non-human transgenic animal model for TIM gene function; and determining the effect of said agent on TIM function.

25

17. The method according to Claim 16, wherein said biologically active agent downregulates or upregulates TIM expression.

18. The method according to Claim 16, wherein said biologically active agent inhibits or increases activity of said TIM polypeptide.

30

19. A method for the diagnosis or staging of Immune dysfunction in an individual, the method comprising:

analyzing said individual for the presence of at least one predisposing TIM polymorphism; wherein the presence of said predisposing polymorphism is indicative of an increased susceptibility to said immune dysfunction.

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20. A method for the diagnosis or staging of cancer in an individual, the method comprising:
analyzing said individual for the presence of at least one predisposing TIM polymorphism; wherein the presence of said predisposing polymorphism is indicative of an increased resistance to cancer therapy.
21. A method for the diagnosis or staging of cancer in an individual, the method comprising:
analyzing said individual for the presence of at least one predisposing TIM-1 expression profile; wherein the expression profile of TIM-1 indicative of an increased resistance to cancer therapy.
22. A method for the diagnosis or staging of cancer in an individual, the method comprising:
analyzing said individual for the presence of at least one predisposing TIM-4 expression profile; wherein the expression profile of TIM-4 indicative of an increased resistance to cancer therapy.
23. A method for the treatment of an immunological disorder in an individual, the method comprising:
administering to said individual an agent that modulates function of a TIM polypeptide.
24. The method according to Claim 23, wherein said TIM polypeptide is TIM-1 or TIM-4.
25. The method according to Claim 24, wherein said agent is an antibody or antibody fragment specific for TIM-1.
26. The method according to Claim 24, wherein said agent is a compound that inhibits TIM-1 function.
27. The method according to Claim 24, wherein said immunological disorder is asthma.
28. The method according to Claim 24, wherein said immunological disorder is an allergy.

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29. The method according to Claim 24, wherein said immunological disorder is eczema.

5 30. The method according to Claim 24, wherein said immunological disorder is an autoimmune disease.

31. The method according to Claim 23, wherein said agent is an antibody or antibody fragment specific for TIM-4.

10

32. The method according to Claim 30, wherein said agent is a compound that inhibits the function of TIM-4.

33. A method for the treatment of malignancy in an individual, the method comprising: administering to said individual an agent that modulates function of a TIM polypeptide.

15

34. The method according to Claim 33, wherein said TIM polypeptide is TIM-1.

20

35. The method according to Claim 34, wherein said agent is an antibody or antibody fragment specific for TIM-1.

36. The method according to Claim 35, wherein said agent is a compound that inhibits the function of TIM-1 and potentiates the effect of chemotherapeutic agents or radiation therapy.

25

37. The method according to Claim 33, wherein said TIM polypeptide is TIM-4.

30

38. The method according to Claim 37, wherein said agent is an antibody or antibody fragment specific for TIM-4.

39. The method according to Claim 38, wherein said agent is a compound that inhibits the function of TIM-4 and potentiates the effect of chemotherapeutic agents or radiation therapy.

35

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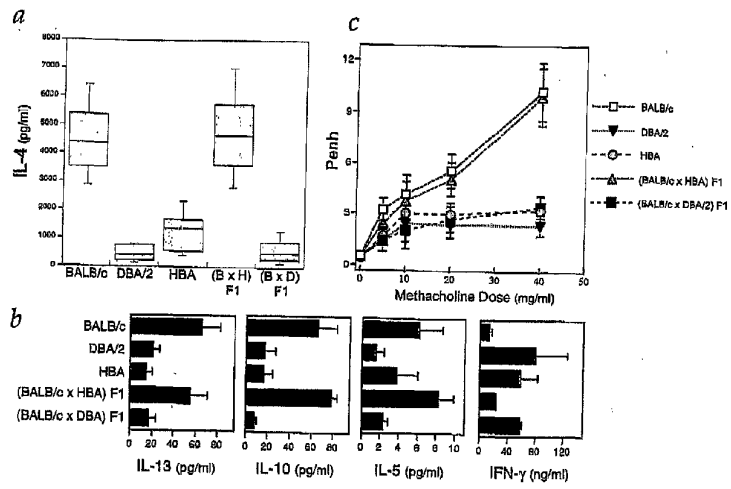


Figure 1



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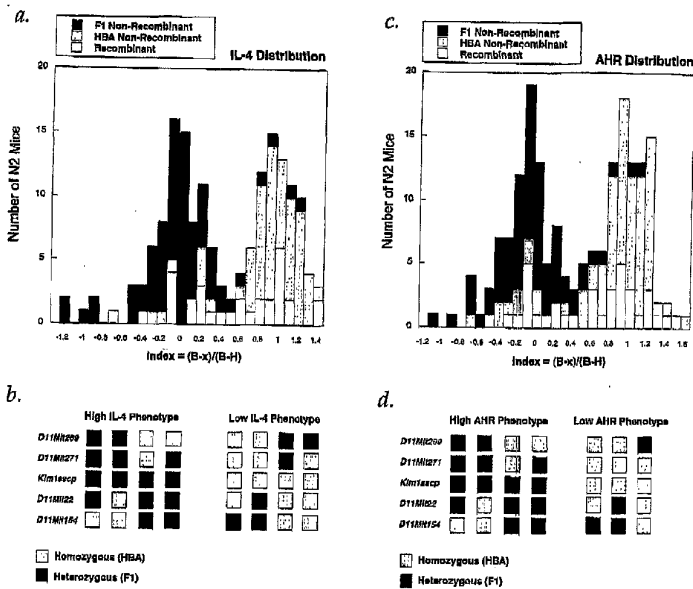


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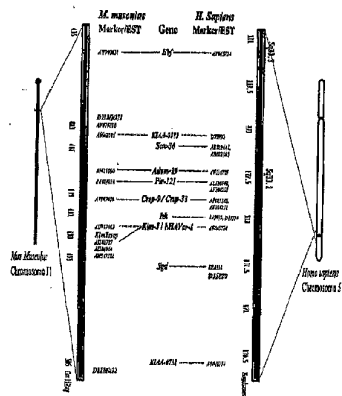


Figure 4

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TIM-1 Variants

A2-11/TIM-3 Variants

[illegible]

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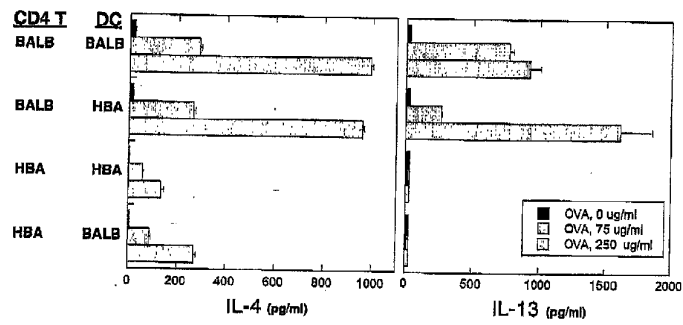
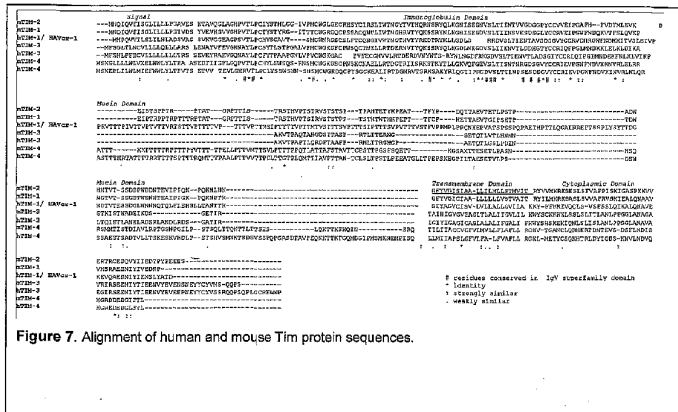


Figure 6

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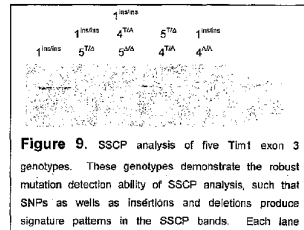
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FIGURE 8

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NM_012206	MTTFVP	
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2		.05
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4		.40
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Umetsu, Dale T.
DeKruyff, Rosemarie
Kuchroo, Vijay
Freeman, Gordon J.

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Use Thereof

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<141> 2002-07-01

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<223> TIM-1 BALB/c allele

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Ile Trp Thr Asn Gly His Arg Val Thr Tyr Gln Lys Ser Ser Arg Tyr
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Pro Thr Ser Ile Arg Val Ser Thr Ser Thr Pro Pro Thr Ser Thr His
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Thr Ala Glu Val Thr Gly Ile Pro Ser His Thr Pro Thr Asp Trp Asn
195    200    205
Gly Thr Val Thr Ser Ser Gly Asp Thr Trp Ser Asn His Thr Glu Ala
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 245 250 255
 Ala Ile Thr Arg Tyr Ile Leu Met Lys Arg Lys Ser Ala Ser Leu Ser
 260 265 270
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 Pro
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 50 55 60
 Ile Trp Thr Asn Gly His Arg Val Thr Tyr Gln Lys Ser Ser Arg Tyr
 65 70 75 80
 Asn Leu Lys Gly His Ile Ser Glu Gly Asp Val Ser Leu Thr Ile Glu
 85 90 95
 Asn Ser Val Glu Ser Asp Ser Gly Leu Tyr Cys Cys Arg Val Glu Ile

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Thr Ala Thr Gly Arg Pro Thr Thr Ile Ser Thr Arg Ser Thr His Val
145 150 155 160
Pro Thr Ser Thr Arg Val Ser Thr Ser Thr Pro Pro Thr Ser Thr His
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Thr Trp Thr Thr His Lys Pro Asp Trp Asn Gly Thr Val Thr Ser Ser Gly
180 185 190
Asp Thr Trp Ser Ser Asn His Thr Glu Ala Ile Pro Pro Gly Lys Pro Gln
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Lys Asn Pro Thr Lys Gly Phe Tyr Val Gly Ile Cys Ile Ala Ala Leu
210 215 220
Leu Leu Leu Leu Leu Val Ser Thr Val Ala Ile Thr Arg Tyr Ile Leu
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Met Lys Arg Lys Ser Ala Ser Leu Ser Val Val Ala Phe Arg Val Ser
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<213> Mus musculus

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 Tyr Gln Leu Lys Gly Asn Ile Ser Glu Gly Asn Val Ser Leu Thr Ile
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 100 105 110
 Ile Pro Gly Ala Phe His Phe Val Asp Tyr Met Leu Glu Val Lys Pro
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 Thr Thr Ile Ser Thr Arg Ser Thr His Val Pro Thr Ser Thr Arg Val
 145 150 155 160
 Ser Thr Ser Thr Ser Pro Thr Pro Ala His Thr Glu Thr Tyr Lys Pro
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 180 185 190
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 195 200 205
 Asp Asp Pro Trp Asp Asp Asn Thr Glu Val Ile Pro Pro Gln Lys Pro
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Leu Ile Trp Thr Asn Gly Tyr Thr Val Thr His Gln Arg Asn Ser Arg
65 70 75
Tyr Gln Leu Lys Gly Asn Ile Ser Glu Gly Asn Val Ser Leu Thr Ile
85 90
Glu Asn Thr Val Val Gly Asp Gly Gly Pro Tyr Cys Cys Val Val Glu
100 105 110
Ile Pro Gly Ala Phe His Phe Val Asp Tyr Met Leu Glu Val Lys Pro
115 120 125
Glu Ile Ser Thr Ser Pro Pro Thr Arg Pro Thr Ala Thr Gly Arg Pro
130 135 140
Thr Thr Ile Ser Thr Arg Ser Thr His Val Pro Thr Ser Thr Arg Val
145 150 155
Ser Thr Ser Thr Ser Pro Thr Pro Ala His Thr Glu Thr Tyr Lys Pro
165 170 175
Glu Ala Thr Thr Phe Tyr Pro Asp Gln Thr Thr Ala Glu Val Thr Glu
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Thr Leu Pro Ser Thr Pro Ala Asp Trp His Asn Thr Val Thr Ser Ser
195 200 205
Asp Asp Pro Trp Asp Asp Asn Thr Glu Val Ile Pro Pro Gln Lys Pro
210 215 220
Gln Lys Asn Leu Asn Lys Gly Phe Tyr Val Gly Ile Ser Ile Ala Ala
225 230 235 240
Leu Leu Ile Leu Met Leu Leu Ser Thr Met Val Ile Thr Arg Tyr Val
245 250 255
Val Met Lys Arg Lys Ser Glu Ser Leu Ser Phe Val Ala Phe Pro Ile
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Val Pro Met Cys Trp Gly Lys Gly Phe Cys Pro Thr Ser Gln Cys Thr
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Asn Glu Leu Leu Arg Thr Asp Glu Arg Asn Val Thr Tyr Gln Lys Ser
65          70          75
Ser Arg Tyr Gln Leu Lys Gly Asp Leu Asn Lys Gly Asp Val Ser Leu
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Ile Ile Lys Asn Val Thr Leu Asp Asp His Gly Thr Tyr Cys Cys Arg
100         105         110
Ile Gln Phe Pro Gly Leu Met Asn Asp Lys Lys Leu Leu Lys Leu
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Asp Ile Lys Ala Ala Lys Val Thr Pro Ala Gln Thr Ala His Gly Asp
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Glu Thr Gln Thr Leu Val Thr Leu His Asn Asn Asn Gly Thr Lys Ile
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Ser Thr Trp Ala Asp Glu Ile Lys Asp Ser Gly Glu Thr Ile Arg Thr
180         185         190
Ala Ile His Ile Gly Val Gly Val Ser Ala Gly Leu Thr Leu Ala Leu
195         200         205
Ile Ile Gly Val Leu Ile Leu Lys Trp Tyr Ser Cys Lys Lys Lys Lys
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tctctttaca caagaaaaag gttaccgcga agcagccttg tctctgtaaa aggaaccctc 2100
gggaaggcca aactttgact gttgtgtgct caaggggaac tgactcagac aacttctcca 2160
ttcctggagg aaactggagc gtttctgac agaagaacaa ccggtgactg ggaacataga 2220
aggcagagct cttgcagcaa tctatatagt cagcaanaata ttctttggga ggaacagtct 2280
caccaaaattg atttccaaag ccgttgaact cagtttctat tggcttacag ctgcctgccc 2340
atgtcccttg atctgtgctg gctccatctc ataacagaat caaattaaat agaccccgag 2400
tgaanaataa aggtgagcg aaagttagct ttgttcaag atttttttgc attggggagc 2460
aactgtgtac atcagagcac atctgttagt gaggacacaa aaactgtgtg tacctgtttt 2520
tcactgtatga abtttgttgt ttaagttgct tctagctagc tgtggaggtc ctgggtttct 2580
taggtgggta tgggaaggcg accatctaac aanaatcatt agagataaca gctctctcgc 2640
agaaggggaa actaatctca aatgttttaa agtaataaaa ctgtactggc aaagtacttt 2700
ggcatatatt aaaaaaaaaa aaaaa 2725

```

<210> 11
 <211> 281
 <212> PRT
 <213> Mus musculus

<220>

<221> VARIANT

<222> (1) ... (281)

<223> TIM-3, C.D2 ES-HBA and DBA/2J allele

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<400> 11
Met Phe Ser Gly Leu Thr Leu Asn Cys Val Leu Leu Leu Gln Leu
1 5 10 15
Leu Leu Ala Arg Ser Leu Glu Asn Ala Tyr Val Phe Glu Val Gly Lys
20 25 30
Asn Ala Tyr Leu Pro Cys Ser Tyr Thr Leu Ser Thr Pro Gly Ala Leu
35 40 45
Val Pro Met Cys Trp Gly Lys Gly Phe Cys Pro Trp Ser Gln Cys Thr
50 55 60
Asn Glu Leu Leu Arg Thr Asp Glu Arg Asn Val Thr Tyr Gln Lys Ser
65 70 75 80
Ser Arg Tyr Gln Leu Lys Gly Asp Leu Asn Lys Gly Asp Val Ser Leu
85 90 95
Ile Ile Lys Asn Val Thr Leu Asp Asp His Gly Thr Tyr Cys Cys Arg
100 105 110
Ile Gln Phe Pro Gly Leu Met Asn Asp Lys Lys Leu Glu Leu Lys Leu
115 120 125
Asp Ile Lys Ala Ala Lys Val Thr Pro Ala Gln Thr Ala His Gly Asp
130 135 140
Ser Thr Thr Ala Ser Pro Arg Thr Leu Thr Thr Glu Arg Asn Gly Ser
145 150 155 160
Glu Thr Gln Thr Leu Val Thr Leu His Asn Asn Asn Gly Thr Lys Ile
165 170 175
Ser Thr Trp Ala Asp Glu Ile Lys Asp Ser Gly Glu Thr Ile Arg Thr
180 185 190
Ala Ile His Ile Gly Val Gly Val Ser Ala Gly Leu Thr Leu Ala Leu
195 200 205
Ile Ile Gly Val Leu Ile Leu Lys Trp Tyr Ser Cys Lys Lys Lys Lys
210 215 220
Leu Ser Ser Leu Ser Leu Ile Thr Leu Ala Asn Leu Pro Pro Gly Gly
225 230 235 240
Leu Ala Asn Ala Gly Ala Val Arg Ile Arg Ser Glu Glu Asn Ile Tyr
245 250 255
Thr Ile Glu Glu Asn Val Tyr Glu Val Glu Asn Ser Asn Glu Tyr Tyr
260 265 270
Cys Tyr Val Asn Ser Gln Gln Pro Ser
275 280

<210> 12

<211> 862

<212> DNA

<213> Mus musculus

<400> 12
cccccccaa gtactcatgt ttccaggtct tacccccaac tgtgtcctgc tgctgctgca 60
actactacttt gcaagggtcat tggaaaatgc ttatgtgttt gaggttggtg acaatgccta 120
ttgcccctgc agttacacac tatctacacc tggggccactt gtgcttatgt gctggggcaa 180
gggallctgt ccttggtcac agtctaccaa cgaattgcct agaacctgat aaagaacagt 240
gacataatcg aaatccagca gataccagct aaagggcgat ctcaacaag gagacgtgtc 300
tttgatcata aagaatgtga ctctggatga ccatgggacc tactgtctgc ggatacagtt 360
ccctggctct atgaatgata aaaaattaga actgaaatta gacatcaag cagccaaggt 420
cactccagct cgaactgcc atggggacac tactacagct tctccaagaa cctaaccac 480
ggagagaaat gggtcagaga cacagacact ggtgaccctc cataataaca atggaacaaa 540
aatttccaca tgggtgatg aaattaagga ctctggagaa acgatcagaa ctgctatcca 600
cattggagtg ggaatctctg ctgggttgac cctggcactt atcattgggt tcttaactct 660
taaatggtat tctgttaaga aaaagaagtt atcaggtttg agccttatta cactggccaa 720
cttgcccca ggaagggttg caaatgcagg agcagtcagg attcgtctg aggaatatat 780
ctacaccatc gaggagaacg tatatgaagt ggaagaattca aatgagtact actgctactg 840
caacagccag cagccatcct ga 862

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<210> 13
 <211> 345
 <212> PRT
 <213> Mus musculus

<220>
 <221> VARIANTE
 <222> (1)...(345)
 <223> TIM-4, BALB/c allele

<400> 13
 Met Ser Lys Gly Leu Leu Leu Leu Trp Leu Val Thr Glu Leu Trp Trp
 1 5 10 15
 Leu Tyr Leu Ser Lys Ser Pro Ala Ala Ser Glu Asp Thr Ile Ile Gly
 20 25 30
 Phe Leu Gly Gln Pro Val Thr Leu Pro Cys His Tyr Leu Ser Trp Ser
 35 40 45
 Gln Ser Arg Asn Ser Met Cys Trp Gly Lys Gly Ser Cys Pro Asn Ser
 50 55 60
 Lys Cys Asn Ala Glu Leu Leu Arg Thr Asp Gly Thr Arg Ile Ile Ser
 65 70 75
 Arg Lys Ser Thr Lys Tyr Thr Leu Leu Gly Lys Val Gln Phe Gly Glu
 80 85 90 95
 Val Ser Leu Thr Ile Ser Asn Thr Asn Arg Gly Asp Ser Gly Val Tyr
 100 105 110
 Cys Cys Arg Ile Glu Val Pro Gly Trp Phe Asn Asp Val Lys Lys Asn
 115 120 125
 Val Arg Leu Glu Leu Arg Ala Thr Thr Thr Lys Lys Pro Thr Thr
 130 135 140
 Thr Thr Arg Pro Thr Thr Thr Pro Tyr Val Thr Thr Thr Pro Glu
 145 150 155 160
 Leu Leu Pro Thr Thr Val Met Thr Thr Ser Val Leu Pro Thr Thr Thr
 165 170 175
 Pro Pro Gln Thr Leu Ala Thr Thr Ala Phe Ser Thr Ala Val Thr Thr
 180 185 190
 Cys Pro Ser Thr Thr Pro Gly Ser Phe Ser Gln Glu Thr Thr Lys Gly
 195 200 205
 Ser Ala Ile Thr Thr Glu Ser Glu Thr Leu Pro Ala Ser Asn His Ser
 210 215 220
 Gln Arg Ser Met Met Thr Ile Ser Thr Asp Ile Ala Val Leu Arg Pro
 225 230 235 240
 Thr Gly Ser Asn Pro Gly Ile Leu Pro Ser Thr Ser Gln Leu Thr Thr
 245 250 255
 Gln Lys Thr Thr Leu Thr Thr Ser Glu Ser Leu Gln Lys Thr Thr Lys
 260 265 270
 Ser His Gln Ile Asn Ser Arg Gln Thr Ile Leu Ile Ile Ala Cys Cys
 275 280 285
 Val Gly Phe Val Leu Met Val Leu Leu Phe Leu Ala Phe Leu Leu Arg
 290 295 300
 Gly Lys Val Thr Gly Ala Asn Cys Leu Gln Arg His Lys Arg Pro Asp
 305 310 315 320
 Asn Thr Glu Val Ser Asp Ser Phe Leu Asn Asp Ile Ser His Gly Arg
 325 330 335
 Asp Asp Glu Asp Gly Ile Phe Thr Leu
 340 345

<210> 14
 <211> 1032
 <212> DNA
 <213> Mus musculus

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<400> 14
atgtccaagg ggcttctcct cctctggctg gtgacggagc tctgggtggct ttatctgaca 60
ccagctgcct cagaggtatc aataataggg tttttgggccc agccgggtgac ttgctcttgt 120
cattacctct cgtggtccca gagccgcaac agtatgtgct ggggcaaggg ttcattgtcc 180
aatccaagt gcaatgcaga gcttctccgt acagatggaa caagaatcat ctccaggaag 240
tcacacaaat atacactttt ggggaaggtc cagtttggtg aagtgtctct gaccatctca 300
aacccaatc gaggtgacag tgggtgtgac tgcgtgcgtc tagaggtgac tgggtgggtc 360
aagatgtca agagaaggt cgtcttggag ctggaggag caacacac caaaaaac 420
acaaacaa caagggcaac caccacccct tatgtaacca caccacccc agagctgctt 480
ccaaacaacg tcatgaccac atctgttctt caaacaccca caccacccc gacactagcc 540
accactgcct tcatgaccag agtgaccacg tgcacctcaa caacacctg ctccctctca 600
caagaaccca caaaagggtc cgcctcact acagaatcag aaactctgcc tgcctccaat 660
cacttccaaa gaagcatgat gaccatctct acagacatag ccgtactcag gccacacagg 720
tctaacctcg ggaattctcc atccacttca cagctgacga cacagaaaac aacattaaca 780
acaagtgagt ctttgcagaa gacaaactaa tcacatcaga tcaacagcag acagacacatc 840
ttgatcattg cctgctgtgt gggatttggg ctaatgggtt tattgtttct ggcgtttctc 900
cttcgagggg aagtcacagg agccaactgt ttgcagagac acaaggagcc agacaacact 960
gaagatagtg acagcgtctc caatgacatg tcacacggga gggatgatga agacggggtc 1020
ttcactctct ga 1032

```

```

<210> 15
<211> 345
<212> PRT
<213> Mus musculus

```

```

<220>
<221> VARIANT
<222> (1)...(345)
<223> C.D2 ES-HBA and DBA/2J allele

```

```

<400> 15
Met Ser Lys Gly Leu Leu Leu Trp Leu Val Met Glu Leu Trp Trp
1 5 10 15
Leu Tyr Leu Ser Lys Ser Pro Ala Ala Ser Glu Asp Thr Ile Ile Gly
20 25 30
Phe Leu Gly Gln Pro Val Thr Leu Pro Cys His Tyr Leu Ser Trp Ser
35 40 45
Gln Ser Arg Asn Ser Met Cys Trp Gly Lys Gly Ser Cys Pro Asn Ser
50 55 60
Lys Cys Asn Ala Glu Leu Leu Arg Thr Asp Gly Thr Arg Ile Ile Ser
65 70 75 80
Arg Lys Ser Thr Lys Tyr Thr Leu Leu Gly Lys Val Gln Phe Gly Glu
85 90 95
Val Ser Leu Thr Ile Ser Asn Thr Asn Arg Gly Asp Ser Gly Val Tyr
100 105 110
Cys Cys Arg Ile Glu Val Pro Gly Trp Phe Asn Asp Val Lys Lys Asn
115 120 125
Val Arg Leu Glu Leu Arg Arg Ala Thr Thr Thr Lys Lys Pro Thr Thr
130 135 140
Thr Thr Arg Pro Thr Thr Pro Tyr Val Thr Thr Thr Thr Pro Glu
145 150 155 160
Leu Leu Pro Thr Thr Val Met Thr Thr Ser Val Leu Pro Thr Thr Thr
165 170 175
Pro Pro Gln Thr Leu Ala Thr Ala Phe Ser Thr Ala Val Thr Thr
180 185 190
Cys Pro Ser Thr Thr Pro Gly Ser Phe Ser Gln Glu Thr Thr Lys Gly
195 200 205
Ser Ala Phe Thr Thr Glu Ser Glu Thr Leu Pro Ala Ser Asn His Ser
210 215 220
Gln Arg Ser Met Met Thr Ile Ser Thr Asp Ile Ala Val Leu Arg Pro
225 230 235 240
Thr Gly Ser Asn Pro Gly Ile Leu Pro Ser Thr Ser Gln Leu Thr Thr

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245 11/25 255
 Gln Lys Thr Thr Leu Thr Thr Ser Glu Ser Leu Gln Lys Thr Thr Lys
 260 265 270
 Ser His Gln Ile Asn Ser Arg Gln Thr Ile Leu Ile Ala Cys Cys
 275 280 285
 Val Gly Phe Val Leu Met Val Leu Leu Phe Leu Ala Phe Leu Leu Arg
 290 295 300
 Gly Lys Val Thr Gly Ala Asn Cys Leu Gln Arg His Lys Arg Pro Asp
 305 310 315 320
 Asn Thr Glu Val Ser Asp Ser Phe Leu Asn Asp Ile Ser His Gly Arg
 325 330 335
 Asp Asp Glu Asp Gly Ile Phe Thr Leu
 340 345

<210> 16
 <211> 1032
 <212> DNA
 <213> Mus musculus

<400> 16
 atgtccagg ggtctctctt cctctggctg gtgatggagc tctggtggct tttatctgaca 60
 ccagctgctt cagaggatac aataataggg tttttgggac agccggtgac ttgtgcttgt 120
 catiaacctt cgtggtccca gagccgcaac agtatgtgct ggggcaaaag ttcatgtccc 180
 aattccaaat gcaatgcaga gcttctccgt acagatggaa caagaatcat ctccaggaa 240
 tcaacaaaat ataacatttt ggggaaggtc cagtttgggt aagtgtctct gaccattcca 300
 aacacnaatc gaggtgacag tggggtgtac tgcgcgcgt tagaggtgct tggctgggtc 360
 aatgatgtca agaagaatgt ggcgttggag ctgaggagag ccacaacaac caaaaaacca 420
 caacaacca cccggccaac caccacctt tatgttaaca ccaccacccc agagctgctt 480
 caacaacag tcaatgaccac atctgttctt ccaaccacca caccaccccc gacactagct 540
 accactgctt tcaatgaccac agtgaccacg tgccctccaa caaaccttgg ctcccttcca 600
 caagaacca caaaagggtc cgccttccat acagaatcag aaactctgct tgcattccat 660
 cacttcaaaa gaagcatgat gaccatatct acagacatag ccgtactcag gccacaggc 720
 tctaacctct ggattctccc atccattcca cagctgacga cacagaaaaa aacattaaca 780
 acaagtgaat otttgcagaa gacaactaaa tcaactcaga tcaacagcag acagaccatc 840
 ttgatcattg cctgtgtggt gggatttggg ctcaatgagt taatgtttct ggcgtttctc 900
 ctccagggg aagtcacagg agccaactgt ttgcagagac acagaggccc agcaaacact 960
 gaagatagtg acagcgtctt caatgacatg tcaacgggga gggatgatga agacgggac 1020
 ttaactctct ga 1032

<210> 17
 <211> 359
 <212> PRT
 <213> H. sapiens

<220>
 <221> VARIANT
 <222> (1)...(360)
 <223> TIM-1 allele 1

<400> 17
 Met His Pro Gln Val Val Ile Leu Ser Leu Ile Leu His Leu Ala Asp
 1 5 10 15
 Ser Val Ala Gly Ser Val Lys Val Gly Gly Glu Ala Gly Pro Ser Val
 20 25 30
 Thr Leu Pro Cys His Tyr Ser Gly Ala Val Thr Ser Met Cys Trp Asn
 35 40 45
 Arg Gly Ser Cys Ser Leu Phe Thr Cys Gln Asn Gly Ile Val Trp Thr
 50 55 60
 Asn Gly Thr His Val Thr Tyr Arg Lys Asp Thr Arg Tyr Lys Leu Leu
 65 70 75 80
 Gly Asp Leu Ser Arg Arg Asp Val Ser Leu Thr Ile Glu Asn Thr Ala

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      85      12/25      95
Val Ser Asp Ser Gly Val Tyr Cys Cys Arg Val Glu His Arg Gly Trp
      100      105      110
Phe Asn Asp Met Lys Ile Thr Val Ser Leu Glu Ile Val Pro Pro Lys
      115      120      125
Val Thr Thr Thr Pro Ile Val Thr Thr Val Pro Thr Val Thr Thr Val
      130      135      140
Arg Thr Ser Thr Thr Val Pro Thr Thr Thr Thr Val Pro Thr Thr Thr
      145      150      155      160
Val Pro Thr Thr Met Ser Ile Pro Thr Thr Thr Thr Val Pro Thr Thr
      165      170      175
Met Thr Val Ser Thr Thr Thr Ser Val Pro Thr Thr Thr Ser Ile Pro
      180      185      190
Thr Thr Thr Ser Val Pro Val Thr Thr Thr Val Ser Thr Phe Val Pro
      195      200      205
Pro Met Pro Leu Pro Arg Gln Asn His Glu Pro Val Ala Thr Ser Pro
      210      215      220
Ser Ser Pro Gln Pro Ala Glu Thr His Pro Thr Thr Leu Gln Gly Ala
      225      230      235
Ile Arg Arg Glu Pro Thr Ser Ser Pro Leu Tyr Ser Tyr Thr Thr Asp
      240      245      250      255
Gly Asn Asp Thr Val Thr Glu Ser Ser Asp Gly Leu Trp Asn Asn Asn
      260      265      270
Gln Thr Gln Leu Phe Leu Glu His Ser Leu Leu Thr Ala Asn Thr Thr
      275      280      285
Lys Gly Ile Tyr Ala Gly Val Cys Ile Ser Val Leu Val Leu Leu Ala
      290      295      300
Leu Leu Gly Val Ile Ile Ala Lys Lys Tyr Phe Phe Lys Lys Glu Val
      305      310      315      320
Gln Gln Leu Ser Val Ser Phe Ser Ser Leu Gln Ile Lys Ala Leu Gln
      325      330      335
Asn Ala Val Glu Lys Glu Val Gln Ala Glu Asp Asn Ile Tyr Ile Glu
      340      345      350
Asn Ser Leu Tyr Ala Thr Asp
      355

```

<210> 18
 <211> 1080
 <212> DNA
 <213> H. sapiens

```

<400> 18
atgcatactc aagtgggtcat cttaagcctc atcctacatc tggcagattc tgtagctggt 60
tctgtaaagg ttggtggaga ggcagggtcca tctgtcacac taccctgcc a ctacagtggg 120
gtctgcacat caatgtgctg gaatagaggc tcatgttctc taitcacatg ccaaaatggc 180
attgtctgga caatgggac ccacgtcacc tctcggaggg acacacgcta taagctattg 240
ggggacottt caagaaggga tgtctctctg accatagaaa atacagctgt gtctgacagt 300
ggcgtatatt gttgcctgtt ttagcacctg ggtggttcca atgacatgaa aatcacogta 360
tcattggaga ttgcgccacc caaggtcacg actactccaa ttgtccaaac tgttccaaac 420
gtcacgactg ttogaacgag caccactgtt ccaacgacaa cgactgttcc aacgacaaac 480
gttccaaacaa caatgagcat tccaacgaca acgactgttc cgacgacaat gactgtttca 540
acgacaacga gcgttccaac gacaacgagc attccaacaa caacaagtgt tccagtgcac 600
acaacgctct ctacotttgt tctccaatg cotttgcoca ggcagaacca tgaaccagta 660
gccacttcac catcttcacc tccagccagca gaaacccacc ctacgacact gcaggagaca 720
ataaggagag aacccaccag ctccaccatt tactcttaca caacagatgg gaatgacacc 780
gtgcagaggt cttcagatgg cctttggaat aacaatcaaa ctcaactgtt cctagaacat 840
agtctactga cggccaatcc cactaaagga atctatgctg gagtctgtat ttctgtcttg 900
gtgttctctg ctcttttggg tgtcatcatt gccaaaaaag attttctcaa aaaggaggtt 960
caacaactaa gtgtttcatt tagcagcctt caaattaaag ctttgcacaa tgcagttgaa 1020
aaggaagtc aagcagaaga caatatctac attgagaata gtctttaigc caggactaa 1080

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<210> 19
 <211> 359
 <212> PRT
 <213> H. sapiens

<220>
 <221> VARIANT
 <222> (1)...(359)
 <223> TIM-1, allele 2

<400> 19
 Met His Pro Gln Val Val Ile Leu Ser Leu Ile Leu His Leu Ala Asp
 1 5 10 15
 Ser Val Ala Gly Ser Val Lys Val Gly Gly Glu Ala Gly Pro Ser Val
 20 25 30
 Thr Leu Pro Cys His Tyr Ser Gly Ala Val Thr Ser Met Cys Trp Asn
 35 40 45
 Arg Gly Ser Cys Ser Leu Phe Thr Cys Gln Asn Gly Ile Val Trp Thr
 50 55 60
 Asn Gly Thr His Val Thr Tyr Arg Lys Asp Thr Arg Tyr Lys Leu Leu
 65 70 75 80
 Gly Asp Leu Ser Arg Arg Asp Val Ser Leu Thr Ile Glu Asn Thr Ala
 85 90 95
 Val Ser Asp Ser Gly Val Tyr Cys Cys Arg Val Glu His Arg Gly Trp
 100 105 110
 Phe Asn Asp Met Lys Ile Thr Val Ser Leu Glu Ile Val Pro Pro Lys
 115 120 125
 Val Thr Thr Thr Pro Ile Val Thr Thr Val Pro Thr Val Thr Thr Val
 130 135 140
 Arg Thr Ser Thr Thr Val Pro Thr Thr Thr Thr Val Pro Thr Thr Thr
 145 150 155 160
 Val Pro Thr Thr Met Ser Ile Pro Thr Thr Thr Thr Val Pro Thr Thr
 165 170 175
 Met Thr Val Ser Thr Thr Thr Ser Val Pro Thr Thr Thr Ser Ile Pro
 180 185 190
 Thr Thr Thr Ser Val Pro Val Thr Thr Ala Val Ser Thr Phe Val Pro
 195 200 205
 Pro Met Pro Leu Pro Arg Gln Asn His Glu Pro Val Ala Thr Ser Pro
 210 215 220
 Ser Ser Pro Gln Pro Ala Glu Thr His Pro Thr Thr Leu Gln Gly Ala
 225 230 235 240
 Ile Arg Arg Glu Pro Thr Ser Ser Pro Leu Tyr Ser Tyr Thr Thr Asp
 245 250 255
 Gly Asn Asp Thr Val Thr Glu Ser Ser Asp Gly Leu Trp Asn Asn Asn
 260 265 270
 Gln Thr Gln Leu Phe Leu Glu His Ser Leu Leu Thr Ala Asn Thr Thr
 275 280 285
 Lys Gly Ile Tyr Ala Gly Val Cys Ile Ser Val Leu Val Leu Leu Ala
 290 295 300
 Leu Leu Gly Val Ile Ile Ala Lys Lys Tyr Phe Phe Lys Lys Glu Val
 305 310 315 320
 Gln Gln Leu Ser Val Ser Phe Ser Ser Leu Gln Ile Lys Ala Leu Gln
 325 330 335
 Asn Ala Val Glu Lys Glu Val Gln Ala Glu Asp Asn Ile Tyr Ile Glu
 340 345 350
 Asn Ser Leu Tyr Ala Thr Asp
 355

<210> 20
 <211> 1080

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<212> DNA

<213> H. sapiens

<400> 20

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atgcacatcc  aagtggtcat  cttangcctc  atccatcac  tggcagattc  tctagctggt  60
tctgtasagg  ttggtggaga  ggcaggtcca  tctgtccac  taccctggca  ctacagtgg  120
gtgtgtacac  caatgtgttg  gaataggagc  tcatgtcttc  tattcacatg  ccaaatggc  180
attgtctggg  caatgggaa  caacgtcaac  tatcggagg  acacacgcta  taagtattg  240
gggacatttc  caagaaggga  tctctctttg  accatagaaa  atacagctgt  gtctgacagt  300
ggcgtatatt  gttgcctgt  tgagccacgt  ggggtggtta  atgacatgaa  aatcaccgta  360
tcattggaga  ttgtgccacc  caagtcacg  actactccaa  ttgtcacac  tgttccacc  420
gtccacacgt  ttggaacgag  caccactgtt  ccaacgacaa  cgactgttcc  aacgacaaot  480
gttccacaaa  caatgagcat  tccaacgaca  acgactgttc  cgacgacaat  gactgtttca  540
acgacaacga  gcgttccaac  gacaacgagc  atttccacaa  caacaagtgt  tccagtgcac  600
acagcggtct  ctacttttgt  tcttccaatg  cttttgccca  ggcagaacca  tgaaccagta  660
ggcacttcac  catcttccac  tcagccagca  gaaacccacc  ctacgacact  gcaggagaca  720
ataaggagag  aacccaccag  ctccaccatt  tactcttaca  caacagatgg  gaatgacacc  780
gtgacagagt  cttcagatgg  cttttggant  aacaatcaaa  ctcaactgtt  cctagaacat  840
agtctactga  cggccaatac  caataaagga  atctatgttg  gactctgtat  tctgtctctg  900
gtgttttttg  cttttttggg  tgcatacatt  gccaaaaagt  atttttccaa  aaggaggtt  960
caacaaactaa  gtgtttcatt  tagcagcctt  caattataag  ctttgcanaa  tgcagttgaa  1020
aaggaagtc  aagcagaaga  caatatctac  attgagaata  gtctttatgc  caccgactaa  1080

```

<210> 21

<211> 365

<212> PRT

<213> H. sapiens

<220>

<221> VARIANT

<222> (1)... (365)

<223> TIM-1, allele 3

<400> 21

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Met His Pro Gln Val Val Ile Leu Ser Leu Ile Leu His Leu Ala Asp
1          5          10          15
Ser Val Ala Gly Ser Val Lys Val Gly Gly Glu Ala Gly Pro Ser Val
20          25          30
Thr Leu Pro Cys His Tyr Ser Gly Ala Val Thr Ser Met Cys Trp Asn
35          40          45
Arg Gly Ser Cys Ser Leu Phe Thr Cys Gln Asn Gly Ile Val Trp Thr
50          55          60
Asn Gly Thr His Val Thr Tyr Arg Lys Asp Thr Arg Tyr Lys Leu Leu
65          70          75
Gly Asp Leu Ser Arg Arg Asp Val Ser Leu Thr Ile Glu Asn Thr Ala
80          85          90
Val Ser Asp Ser Gly Val Tyr Cys Cys Arg Val Glu His Arg Gly Trp
100          105          110
Phe Asn Asp Met Lys Ile Thr Val Ser Leu Glu Ile Val Pro Pro Lys
115          120          125
Val Thr Thr Thr Pro Ile Val Thr Thr Val Pro Thr Val Thr Thr Val
130          135          140
Arg Thr Ser Thr Thr Val Pro Thr Thr Thr Thr Val Pro Met Thr Thr
145          150          155          160
Thr Val Pro Thr Thr Thr Val Pro Thr Thr Met Ser Ile Pro Thr Thr
165          170          175
Thr Thr Val Pro Thr Thr Met Thr Val Ser Thr Thr Thr Ser Val Pro
180          185          190
Thr Thr Thr Ser Ile Pro Thr Thr Thr Ser Val Pro Val Thr Thr Ala
195          200          205
Val Ser Thr Phe Val Pro Pro Met Pro Leu Pro Arg Gln Asn His Glu

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210          215          220
Pro Val Ala Thr Ser Pro Ser Ser Pro Gln Pro Ala Glu Thr His Pro
225          230          235
Thr Thr Leu Gln Gly Ala Ile Arg Arg Glu Pro Thr Ser Ser Pro Leu
245          250          255
Tyr Ser Tyr Thr Thr Asp Gly Asn Asp Thr Val Thr Glu Ser Ser Asp
260          265          270
Gly Leu Trp Asn Asn Asn Gln Thr Gln Leu Phe Leu Glu His Ser Leu
275          280          285
Leu Thr Ala Asn Thr Thr Lys Gly Ile Tyr Ala Gly Val Cys Ile Ser
290          295          300
Val Leu Val Leu Leu Ala Leu Leu Gly Val Ile Ile Ala Lys Lys Tyr
305          310          315
Phe Phe Lys Lys Glu Val Gln Gln Leu Ser Val Ser Phe Ser Ser Leu
325          330          335
Gln Ile Lys Ala Leu Gln Asn Ala Val Glu Lys Glu Val Gln Ala Glu
340          345          350
Asp Asn Ile Tyr Ile Glu Asn Ser Leu Tyr Ala Thr Asp
355          360          365

```

<210> 22

<211> 1098

<212> DNA

<213> H. sapiens

<400> 22

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atgcatcttc aagtggatc cttaagcttc atctacatc tggcagatc ttagctggg 60
tcgttaagg ttgggggaga ggcaggtcca tctgtcacac taccctgcca ctacagtgg 120
gtgtcacat caatgtgctg gaatagaggc tcatgttttc tattcacatg ccaaatggc 180
attgtctgga ccaatgggac ccacgtcacc tatcggaagg acacacgtta taagctattg 240
ggggaccttt caagaaggga tgtctctttg accatagaaa atacagctgt gtctgacagt 300
ggcgtatatt gtgcgcgigt ttagcacagt ggtgtgttca atgacatgaa aatcacctga 360
tcattggaga ttgtgcccac caaggtcacg actactccaa ttgtcacacc tgttccacc 420
gtcacgacg ttggaacag ccaactgtgt ccaacgacaa cgactgttcc aatgaacag 480
actgttccaa cgacaactgt tccaacaaca atgagcattc caacgacaa gactgttcc 540
acgacaatga ctgtttccaa gacaacgagc gtccaacga caacgagcat tccaacaaca 600
acnagtgttc cagtacaaac arogtctctc acctttgttc ctccaatgcc ttgtcccagg 660
cagaacatgc aaccagtgc cacttcacca tcttcacctc agccacgaga aacccacct 720
acgacactgc agggagcaat aaggagagaa cccaccagct caccattgta ctcttacaca 780
acagatggga atgacacctg gacagagtct tccagtggcc ttgggaataa caatcaaac 840
caactgttcc tagaacatag tctactgacg gccaatacca ctaaaggaat ctatgctgga 900
gtctgtattt ctgtcttggt gcttcttgct cttttgggtg tcatcattgc caaaaagtat 960
ttcttcaaaa agggagttca acaactaagt gtttcattta gcagccttca aattaaagt 1020
ttgcanaatg cagttgaaaa ggaagtccaa gcagaagaca atatctacat tgagaatagt 1080
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1098

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<210> 23

<211> 359

<212> PRT

<213> H. sapiens

<220>

<221> VARIANT

<222> (1)...(359)

<223> TIM-1, allele 4

<400> 23

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Met His Pro Gln Val Val Ile Leu Ser Leu Ile Leu His Leu Ala Asp
1          5          10          15
Ser Val Ala Gly Ser Val Lys Val Gly Gly Glu Ala Gly Pro Ser Val
20          25          30

```

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Thr Leu Pro Cys His Tyr Ser Gly Ala Val Thr Ser Met Cys Trp Asn
 35 40 45
 Arg Gly Ser Cys Ser Leu Phe Thr Cys Gln Asn Gly Ile Val Trp Thr
 50 55 60
 Asn Gly Thr His Val Thr Tyr Arg Lys Asp Thr Arg Tyr Lys Leu Leu
 65 70 75 80
 Gly Asp Leu Ser Arg Arg Asp Val Ser Leu Thr Ile Glu Asn Thr Ala
 85 90 95
 Val Ser Asp Ser Gly Val Tyr Cys Cys Arg Val Glu His Arg Gly Trp
 100 105 110
 Phe Asn Asp Met Lys Ile Thr Val Ser Leu Glu Ile Val Pro Pro Lys
 115 120 125
 Val Thr Thr Thr Pro Ile Val Thr Thr Val Pro Thr Val Thr Thr Val
 130 135 140
 Arg Thr Ser Thr Thr Val Pro Thr Thr Thr Thr Val Pro Thr Thr Thr
 145 150 155 160
 Val Pro Thr Thr Met Ser Ile Pro Thr Thr Thr Thr Val Pro Thr Thr Thr
 165 170 175
 Met Thr Val Ser Thr Thr Thr Ser Val Pro Thr Thr Thr Ser Ile Pro
 180 185 190
 Thr Thr Thr Ser Val Pro Val Thr Thr Ser Val Ser Thr Phe Val Pro
 195 200 205
 Pro Met Pro Leu Pro Arg Gln Asn His Glu Pro Val Ala Thr Ser Pro
 210 215 220
 Ser Ser Pro Gln Pro Ala Glu Thr His Pro Thr Thr Leu Gln Gly Thr
 225 230 235 240
 Ile Arg Arg Glu Pro Thr Ser Ser Pro Leu Tyr Ser Tyr Thr Thr Asp
 245 250 255
 Gly Asn Asp Thr Val Thr Glu Ser Ser Asp Gly Leu Trp Ser Asn Asn
 260 265 270
 Gln Thr Gln Leu Phe Leu Glu His Ser Leu Leu Thr Ala Asn Thr Thr
 275 280 285
 Lys Gly Ile Tyr Ala Gly Val Cys Ile Ser Val Leu Val Leu Leu Ala
 290 295 300
 Leu Leu Gly Val Ile Ile Ala Lys Lys Tyr Phe Phe Lys Lys Glu Val
 305 310 315 320
 Gln Gln Leu Ser Val Ser Phe Ser Ser Leu Gln Ile Lys Ala Leu Gln
 325 330 335
 Asn Ala Val Glu Lys Glu Val Gln Ala Glu Asp Asn Ile Tyr Ile Glu
 340 345 350
 Asn Ser Leu Tyr Ala Thr Asp
 355

<210> 24

<211> 1079

<212> DNA

<213> H. sapiens

<400> 24

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gctgtcacat caatgtgctg gaatagaggc tcatgtcttc tattcacatg ccaaaatggc 180
attgtctgga ccaatgggac ccacgtcacc tatcggaagg acacacgcta taagctattg 240
ggggaaccttt caagaaggga tgtctctttg accatagaaa atacagctgt gctcgacagt 300
ggcgatatatt gttgccgtgt ttagcaccgt ggggtggttc atgacatgaa aatcaccgta 360
tcattggaga ttgtgccacc caaggtcacc actactccaa ttgtcacaaac tgttccaacc 420
gtcacgactg ttgcgaacgag caccactgtt ccaacgacaa cgaactgttcc aacgacaact 480
gttccaaacaa caatgagcat tccaacgaca acggaactgt ccgacgacaa tgaactgttc 540
aacgacaacg agcgtttccaa cyacaacgag catbccaaca acacacagtg ttccagtgac 600
aacatgtctc taccattgtt cttccaatgc ctttgcccag gcagacacat gaaccagtag 660
ccacttcaac atcttcacct cagccagcag aaacccaacc taagacactg cagggagcaa 720

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 taaggagaga acccaccagc tcaccattgt actcttacac aacagatggg aatgacacg 780
 tgcagagctc ttcagatggc ctttggarta acaatcaaac tcaactgttc ctagaacata 840
 gtctactgac ggccaatacc actaaaggaa tctatgctgg agtctgtatt tctgtcttgg 900
 tgcctcttgc tcttttgggt gtcacattg ccaaanagta tttcttcaaa aaggaggttc 960
 aaccaactag tgttttcatt agcagccttc aaattanagc ttgcaaaat gcagttgaaa 1020
 aggaagtcca agcagaagac aatatctaca ttgagaatag tctttatgac acggactaa 1079

<210> 25
 <211> 364
 <212> PR1
 <213> H. sapiens

<220>
 <221> VARIANT
 <222> (1)...(364)
 <223> TIM-1 allele 5

<400> 25
 Met His Pro Gln Val Val Ile Leu Ser Leu Ile Leu His Leu Ala Asp
 1 5 10 15
 Ser Val Ala Gly Ser Val Lys Val Gly Glu Ala Gly Pro Ser Val
 20 25 30
 Thr Leu Pro Cys His Tyr Ser Gly Ala Val Thr Ser Met Cys Trp Asn
 35 40 45
 Arg Gly Ser Cys Ser Leu Phe Thr Cys Gln Asn Gly Ile Val Trp Thr
 50 55 60
 Asn Gly Thr His Val Thr Tyr Arg Lys Asp Thr Arg Tyr Lys Leu Leu
 65 70 75 80
 Gly Asp Leu Ser Arg Arg Asp Val Ser Leu Thr Ile Glu Asn Thr Ala
 85 90 95
 Val Ser Asp Ser Gly Val Tyr Cys Cys Arg Val Glu His Arg Gly Trp
 100 105 110
 Phe Asn Asp Met Lys Ile Thr Val Ser Leu Glu Ile Val Pro Pro Lys
 115 120 125
 Val Thr Thr Thr Pro Ile Val Thr Thr Val Pro Thr Val Thr Thr Val
 130 135 140
 Arg Thr Ser Thr Thr Val Pro Thr Thr Thr Val Pro Met Thr Thr
 145 150 155 160
 Thr Val Pro Thr Thr Thr Val Pro Thr Met Ser Ile Pro Thr Thr
 165 170 175
 Thr Thr Val Pro Thr Thr Met Thr Val Ser Thr Thr Thr Ser Val Pro
 180 185 190
 Thr Thr Thr Ser Ile Pro Thr Thr Ser Val Pro Val Thr Thr Thr Val
 195 200 205
 Ser Thr Phe Val Pro Pro Met Pro Leu Pro Arg Gln Asn His Glu Pro
 210 215 220
 Val Ala Thr Ser Pro Ser Ser Pro Gln Pro Ala Glu Thr His Pro Thr
 225 230 235 240
 Thr Leu Gln Gly Ala Ile Arg Arg Glu Pro Thr Ser Ser Pro Leu Tyr
 245 250 255
 Ser Tyr Thr Thr Asp Gly Asn Asp Thr Val Thr Glu Ser Ser Asp Gly
 260 265 270
 Leu Trp Asn Asn Asn Gln Thr Gln Leu Phe Leu Glu His Ser Leu Leu
 275 280 285
 Thr Ala Asn Thr Thr Lys Gly Ile Tyr Ala Gly Val Cys Ile Ser Val
 290 295 300
 Leu Val Leu Leu Ala Leu Leu Gly Val Ile Ile Ala Lys Lys Tyr Phe
 305 310 315 320
 Phe Lys Lys Glu Val Gln Gln Leu Ser Val Ser Phe Ser Ser Leu Gln
 325 330 335
 Ile Lys Ala Leu Gln Asn Ala Val Glu Lys Glu Val Gln Ala Glu Asp
 340 345 350

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Asn Ile Tyr Ile Glu Asn Ser Leu Tyr Ala Thr Asp
 355 360

<210> 26

<211> 1095

<212> DNA

<213> H. sapiens

<400> 26

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totgtaaagg ttggtggaga ggcaggtcca tctgtcacac taccctgcca ctacagtggg 120
gctgtcacat caatgtgctg gaatagaggc toatgttctc tattcacatg ccaaaatggc 180
attgtctcga ccaatgggaa ccacgtcacc tatcggaagg acacacgcta taagctattg 240
ggggaccttt caagaaggga tgtctctttg accatagaaa atacagctgt gtctgacagt 300
ggcgtatatt gttggcgtgt tggacacgtt ggttggttca atgacatgaa aatcacgta 360
tcattggaga ttgtgccacc caaggtcacc actactccaa ttgtcacaa tggtccaaac 420
gtcacgactg ttgaacgag caccactgtt ccaacgacaa cgactgttcc aatgacaacg 480
actgttccaa cgcacaactg tccaacaaca atgagcattc caacgacaa gactgttccg 540
acgacaatga ctgtttcacc gacaacgagc gtctccacga ccaacgacat tccaacaaca 600
agtgttccag tgacaacaac ggtctctacc ttgttctcct caatgccttt gccacggcag 660
aaccatgaac cagttagccac ttacacatct tcacttcacc cagcagaaac ccacctacg 720
acactggcgg gagcaataag gagagaaccc accagctcac cattgtactc ttacacaaca 780
gatgggaatg acaccgtgac agagtcttca gatgccttt ggaataacaa tcaaaactca 840
ctgttctctag aacatagtct actgacggcc aataccacta aaggaatcta tgctggagtc 900
tgtattctct tcttggtgct tcttgctctt ttgggtgtca toattgccaa aaagtatttc 960
ttcaaaaagg aggttcaaca actaagtggt tcatttagca gccttcaaat taaagctttg 1020
caaatgcag ttgaaaagga agtccaagca gaagacaata tctacattga gaatagtctt 1080
tatgccacgg actaa 1095

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<210> 27

<211> 364

<212> PRT

<213> H. sapiens

<220>

<221> VARIANT

<222> (1)...(364)

<223> TIM-1, allele 6

<400> 27

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Met His Pro Gln Val Val Ile Leu Ser Leu Ile Leu His Leu Ala Asp
1 5 10 15
Ser Val Ala Gly Ser Val Lys Val Gly Gly Glu Ala Gly Pro Ser Val
20 25 30
Thr Leu Pro Cys His Tyr Ser Gly Ala Val Thr Ser Met Cys Trp Asn
35 40 45
Arg Gly Ser Cys Ser Leu Phe Thr Cys Gln Asn Gly Ile Val Trp Thr
50 55 60
Asn Gly Thr His Val Thr Tyr Arg Lys Asp Thr Arg Tyr Lys Leu Leu
65 70 75 80
Gly Asp Leu Ser Arg Arg Asp Val Ser Leu Thr Ile Glu Asn Thr Ala
85 90 95
Val Ser Asp Ser Gly Val Tyr Cys Cys Arg Val Glu His Arg Gly Trp
100 105 110
Phe Asn Asp Met Lys Ile Thr Val Ser Leu Gly Ile Val Pro Pro Lys
115 120 125
Val Thr Thr Thr Pro Ile Val Thr Thr Val Pro Thr Val Thr Thr Val
130 135 140
Arg Thr Ser Thr Thr Val Pro Thr Thr Thr Thr Val Pro Met Thr Thr
145 150 155 160
Thr Val Pro Thr Thr Thr Val Pro Thr Thr Met Ser Ile Pro Thr Thr

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	165	170	175
Thr Thr Val	Pro Thr Thr Met Thr	Val Ser Thr Thr Thr	Ser Val Pro
180	185	190	
Thr Thr Thr	Ser Ile Pro Thr Thr	Ser Val Pro Val Thr Thr	Val
195	200	205	
Ser Thr Phe	Val Pro Pro Met Pro	Leu Pro Arg Gln Asn His	Glu Pro
210	215	220	
Val Ala Thr	Ser Pro Ser Ser Pro	Gln Pro Ala Glu Thr His	Pro Thr
225	230	235	240
Thr Leu Gln	Gly Ala Ile Arg Arg	Glu Pro Thr Ser Ser Pro	Leu Tyr
245	250	255	
Ser Tyr Thr	Thr Asp Gly Asp Asp	Thr Val Thr Glu Ser Ser	Asp Gly
260	265	270	
Leu Trp Asn	Asn Asn Gln Thr Gln	Leu Phe Leu Glu His	Ser Leu Leu
275	280	285	
Thr Ala Asn	Thr Thr Lys Gly Ile Tyr	Ala Gly Val Cys Ile	Ser Val
290	295	300	
Leu Val Leu	Leu Ala Leu Leu Gly	Val Ile Ile Ala Lys Lys	Tyr Phe
305	310	315	320
Phe Lys Lys	Glu Val Gln Gln Leu	Ser Val Ser Phe Ser Ser	Leu Gln
325	330	335	
Ile Lys Ala	Leu Gln Asn Ala Val	Glu Lys Glu Val Gln Ala	Glu Asp
340	345	350	
Asn Ile Tyr	Ile Glu Asn Ser Leu	Tyr Ala Thr Asp	
355	360		

<210> 28

<211> 1099

<212> DNA

<213> H. sapiens

<400> 28

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gcgtgcacacat caatgtgctg gaatagaggc tcatgtcttc tattcacatg ccaaaatggc 180
atgtcttgga ccaatggaac ccacgtcacc tatcggaagg acacacgcta taagctattg 240
ggggaccttt caagaaggga tgtctctttg accatagaaa atacacgtgt gtctgacagt 300
ggcgctatatt gttcccggtt tgagcacctg ggggtggtca atgacatgaa aatcaccgta 360
tcattggaga ttgtgccacc caaggtcacg actactccaa ttgtcacaa tggtccaacc 420
gtcacgactg ttogaacagag caccactgtt ccaacgacaa cgactgttcc aatgacaacc 480
gactgttcca acgacaactg ttccacaac aatgagcatt ccaacgacaa cgactgttcc 540
gactgacaatg actgtttcaa cgacaacgag cgttccaacg acaacgagca ttccaacaac 600
aacaagtgtt ccagtgacaa caacggtctc tacctttgtt cctccaatgc ctttgccag 660
gcagaacctat gaaccagtag coacttcacc atcttcacct cagccagcag aaacccacc 720
tacgacactg caggagacaa taaggagaga acccaccago tcaccattgt actcttacac 780
aacagatgga gatgacacag tgacagagtc ttccagatgg clttggaata acatcacac 840
tcacactgttc ctagaacata gtctactgac ggcacatacc actaaagaa tctatgtgg 900
agtctgtatc tctgtcttgg tgcctcttgc tcttttgggt gtcatacttg ccaaaaagta 960
ttcttccaaa aaggaggttc aacacctaag tgtttctatt gcgcgccttc aaattaaagc 1020
tttgcanaat gcagttgaaa aggaagtcca agcagaagac aatatctaca ttgagaatag 1080
tccttatgcc acgactaa 1099

```

<210> 29

<211> 301

<212> PRT

<213> H. sapiens

<220>

<221> VARIANT

<222> (1)...(301)

<223> TIM-3, allele 1

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```

<400> 29
Met Phe Ser His Leu Pro Phe Asp Cys Val Leu Leu Leu Leu Leu
1      5      10      15
Leu Leu Thr Arg Ser Ser Glu Val Glu Tyr Arg Ala Glu Val Gly Gln
20      25      30
Asn Ala Tyr Leu Pro Cys Phe Tyr Thr Pro Ala Ala Pro Gly Asn Leu
35      40      45
Val Pro Val Cys Trp Gly Lys Gly Ala Cys Pro Val Phe Glu Cys Gly
50      55      60
Asn Val Val Leu Arg Thr Asp Glu Arg Asp Val Asn Tyr Trp Thr Ser
65      70      75      80
Arg Tyr Trp Leu Asn Gly Asp Phe Arg Lys Gly Asp Val Ser Leu Thr
85      90      95
Ile Glu Asn Val Thr Leu Ala Asp Ser Gly Ile Tyr Cys Cys Arg Ile
100     105     110
Gln Ile Pro Gly Ile Met Asn Asp Glu Lys Phe Asn Leu Lys Leu Val
115     120     125
Ile Lys Pro Ala Lys Val Thr Pro Ala Pro Thr Arg Gln Arg Asp Phe
130     135     140
Thr Ala Ala Phe Pro Arg Met Leu Thr Thr Arg Gly His Gly Pro Ala
145     150     155     160
Glu Thr Gln Thr Leu Gly Ser Leu Pro Asp Ile Asn Leu Thr Gln Ile
165     170     175
Ser Thr Leu Ala Asn Glu Leu Arg Asp Ser Arg Leu Ala Asn Asp Leu
180     185     190
Arg Asp Ser Gly Ala Thr Ile Arg Ile Gly Ile Tyr Ile Gly Ala Gly
195     200     205
Ile Cys Ala Gly Leu Ala Leu Ala Leu Ile Phe Gly Ala Leu Ile Phe
210     215     220
Lys Trp Tyr Ser His Ser Lys Glu Lys Ile Gln Asn Leu Ser Leu Ile
225     230     235
Ser Leu Ala Asn Leu Pro Pro Ser Gly Leu Ala Asn Ala Val Ala Glu
240     245     250     255
Gly Ile Arg Ser Glu Glu Asn Ile Tyr Thr Ile Glu Glu Asn Val Tyr
260     265     270
Glu Val Glu Glu Pro Asn Glu Tyr Tyr Cys Tyr Val Ser Arg Gln
275     280     285
Gln Pro Ser Gln Pro Leu Gly Cys Arg Phe Ala Met Pro
290     295     300

```

```

<210> 30
<211> 1116
<212> DNA
<213> H. sapiens

```

```

<400> 30
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ttttcacatc ttccctttga ctgtgtctctg ctgtgtctgc tgctactact tacaaggtcc 120
tcagaagtgg aatacagagc ggaggtcgtt cagaatgcct atctgacctg cttctacacc 180
ccagccgccc caggggaacct cgtgcccgct tgctggggca aaggagcctg tctctgtttt 240
gaatgtggca acgtgtgtgt caggactgat gaaagggatg tgaattattg gacatccaga 300
tactgtgtaa atggggattt ccgcaaagga gatgtgtccc tgacctatga gaattgtact 360
ctagcagaca gtggggtcta ctgctgcctg atccaatcc caggcatant gaatgatgan 420
aaatttaacc tgaagtgggt catcaancca gccaaaggtca cccctgcacc gactctgcag 480
agagacttca ctgcagcctt tccaaggatg cttaccacca ggggacatgg cccagcagag 540
acacagacac tggggagcct cctgatata aatctaacac aaatatccac attggccaat 600
gagttacggg actctagatt ggccaatgac ttaacgggact ctggagcaac caacagaata 660
ggcactctaca tgggagcagg gactctgtgt gggctggctc tggctcttat cttcggcgct 720
ttaattttca aatgggtatt tcatagcaaa gagaagatag agatttaag cctcattcct 780
ttggccaacc tccctccctc aggattggca aatgcagtag cagagggatg togtccagaa 840

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21/25
 gaaaacatct ataccattga agagaacgta tatgaagtgg aggaacccaa tgagtattat 900
 tgctatgtca gcagcaggca gcaacccctca caacctttgg gttgtcgctt tgcgaatgca 960
 tagatccaac cacccttatt ttgagcttgg tgtttgtct tttcagaaa ctatgagctg 1020
 tgtcaacctga ctggttttgg aggtttctgt cactgctatg gacgagagtt ttaccatttt 1080
 cagaagataa tgactcacat gggaattgaa ctggga 1116

<210> 31
 <211> 301
 <212> PRT
 <213> H. sapiens
 <220>
 <221> VARIANT
 <222> (1)...(301)
 <223> TIM-3, allele 2

<400> 31
 Met Phe Ser His Leu Pro Phe Asp Cys Val Leu Leu Leu Leu Leu Leu
 1 5 10 15
 Leu Leu Thr Arg Ser Ser Glu Val Glu Tyr Arg Ala Glu Val Gly Gln
 20 25 30
 Asn Ala Tyr Leu Pro Cys Phe Tyr Thr Pro Ala Ala Pro Gly Asn Leu
 35 40 45
 Val Pro Val Cys Trp Gly Lys Gly Ala Cys Pro Val Phe Glu Cys Gly
 50 55 60
 Asn Val Val Leu Arg Thr Asp Glu Arg Asp Val Asn Tyr Trp Thr Ser
 65 70 75 80
 Arg Tyr Trp Leu Asn Gly Asp Phe Arg Lys Gly Asp Val Ser Leu Thr
 85 90 95
 Ile Glu Asn Val Thr Leu Ala Asp Ser Gly Ile Tyr Cys Cys Arg Ile
 100 105 110
 Gln Ile Pro Gly Ile Met Asn Asp Glu Lys Phe Asn Leu Lys Leu Val
 115 120 125
 Ile Lys Pro Ala Lys Val Thr Pro Ala Pro Thr Leu Gln Arg Asp Phe
 130 135 140
 Thr Ala Ala Phe Pro Arg Met Leu Thr Thr Arg Gly His Gly Pro Ala
 145 150 155 160
 Glu Thr Gln Thr Leu Gly Ser Leu Pro Asp Ile Asn Leu Thr Gln Ile
 165 170 175
 Ser Thr Leu Ala Asn Glu Leu Arg Asp Ser Arg Leu Ala Asn Asp Leu
 180 185 190
 Arg Asp Ser Gly Ala Thr Ile Arg Ile Gly Ile Tyr Ile Gly Ala Gly
 195 200 205
 Ile Cys Ala Gly Leu Ala Leu Ala Leu Ile Phe Gly Ala Leu Ile Phe
 210 215 220
 Lys Trp Tyr Ser His Ser Lys Glu Lys Ile Gln Asn Leu Ser Leu Ile
 225 230 235 240
 Ser Leu Ala Asn Leu Pro Pro Ser Gly Leu Ala Asn Ala Val Ala Glu
 245 250 255
 Gly Ile Arg Ser Glu Glu Asn Ile Tyr Thr Ile Glu Glu Asn Val Tyr
 260 265 270
 Glu Val Glu Glu Pro Asn Glu Tyr Tyr Cys Tyr Val Ser Ser Arg Gln
 275 280 285
 Gln Pro Ser Gln Pro Leu Gly Cys Arg Phe Ala Met Pro
 290 295 300

<210> 32
 <211> 1116
 <212> DNA
 <213> H. sapiens

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<400> 32
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tcggaagtggt aatacagagc ggaggtcggt cagaatgcct atctgccctg ctcttaoacc 180
ccagccgccc cagggaaacct cgtgcccgte tgctggggca aaggagcctg tctgtgtttt 240
gaatgtggca acgtggtgct caggactgat gaaagggatg tgaattattg gaaatccaga 300
tactggctaa atggggattt ccgcaaggga gatgtgtccc tgacctaga gaattgact 360
ctagagaaat gtgggatact ctgtgtcgcg atccaaatcc caggcaatat gaattgaa 420
aaatttaacc tgaagtttgt catcaaaacca gccaaagtca cccctgcacc gaetggcag 480
agagacttca ctgcagcctt tccaaggatg cttaccaaca ggggacatgy cccagcagag 540
acacagacac tggggagcct ccttgatata aatctaacac aatatccac attggccaat 600
gagttacggg actctagatt gcccaatgac ttacgggact ctggagcaac catcagaata 660
ggcatctaca tcggagcagg gatctgtgct gggctggctc tggctcttat cttcggcgct 720
ttaattttca aatggtattc tcatagcaaa gagaagatac agaatttaag cctcatctct 780
ttggccaacc tccctccttc aggatgggca aatgcagtag cagagggaaat tgcctcagaa 840
gaaacatct ataccattga aggaacgta tatgaagtgy aggagcccaa tgagtattat 900
tgctatgta gcagcaggca gcaaccccta caacctttgg gttgtogctt tgcattgcaa 960
tgatcccaac caocttattt ttgagcttgg tgttttgtct ttttcagaaa ctatgagctg 1020
tgcacactga ctggttttgg aggttctgtc cactgctatg gacagagatt ttccactttt 1080
cagaagataa tgactcacat ggggaattgaa ctggga 1116

```

<210> 33

<211> 378

<212> FRU

<213> H. sapiens

<220>

<221> VARIANT

<222> (1)... (378)

<223> TIM-4, allele 1

<400> 33

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Met Ser Lys Glu Pro Leu Ile Leu Trp Leu Met Ile Glu Phe Trp Trp
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Leu Tyr Leu Thr Pro Val Thr Ser Glu Thr Val Val Thr Glu Val Leu
20     25     30
Gly His Arg Val Thr Leu Pro Cys Leu Tyr Ser Ser Trp Ser His Asn
35     40     45
Ser Asn Ser Met Cys Trp Gly Lys Asp Gln Cys Pro Tyr Ser Gly Cys
50     55     60
Lys Glu Ala Leu Ile Arg Thr Asp Gly Met Arg Val Thr Ser Arg Lys
65     70     75     80
Ser Ala Lys Tyr Arg Leu Gln Gly Thr Ile Pro Arg Gly Asp Val Ser
85     90     95
Leu Thr Ile Leu Asn Pro Ser Glu Ser Asp Ser Gly Val Tyr Cys Cys
100    105    110
Arg Ile Glu Val Pro Gly Trp Phe Asn Asp Val Lys Ile Asn Val Arg
115    120    125
Leu Asn Leu Gln Arg Ala Ser Thr Thr Thr His Arg Thr Ala Thr Thr
130    135    140
Thr Thr Arg Arg Thr Thr Thr Thr Ser Pro Thr Thr Thr Arg Gln Met
145    150    155    160
Thr Thr Thr Pro Ala Ala Leu Pro Thr Thr Val Val Thr Thr Pro Asp
165    170    175
Leu Thr Thr Gly Thr Pro Leu Gln Met Thr Thr Ile Ala Val Phe Thr
180    185    190
Thr Ala Asn Thr Cys Leu Ser Leu Thr Pro Ser Thr Leu Pro Glu Glu
195    200    205
Ala Thr Gly Leu Leu Thr Pro Glu Pro Ser Lys Glu Gly Pro Ile Leu
210    215    220
Thr Ala Glu Ser Glu Thr Val Leu Pro Ser Asp Ser Trp Ser Ser Ala
225    230    235    240

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23/25

Glu Ser Thr Ser Ala Asp Thr Val	Leu Leu Thr Ser Lys Glu Ser Lys
245	250
Val Trp Asp Leu Pro Ser Thr Ser His Val Ser Met Trp Lys Thr Ser	
260	265
Asp Ser Val Ser Ser Pro Gln Pro Gly Ala Ser Asp Thr Ala Val Pro	
275	280
Glu Gln Asn Lys Thr Thr Lys Thr Gly Gln Met Asp Gly Ile Pro Met	
290	295
Ser Met Lys Asn Glu Met Pro Ile Ser Gln Leu Leu Met Ile Ile Ala	
305	310
Pro Ser Leu Gly Phe Val Leu Phe Ala Leu Phe Val Ala Phe Leu Leu	
325	330
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Boston, MA 02115 (US). KUCHROO, Vijay; 1249
Boylston Street, 2nd Street, Boston, MA 02115 (US).

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(74) Agent: SHERWOOD, Pamela, J.; Bozicovic, Field &
Francis, L.L.P., 200 Middlefield Road, Suite 200, Menlo
Park, CA 94025 (US).

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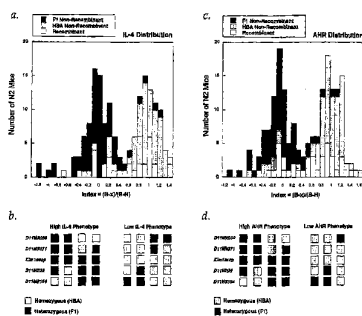
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LAND STANFORD JUNIOR UNIVERSITY [US/US];
900 Welch Road, Suite 350, Palo Alto, CA 94304 (US).

(72) Inventors: MCINTIRE, Jennifer, Jones; 1106 Clark
Way, Palo Alto, CA 94304 (US), DEKRUYFF, Rose-
marie, H.; 825 Allardice Way, Stanford, CA 94305 (US),
UMETSU, Dale, T.; 825 Allardice Way, Stanford, CA
94305 (US), FREEMAN, Gordon, J.; 44 Binney Street,
Boston, MA 02115 (US), KUCHROO, Vijay; 1249
Boylston Street, 2nd Street, Boston, MA 02115 (US).

(74) Agent: SHERWOOD, Pamela, J.; Bozicevic, Field &
Francis, L.L.P., 200 Middlefield Road, Suite 200, Menlo
Park, CA 94025 (US).

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(54) Title: T CELL REGULATORY GENES AND METHODS OF USE THEREOF

(57) Abstract: A genetic locus and corresponding family of proteins associated with regulation of immune function and cell survival are provided. These genes encode cell surface molecules with conserved IgV and mucin domains. The locus comprising the TIM family is genetically associated with immune dysfunction, including asthma. Furthermore, the TIM gene family is located within a region of human chromosome 5 that is commonly deleted in malignancies and myelodysplastic syndrome. Polymorphisms in the gene sequences are associated with the development of airway hyperactivity and allergic inflammation, and T cell production of IL-4 and IL-13. The proteins include the human hepatitis A cellular receptor, hHAVcr-1.



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T CELL REGULATORY GENES AND METHODS OF USE THEREOF

BACKGROUND OF THE INVENTION

The astonishing complexity of the immune system is its greatest strength.

5 The 10^{12} - 10^{14} possible antibody specificities, the delicate interplay between the various regulatory and effector cells, the restriction of T cell responses according to MHC antigens; all these contribute to the ability of the host to effectively react against infectious agents and other antigens perceived as foreign. But this diversity has its drawbacks. Mistakes happen: the target of a response may turn out to be a normal self protein; inflammatory
10 responses are misregulated; and normal responses are undesirably directed against grafts and transplanted cells. Under these circumstances, the complexity of the system makes diagnosis and therapy extremely difficult.

The profile of cytokines produced by $CD4^+$ T cells during an immune response determines the nature of effector functions which develop and regulates the
15 outcome of an immune response. Production of IL-2 and IFN- γ during Th1-dominated responses is associated with vigorous cell-mediated immunity, the induction of IgG2a and inhibition of IgE synthesis, and with resistance to intracellular pathogens. In contrast, the production of IL-4, IL-5 and IL-10 during Th2-dominated responses is associated with humoral immunity and protection from autoimmune pathology. Overproduction of Th2-
20 cytokines by allergen-specific $CD4^+$ T cells can result in the development of allergic disease and asthma, while Th1 cells have been associated with a variety of pro-inflammatory diseases.

One approach to immune associated diseases is immunotherapy. Immunotherapy has proven to be effective when used properly, and it is hoped that
25 advances in immunologic intervention will further improve the efficacy. Alternative approaches have attempted to use cytokines to shift the immune response. IL-12, a heterodimeric cytokine produced by macrophages and dendritic cells, is potent in driving the development of Th1 cytokine synthesis in naive and memory $CD4^+$ T cells. Other cytokines, such as IL-13 and IL-4, have been associated with the differentiation of T cells to
30 a Th2 type.

Atopy, which includes asthma, allergic rhinitis, and atopic dermatitis, is a complex trait that arises as a result of environmentally induced immune responses in genetically susceptible individuals. The prevalence of all atopic diseases has dramatically increased in industrialized countries over the past two decades. Asthma is the most
35 common chronic disease of childhood and affects more than 15 million individuals in the United States, leading to direct treatment costs exceeding \$11 billion per annum. Epidemiological studies have suggested that the increase in asthma prevalence results

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from changes in hygiene and from reduced frequency of infections (e.g., tuberculosis or hepatitis A) within industrialized society. However, the specific molecular pathways that result in the increased asthma prevalence, and the genetic polymorphisms that confer asthma susceptibility are poorly understood.

5 Expression of asthma is influenced by multiple environmental and genetic factors that interact with each other in non-additive ways, complicating the identification of asthma susceptibility genes. Asthma susceptibility has been linked to several chromosomal regions, but with resolution no better than 5-10 cM, in which there are usually hundreds of candidate genes. Moreover, because the effects of genetic variation in any single gene are
10 likely to have only modest effects in the overall pathogenesis of asthma, and because gene-gene and gene-environment interactions confound the analysis, the location of putative susceptibility genes to regions amenable to positional cloning has proven difficult to refine. Nevertheless, asthma susceptibility has been linked to chromosomes 5, 6, 11, 14, and 12. Of these, chromosome 5q23-35 has received the greatest attention because it
15 contains a large number of candidate genes(11, 12, 13, 14, 15, 16, 17, 18), including IL-9, IL-12p40, the β -adrenergic receptor, and the IL-4 cytokine cluster, which contains the genes for IL-4, IL-5, and IL-13. However, the large size of the linked region of 5q complicates its analysis, and a gene for asthma from this site has not yet been conclusively identified.

20

Related Publications

The genetic sequence of the human hepatitis virus A cellular receptor may be found in Genbank, accession number XM_011327. A related sequence is provided in Genbank, accession number BAB55044. Monney *et al.* (2002) Nature 415:436 describe
25 cell surface molecules expressed on Th1 cells. U.S. Patent nos. 5721351, US 6204371, US 6288218 relate to sequences corresponding to a mouse TIM-3 allele.

SUMMARY OF THE INVENTION

Genetic sequences of a gene family encoding polypeptides associated with
30 immune function and cell survival are provided. These genes encode cell surface molecules with conserved IgV and mucin domains, herein referred to as T cell Immunoglobulin domain and Mucin domain (TIM) proteins. The locus comprising the TIM family is genetically associated with immune dysfunction, including asthma. Furthermore, the TIM gene family is located within a region of human chromosome 5 that is commonly
35 deleted in malignancies and myelodysplastic syndrome. Polymorphisms are identified in TIM-1, TIM-3 and TIM-4, which can be associated with Th1/Th2 differentiation and airway hyperresponsiveness (AHR).

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The nucleic acid compositions are used to produce the encoded proteins, which may be employed for functional studies, as a therapeutic, and in studying associated physiological pathways. TIM specific binding agents, including nucleic acids, antibodies, and the like, are useful as diagnostics for determining genetic susceptibility to atopy and asthma and as diagnostics for assessing tumor resistance to cancer therapy. TIM blocking agents find use as therapeutics in the treatment of immune dysfunction and disorders of cell survival, including malignancies.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1a, *HBA mice produce significantly less IL-4 than do BALB/c mice.* Lymph node cells of mice immunized with 150 μ g of KLH were harvested, B-cell depleted, and cultured in cDME with 10 μ g/ml KLH. Supernatants were harvested after 96 hours and assessed for IL-4 levels by ELISA. Shown is a box plot of IL-4 levels ($n=10$ in each group), representing the full range of data, with the boxes encompassing the upper and lower quartiles, with the median of the data set shown inside each box. IL-4 levels produced by BALB/c cells and by (BALB/c x HBA) F1 cells are significantly higher than HBA IL-4 levels $P<0.0001$ (student's t-test). *b, HBA mice produce significantly less IL-13 and IL-10 than do BALB/c mice.* Data shown are the average values of cytokines produced by lymph node cell cultures from ten individual mice in each experimental group, $\pm$ S.D. HBA IL-13 and IL-10 levels were lower than either the BALB/c or (BALB/c x HBA) F1 values, $P<0.0001$. HBA IL-5 versus BALB/c, $P<0.05$. HBA IFN- γ versus BALB/c, $P<0.001$. *c, Allergen-induced airway hyperreactivity is significantly greater in BALB/c than do HBA mice.* Pulmonary airflow obstruction was measured, and data shown represent peak enhanced pause (Penh) values averaged among sensitized mice in each group at various methacholine concentrations, $\pm$ S.E.M. (BALB x HBA) F1 demonstrate BALB/c phenotypes, while (BALB x DBA) F1 mice demonstrate DBA/2-like phenotypes.

Figure 2. Regions of HBA chromosome 11 were inherited from DBA/2. HBA chromosome 11 contains two regions derived from DBA between *hba- α 2* and *es-3*, as delineated by SSLP markers. The regions of HBA chromosome 11 with DBA/2 genotypes are highlighted (in blue) in the diagram to the left. The markers (left) provide 2-5 cM resolution distal to *D11Mit230*. Where the mouse chromosome 11 has regions of highly conserved synteny with 5q23-35, additional markers were identified with informative polymorphisms between BALB/c and DBA/2, to provide 0.01-2 cM resolution in this region (right column markers). Single-stranded confirmation polymorphisms (SSCP) markers are shown in green to distinguish them from the SSLP markers, and the positions of particular genes of interest, denoted in red, are also shown. Where the arrangement of our marker map differs from the Chromosome Committee Reports and the MIT linkage map, our map

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concur with previous linkage and physical maps.

Figure 3a. *IL-4 production by N2 mice is bimodal, with peaks corresponding to F1 and HBA phenotypes.* As a means of evaluating the relative phenotypes of recombinant N2 mice in multiple experiments, we utilized an indexing function that allowed us to consolidate data from multiple experiments. A histogram shows a bimodal distribution of IL-4 index values for N2 mice with (BALB x HBA) F1, HBA homozygous, and recombinant haplotypes indicated. Distributions associated with the F1 and HBA haplotypes are distinct ($P < 0.0001$, paired Student's t-test). *b. IL-4 regulation segregates with Kim1sscp.* Recombinant N2 haplotypes were sorted by IL-4 index values into groups associated with high IL-4 phenotypes (index < 0.35) and low IL-4 phenotypes (index > 0.65). Each column of boxes represents a recombinant haplotype. Alleles for these haplotypes at loci between D11Mit269 to D11Mit154 are shaded according to genotype (dark = F1; pale = HBA). High IL-4 production segregates with four haplotypes (left), and low IL-4 production segregates with four haplotypes (right). The IL-4 phenotype is linked to *Kim1sscp*. *c. AHR of N2 mice is bimodal, with peaks corresponding to F1 and HBA phenotypes.* Index values calculated from Penh values are shown. The histogram shows a bimodal distribution of AHR index values for N2 mice. (BALB x HBA) F1, HBA homozygous, and recombinant haplotypes are indicated. Distributions associated with the F1 and HBA haplotypes are distinct ($P < 0.0001$, paired student's t-test). *d. AHR Regulatory Locus cosegregates with the IL-4 Regulatory Locus between D11Mit22 and D11Mit271.* Recombinant N2 haplotypes were sorted by AHR index values into groups associated with high AHR phenotypes (index < 0.35) and low AHR phenotypes (index > 0.65). Each column of boxes represents a recombinant haplotype. Alleles for these haplotypes at loci between D11Mit269 to D11Mit154 are shaded according to genotype (dark = F1; pale = HBA). High AHR segregates with four haplotypes (left), and low IL-4 production segregates with three haplotypes (right). The AHR regulatory locus is linked to *Kim1sscp*, between *D11Mit271* and *D11Mit22*.

Figure 4 *Mouse chromosome 11 interval containing Tapr is highly homologous to 5q33.* In order to construct a composite map around the *Tapr* locus, we integrated available information from the mouse linkage, backcross, and radiation hybrid maps and identified a region of highly conserved synteny in current maps of the human genome (Human Genome Browser v3, UCSC, March 2001). ESTs located on a physical map of mouse chromosome 11 (left) are denoted by their accession numbers and aligned by homology to genes (center), which correspond to ESTs on human chromosome 5 (right). All known genes between *KIAA0171* and *Sgd* are shown.

Figure 5a, b, c. *Identification novel TIM gene family and major polymorphisms in TIM-1 and TIM-3.* Cloning of mouse TIM-1 and mouse TIM-2. Members of a gene family.

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Sequences of the mouse TIM gene family members are shown. Shaded boxes illustrate identity between two of the mouse TIM genes. Total RNA from conA-stimulated splenocytes was reverse transcribed using Gibco Superscript II. PCR products of full length *Tim-3* cDNA were amplified, purified with Qiagen QIAquick PCR Purification reagents, and sequenced directly by Biotech Core (Mountain View, CA). PCR products for *Tim-1* and *Tim-2* cDNA were cloned into electrocompetent TOP10 cells with TOPO-XL cloning reagents (Invitrogen). Plasmids were purified with a standard alkaline lysis protocol. BALB/c and HBA plasmids were sequenced, as described. Homology of mouse TIM-1, rat KIM-1, and HAVcr-1. Identity with mouse TIM-1 is denoted by the shaded boxes.

The approximate signal site is denoted by an open, inverted triangle and the Ig domain/mucin domain boundary is shown with a filled diamond. The predicted transmembrane domains are underlined. TIM-1 and TIM-3 sequences with major polymorphisms between BALB/c and HBA TIM-1 and TIM-3 shown.

Figure 6. *Tapr Regulates CD4 T cell IL-4 and IL-13 Responses.* T cells from BALB/c DO11.10 mice produce higher levels of IL-4 and IL-13 in response to antigen than do T cells from HBA DO11.10 mice. Splenic CD4⁺ cells were isolated by positive selection with anti-CD4 magnetic beads and then cocultured with bone marrow-derived DCs and OVA. After seven days, the cells were restimulated. Supernatants were harvested after 18-24 hours of the secondary culture. Data represent mean cytokine levels detected at increasing concentrations of OVA, $\pm$ S.D. Detection of expression of TIM-1 mRNA in purified CD 4 T cells during priming and differentiation.

Figure 7. Sequence alignment of Human and Mouse TIM protein sequences.

Figure 8. Polymorphisms in human TIM-1

Figure 9. SSCP polymorphism analysis of human TIM-1.

DETAILED DESCRIPTION OF THE EMBODIMENTS

Genetic sequences associated with immune function, including susceptibility to asthma, are provided. The sequences of murine Tim-1, Tim-2, Tim-3, and Tim-4, and the human counterpart sequences, are provided herein. The sequence of major polymorphisms are also provided. Genomic sequences demonstrate that these polymorphisms, including a deletion, are true polymorphisms, not splicing variants. Variants of the TIM-1 and TIM-3 sequences are associated with susceptibility to airway hyperreactivity and allergic T cell responses, and other variants associated with protection against these responses.

The extracellular domain of TIM gene family members contains two domains, an IgV domain and a mucin domain. This Ig/mucin structure is also found in

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MAdCAM (mucosal addressin cell adhesion molecule), which contains two Ig domains and a mucin domain; however, there is only a low level of homology between TIMs and MAdCAM. A similar degree of homology is also present between TIM-1 and TOSO (NP_005440.1 GI:4885641), a protein which, like TIM-1 is expressed on activated T cells, and which protects T cells from Fas-mediated apoptosis.

T cells express the TIM family of genes, which critically regulates CD4 T cell differentiation. Th1 cells preferentially express the TIM-3 protein, while Th2 cells preferentially express the TIM-1 protein. TIM-1 has been linked to airway hyperreactivity and TIM-3 to autoimmune disease, therefore the expression pattern on differentiating lymphoid cells and the kinetics of expression of TIM-1 on lymphoid cells reflect the function of these molecules.

In another aspect of the invention, methods are provided for determining susceptibility of an individual to developing asthma and atopic diseases, by determining the genotype at the Tim locus. Screening may analyze, for example, polymorphisms in any one of the TIM-1, TIM-3 or TIM-4 alleles provided herein, or otherwise determined. Methods are provided for screening such polymorphisms, e.g. SSCP analysis, size polymorphisms, and the like.

In another aspect of the invention, a method of screening for biologically active agents that modulate Tim gene or polypeptide function is provided, where the method comprises combining a candidate biologically active agent with any one of: (a) a Tim polypeptide; (b) a cell comprising a nucleic acid encoding a Tim polypeptide; or (c) a non-human transgenic animal model for Tim gene function comprising one of: (i) a knockout of an *Tim* gene; (ii) an exogenous and stably transmitted *Tim* gene sequence; or (iii) a *Tim* promoter sequence operably linked to a reporter gene; and determining the effect of said agent on Tim function.

The activity of TIM polypeptides may be modulated in order to direct immune function. TIM-1 is preferentially expressed in Th2 cells, and agents that modulate TIM-1 activity find use in the treatment of Th2 related disorders, including allergies, asthma, and the like. TIM-3 is preferentially expressed in Th1 cells, and agents that modulate TIM-3 activity find use in the treatment of pro-inflammatory immune diseases, including autoimmune diseases, graft rejection and the like.

CONDITIONS OF INTEREST

Atopic diseases are complex genetic traits that develop as a result of environmentally induced immune responses in genetically predisposed individuals. Both atopic and non-atopic individuals are exposed to the same environmental factors, but genetic differences that distinguish atopic from non-atopic individuals result in atopic

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disease in some individuals, manifested by allergic inflammation in the respiratory tract, skin or gastrointestinal tract, as well as by elevated serum IgE, eosinophilia and the symptoms of wheezing, sneezing or hives. In addition, allergic inflammatory responses are characterized by the presence of Th2 lymphocytes producing high levels of IL-4, IL-5, IL-9 and IL-13, which enhance the growth, differentiation and/or recruitment of eosinophils, mast cells, basophils and B cells producing IgE.

Allergens of interest include antigens found in food, such as strawberries, peanuts, milk proteins, egg whites, etc. Other allergens of interest include various airborne antigens, such as grass pollens, animal danders, house mite feces, etc. Molecularly cloned allergens include *Dermatophagoides pteronyssinus* (Der P1); Lol p1-V from rye grass pollen; a number of insect venoms, including venom from jumper ant *Myrmecia pilosula*; *Apis mellifera* bee venom phospholipase A2 (PLA₂ and antigen 5S; phospholipases from the yellow jacket *Vespula maculifrons* and white faced hornet *Dolichovespula maculata*; a large number of pollen proteins, including birch pollen, ragweed pollen, *Parol* (the major allergen of *Parietaria officinalis*) and the cross-reactive allergen *Parl* (from *Parietaria judaica*), and other atmospheric pollens including *Olea europaea*, *Artemisia* sp., *gramineae*, etc. Other allergens of interest are those responsible for allergic dermatitis caused by blood sucking arthropods, e.g. *Diptera*, including mosquitos (*Anopheles* sp., *Aedes* sp., *Culiseta* sp., *Culex* sp.); flies (*Phlebotomus* sp., *Culicoides* sp.) particularly black flies, deer flies and biting midges; ticks (*Dermacentor* sp., *Ornithodoros* sp., *Otobius* sp.); fleas, e.g. the order *Siphonaptera*, including the genera *Xenopsylla*, *Pulex* and *Ctenocephalides felis felis*. The specific allergen may be a polysaccharide, fatty acid moiety, protein, etc.

Polymorphisms in a number of interacting/epistatic atopy genes are thought to result in enhanced susceptibility to allergic disorders. Genome wide scans have sought to identify the responsible genes by linking various parameters of allergy and asthma with polymorphic DNA markers of specific genes, usually repeat sequences of DNA (microsatellites that contain di- tri- and tetra-nucleotide repeats). These studies have identified several chromosomal regions as likely to be involved in the pathogenesis of atopy, but with resolution no better than 5-10 cM in which there are typically hundreds of candidate genes. Nevertheless, asthma susceptibility has been linked to chromosomes 5q23-31, chromosome 6p21, chromosome 11q13, and chromosome 12q (9-13) by two or more of these genome wide scanning studies.

Asthma, as defined herein, is reversible airflow limitation in an individual over a period of time. Asthma is characterized by the presence of cells such as eosinophils, mast cells, basophils, and CD25⁺ T lymphocytes in the airway walls. There is a close interaction between these cells, because of the activity of cytokines which have a variety of communication and biological effector properties. Chemokines attract cells to the site of

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inflammation and cytokines activate them, resulting in inflammation and damage to the mucosa. With chronicity of the process, secondary changes occur, such as thickening of basement membranes and fibrosis. The disease is characterized by increased airway hyperresponsiveness to a variety of stimuli, and airway inflammation. A patient diagnosed as asthmatic will generally have multiple indications over time, including wheezing, asthmatic attacks, and a positive response to methacholine challenge, i.e., a PC20 on methacholine challenge of less than about 4 mg/ml. Guidelines for diagnosis may be found, for example, in the National Asthma Education Program Expert Panel Guidelines for Diagnosis and Management of Asthma, National Institutes of Health, 1991, Pub. No. 91-3042.

Asthma, allergic rhinitis (hay fever), atopic dermatitis (eczema) and food allergy are diseases that occur in the same families, implying common genetic mechanisms. These atopic diseases are exceedingly prevalent, affecting 20-40% of the general population and constitute a major public health problem. The economic costs for these disorders are enormous. For asthma alone, the estimated health care costs in 1996 were \$14 billion. In addition, the prevalence of all of the atopic diseases has increased dramatically in industrialized countries over the past two decades for reasons that are not yet clear. The prevalence of asthma in industrialized countries, for which the numbers are the most accurate, has doubled since 1982, and is projected to double again in prevalence by the year 2020.

Pro-inflammatory diseases associated with Th1 type T cells include autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, type I diabetes mellitus, etc. (RA) is a chronic autoimmune inflammatory synovitis affecting 0.8% of the world population. Current therapy for RA utilizes therapeutic agents that non-specifically suppress or modulate immune function. Such therapeutics, including the recently developed TNF α antagonists, are not fundamentally curative, and disease activity rapidly returns following discontinuation of therapy. Tremendous clinical need exists for fundamentally curative therapies that do not cause systemic immune suppression or modulation.

A quantitative increase in myelin-autoreactive T cells with the capacity to secrete IFN-gamma is associated with the pathogenesis of MS and EAE, suggesting that autoimmune inducer/helper T lymphocytes in the peripheral blood of MS patients may initiate and/or regulate the demyelination process in patients with MS. The overt disease is associated with muscle weakness, loss of abdominal reflexes, visual defects and paresthesias. During the presymptomatic period there is infiltration of leukocytes into the cerebrospinal fluid, inflammation and demyelination.

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10 IDDM is a cell-mediated autoimmune disorder leading to destruction of
insulin-secreting beta cells and overt hyperglycemia. T lymphocytes invade the islets of
Langerhans, and specifically destroy insulin-producing β -cells. The depletion of β cells
results in an inability to regulate levels of glucose in the blood. The disease progression
5 may be monitored in individuals diagnosed by family history and genetic analysis as being
susceptible. The most important genetic effect is seen with genes of the major
histocompatibility locus (*IDDM1*), although other loci, including the insulin gene region
(*IDDM2*) also show linkage to the disease (see Davies *et al, supra* and Kennedy *et al.*
(1995) *Nature Genetics* 9:293-298).

10

TIM GENE FAMILY

The provided TIM family genes and fragments thereof, encoded proteins,
genomic regulatory regions, and specific antibodies are useful in the identification of
individuals predisposed to development or resistance to asthma, and for the modulation of
15 gene activity in vivo for prophylactic and therapeutic purposes. The encoded proteins are
useful as an immunogen to raise specific antibodies, in drug screening for compositions
that mimic or modulate activity or expression, including altered forms of the proteins, and as
a therapeutic.

20 The TIM family genes are immediately adjacent to each other on human
chromosome 5, in the order TIM-4, TIM-1, TIM-3, with no intervening genes. This segment
of human chromosome 5 is commonly deleted in malignancies and dysplastic cell
populations, as in myelodysplastic syndrome (see Boultonwood, *et al*, (1997) *Genomics*
45:88-96). There are TIM pseudogenes on chromosomes 5, 12, and 19. Each TIM
protein, except TIM-4, contains a distinct predicted tyrosine signaling motif. The
25 cytoplasmic region of TIM-1 contains two tyrosine residues and includes a highly conserved
tyrosine kinase phosphorylation motif, RAEDNIY. The expanded region,
SRAEDNIYVEDRP, contains a predicted site for Itk phosphorylation and for EGF-receptor
phosphorylation.

30 The mouse *Tim1* gene encodes a 305 amino acid membrane protein. The
cytoplasmic region of TIM-1 contains two tyrosine residues and includes a highly conserved
tyrosine kinase phosphorylation motif, RAEDNIY. The mucin domain of TIM-1 has multiple
sites for O-linked glycosylation, and there two sites for N-linked glycosylation found in the
immunoglobulin domain.

35 Mouse TIM-2, a similar 305 amino acid membrane protein, has 64% identity
to mouse TIM-1, 60% identity to rat KIM-1, and 32% identity to hHAVcr-1. Like TIM-1, TIM-
2 has two extracellular N-linked glycosylation sites and a serine, threonine- rich mucin
domain with many O-linked glycosylation sites. TIM-2 also has an intracellular tyrosine

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kinase phosphorylation motif, RTRCEDQVY.

Tim3 encodes a 281 amino acid membrane protein in mice, and a 301 amino acid protein in humans, that has a similar, integral membrane glycoprotein structure with multiple extracellular glycosylation sites and an intracellular tyrosine phosphorylation motif.

- 5 Although the mucin domain is not as prominent in TIM-3 as it is in TIM-1 and TIM-2, TIM-3 expressed on T cells interacts with a ligand on APCs and alters APC activation. TIM-3 has four sites for N-linked and five sites for O-linked glycosylation, suggesting that TIM-3, like TIM-1 and TIM-2, is heavily glycosylated and might interact with a ligand present on other cells, such as antigen presenting cells.

- 10 *Tim4* encodes a 344 amino acid protein in mice, and a 378 amino acid protein in humans. The predicted TIM-4 also shares the general membrane glycoprotein structural motifs of the other TIM proteins, a with an IgV-like domain with highly conserved cysteine residues, a threonine-rich mucin-like domain, and a short intracellular tail.

- Polymorphisms in the murine sequences are provided in the sequence listing
15 for the BALB/c and HBA/DBA strains. In TIM-1, these polymorphisms encode three amino acid differences and a fifteen amino acid deletion in HBA/DBA. Seven predicted amino acid differences were identified in TIM-3. The polymorphisms in TIM-1 and TIM-4 are located in the signal and mucin-like domains, while the polymorphisms identified in TIM-3 are clustered in the Ig domain.

- 20 Variants in coding regions of human *Tim1* are provided in the seqlist and Figure 8. Variations include an insertion (labeled polymorphism 1), 157insMTTTP, observed in 65% of the chromosomes, and a deletion (polymorphism 5), 187ΔThr, observed in 65% of the chromosomes. Other polymorphisms are T140A (polymorphism 7); V161A; (polymorphism 2); V167I (polymorphism 3); T172A (polymorphism 4); N258D
25 (polymorphism 6). Polymorphism 4 was observed in 40% of the chromosomes, and the other polymorphisms were each observed in ≤5% of the chromosomes. Most of these variations (2-6) are located within exon 3, the first mucin-encoding exon, and all of the variants occur at the genomic level and are not splice variants. The association between *Tim1* and asthma susceptibility is further supported by reports of significant linkage of mite-sensitive childhood asthma to D5S820 (mean LOD score = 4.8), a marker which is
30 approximately 0.5 megabases from *Tim1*.

- In human tissues, a 4.4 kb TIM-1 mRNA is present in almost all tissues, though it is faint in most. A 5.5-kb band was observed in colon and liver. A 7.5-kb band was observed in spleen, thymus, and peripheral blood leukocytes, and smaller than 4.4-kb
35 bands were observed in some organs. TIM-1 mRNA is expressed with alternate 5' untranslated regions, in different cell populations. Hypoxia and ischemia induces TIM-1 expression in epithelial cells, and radiation induces expression of TIM gene family mRNA.

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The TIM genes are expressed in tumor specimens. Human TIM-4 mRNA is expressed in glioblastoma tissue, and is also detected in mitogen stimulated or irradiated peripheral blood monocytes.

In one aspect, the invention provides for an isolated nucleic acid molecule other than a naturally occurring chromosome comprising a sequence encoding a TIM-1, TIM-2, TIM-3 or TIM-4 protein, or a homolog or variant thereof, which variant may be associated with susceptibility to airway hyperreactivity and allergic T cell responses. The nucleic acid may be operably linked to a vector and/or control sequences for expression in a homologous or heterologous host cell. Such a host cell can find use in the production of the encoded protein. In another aspect of the invention, a purified polypeptide is provided of TIM-1, TIM-2, TIM-3 or TIM-4 protein, or a homolog or variant thereof, which variant may be associated with susceptibility to airway hyperreactivity and allergic T cell responses. In another aspect, an antibody or other specific binding member that binds to the TIM-1, TIM-2, TIM-3 or TIM-4 polypeptide is provided.

Sequences of human and murine TIM sequences are provided in the sequence listing, as follows:

SEQ ID NO	Name	Length	Type
1	BALB/c TIM-1	305	Protein
2	BALB/c TIM-1	918	DNA
3	ES-HBA TIM-1	282	Protein
4	ES-HBA TIM-1	849	DNA
5	BALB/c TIM-2	305	Protein
6	BALB/c TIM-1	958	DNA
7	ES-HBA TIM-2	305	Protein
8	ES-HBA TIM-2	958	DNA
9	BALB/c TIM-3	281	Protein
10	BALB/c TIM-3	2725	DNA
11	ES-HBA TIM-3	281	Protein
12	ES-HBA TIM-3	882	DNA
13	BALB/c TIM-4	345	Protein
14	BALB/c TIM-4	1032	DNA
15	ES-HBA TIM-4	345	Protein
16	ES-HBA TIM-4	1032	DNA
17	hTIM-1 allele 1	359	Protein
18	hTIM-1 allele 1	1080	DNA
19	hTIM-1 allele 2	359	Protein
20	hTIM-1 allele 2	1080	DNA
21	hTIM-1 allele 3	365	Protein
22	hTIM-1 allele 3	1098	DNA
23	hTIM-1 allele 4	359	Protein
24	hTIM-1 allele 4	1079	DNA
25	hTIM-1 allele 5	364	Protein
26	hTIM-1 allele 5	1095	DNA
27	hTIM-1 allele 6	364	Protein
28	hTIM-1 allele 6	1099	DNA
29	hTIM-3 allele 1	301	Protein
30	hTIM-3 allele 1	1116	DNA

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31	hTIM-3 allele 2	301	Protein
32	hTIM-3 allele 2	1116	DNA
33	hTIM-4 allele 1	378	Protein
34	hTIM-4 allele 1	1156	DNA
35	hTIM-4 allele 2	378	Protein
36	hTIM-4 allele 2	1156	DNA

The DNA sequence encoding Tim polypeptides may be cDNA or genomic DNA or a fragment thereof. Fragments of interest for probes, producing polypeptides, *etc.* may comprise one or more polymorphic residues. The term Tim gene shall be intended to mean the open reading frame encoding any one of the specific Tim polypeptides, introns, as well as adjacent 5' and 3' non-coding nucleotide sequences involved in the regulation of expression, up to about 1 kb beyond the coding region, but possibly further in either direction. The gene may be introduced into an appropriate vector for extrachromosomal maintenance or for integration into the host.

In some embodiments, the Tim gene sequence is other than human TIM-1 allele 1, as set forth in the sequence listing; and/or other than mouse TIM-3 DBA allele.

The term "cDNA" as used herein is intended to include all nucleic acids that share the arrangement of sequence elements found in native mature mRNA species, where sequence elements are exons and 3' and 5' non-coding regions. Normally mRNA species have contiguous exons, with the intervening introns removed by nuclear RNA splicing, to create a continuous open reading frame encoding a Tim protein.

A genomic sequence of interest comprises the nucleic acid present between the initiation codon and the stop codon, as defined in the listed sequences, including all of the introns that are normally present in a native chromosome. It may further include the 3' and 5' untranslated regions found in the mature mRNA. It may further include specific transcriptional and translational regulatory sequences, such as promoters, enhancers, *etc.*, including about 1 kb, but possibly more, of flanking genomic DNA at either the 5' or 3' end of the transcribed region. The genomic DNA may be isolated as a fragment of 100 kbp or smaller; and substantially free of flanking chromosomal sequence.

The sequence of the 5' region, and further 5' upstream sequences and 3' downstream sequences, may be utilized for promoter elements, including enhancer binding sites, that provide for expression in tissues where *Tim* genes are expressed. The tissue specific expression is useful for determining the pattern of expression, and for providing promoters that mimic the native pattern of expression. Naturally occurring polymorphisms in the promoter region are useful for determining natural variations in expression, particularly those that may be associated with disease. Alternatively, mutations may be introduced into the promoter region to determine the effect of altering expression in experimentally defined systems. Methods for the identification of specific DNA motifs

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involved in the binding of transcriptional factors are known in the art, *e.g.* sequence similarity to known binding motifs, gel retardation studies, *etc.* For examples, see Blackwell *et al.* (1995) Mol Med 1: 194-205; Mortlock *et al.* (1996) Genome Res. 6: 327-33; and Joulin and Richard-Foy (1995) Eur J Biochem 232: 620-626.

5 The regulatory sequences may be used to identify *cis* acting sequences required for transcriptional or translational regulation of TIM expression, especially in different tissues or stages of development, and to identify *cis* acting sequences and *trans* acting factors that regulate or mediate TIM expression. Such transcription or translational control regions may be operably linked to a TIM gene in order to promote expression of wild
10 type or altered TIM or other proteins of interest in cultured cells, or in embryonic, fetal or adult tissues, and for gene therapy.

The nucleic acid compositions of the subject invention may encode all or a part of the subject polypeptides. Fragments may be obtained of the DNA sequence by chemically synthesizing oligonucleotides in accordance with conventional methods, by
15 restriction enzyme digestion, by PCR amplification, *etc.* For the most part, DNA fragments will be of at least 15 nt, usually at least 18 nt, more usually at least about 50 nt. Such small DNA fragments are useful as primers for PCR, hybridization screening, *etc.* Larger DNA fragments, *i.e.* greater than 100 nt are useful for production of the encoded polypeptide. For use in amplification reactions, such as PCR, a pair of primers will be used. The exact
20 composition of the primer sequences is not critical to the invention, but for most applications the primers will hybridize to the subject sequence under stringent conditions, as known in the art. It is preferable to choose a pair of primers that will generate an amplification product of at least about 50 nt, preferably at least about 100 nt. Algorithms for the selection of primer sequences are generally known, and are available in commercial
25 software packages. Amplification primers hybridize to complementary strands of DNA, and will prime towards each other.

The *TIM* genes are isolated and obtained in substantial purity, generally as other than an intact mammalian chromosome. Usually, the DNA will be obtained substantially free of other nucleic acid sequences that do not include an *TIM* sequence or
30 fragment thereof, generally being at least about 50%, usually at least about 90% pure and are typically "recombinant", *i.e.* flanked by one or more nucleotides with which it is not normally associated on a naturally occurring chromosome.

The DNA sequences are used in a variety of ways. They may be used as probes for identifying *TIM* related genes. Mammalian homologs have substantial sequence similarity to the subject sequences, *i.e.* at least 75%, usually at least 90%, more usually at
35 least 95% sequence identity with the nucleotide sequence of the subject DNA sequence. Sequence similarity is calculated based on a reference sequence, which may be a subset

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of a larger sequence, such as a conserved motif, coding region, flanking region, *etc.* A reference sequence will usually be at least about 18 nt long, more usually at least about 30 nt long, and may extend to the complete sequence that is being compared. Algorithms for sequence analysis are known in the art, such as BLAST, described in Altschul *et al.* (1990)

5 J Mol Biol **215**:403-10.

Nucleic acids having sequence similarity are detected by hybridization under low stringency conditions, for example, at 50°C and 10XSSC (0.9 M saline/0.09 M sodium citrate) and remain bound when subjected to washing at 55°C in 1XSSC. Sequence identity may be determined by hybridization under stringent conditions, for example, at 10 50°C or higher and 0.1XSSC (9 mM saline/0.9 mM sodium citrate). By using probes, particularly labeled probes of DNA sequences, one can isolate homologous or related genes. The source of homologous genes may be any species, *e.g.* primate species, particularly human; rodents, such as rats and mice, canines, felines, bovines, ovines, equines, yeast, *Drosophila*, *Caenorhabditis*, *etc.*

15 The DNA may also be used to identify expression of the gene in a biological specimen. The manner in which one probes cells for the presence of particular nucleotide sequences, as genomic DNA or RNA, is well established in the literature and does not require elaboration here. mRNA is isolated from a cell sample. mRNA may be amplified by RT-PCR, using reverse transcriptase to form a complementary DNA strand, followed by 20 polymerase chain reaction amplification using primers specific for the subject DNA sequences. Alternatively, mRNA sample is separated by gel electrophoresis, transferred to a suitable support, *e.g.* nitrocellulose, nylon, *etc.*, and then probed with a fragment of the subject DNA as a probe. Other techniques, such as oligonucleotide ligation assays, *in situ* hybridizations, and hybridization to DNA probes arrayed on a solid chip may also find use. 25 Detection of mRNA hybridizing to the subject sequence is indicative of *TIM* gene expression in the sample.

The subject nucleic acid sequences may be modified for a number of purposes, particularly where they will be used intracellularly, for example, by being joined to a nucleic acid cleaving agent, *e.g.* a chelated metal ion, such as iron or chromium for 30 cleavage of the gene; or the like.

The sequence of the *TIM* locus, including flanking promoter regions and coding regions, may be mutated in various ways known in the art to generate targeted changes in promoter strength, sequence of the encoded protein, *etc.* The DNA sequence or product of such a mutation will be substantially similar to the sequences provided herein, 35 *i.e.* will differ by at least one nucleotide or amino acid, respectively, and may differ by at least two but not more than about ten nucleotides or amino acids. The sequence changes may be substitutions, insertions or deletions. Deletions may further include larger changes,

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such as deletions of a domain or exon. Other modifications of interest include epitope tagging, e.g. with the FLAG system, HA, etc. For studies of subcellular localization, fusion proteins with green fluorescent proteins (GFP) may be used. Such mutated genes may be used to study structure-function relationships of TIM polypeptides, or to alter properties of the protein that affect its function or regulation. For example, constitutively active transcription factors, or a dominant negatively active protein that binds to the TIM DNA target site without activating transcription, may be created in this manner.

Techniques for *in vitro* mutagenesis of cloned genes are known. Examples of protocols for scanning mutations may be found in Gustin *et al.*, *Biotechniques* 14:22 (1993); Barany, *Gene* 37:111-23 (1985); Colicelli *et al.*, *Mol Gen Genet* 199:537-9 (1985); and Prentki *et al.*, *Gene* 29:303-13 (1984). Methods for site specific mutagenesis can be found in Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, CSH Press 1989, pp. 15.3-15.108; Weiner *et al.*, *Gene* 126:35-41 (1993); Sayers *et al.*, *Biotechniques* 13:592-6 (1992); Jones and Winistorfer, *Biotechniques* 12:528-30 (1992); Barton *et al.*, *Nucleic Acids Res* 18:7349-55 (1990); Marotti and Tomich, *Gene Anal Tech* 6:87-70 (1989); and Zhu *Anal Biochem* 177:120-4 (1989).

Arrays provide a high throughput technique that can assay a large number of polynucleotides in a sample. In one aspect of the invention, an array is constructed comprising one or more of the TIM genes, proteins or antibodies, preferably comprising all of these sequences, which array may further comprise other sequences known to be up- or down-regulated in T cells, monocytes, and the like. This technology can be used as a tool to test for differential expression, or for genotyping. Arrays can be created by spotting polynucleotide probes onto a substrate (e.g., glass, nitrocellulose, etc.) in a two-dimensional matrix or array having bound probes. The probes can be bound to the substrate by either covalent bonds or by non-specific interactions, such as hydrophobic interactions. Techniques for constructing arrays and methods of using these arrays are described in, for example, Schena *et al.* (1996) *Proc Natl Acad Sci U S A*, 93(20):10614-9; Schena *et al.* (1995) *Science* 270(5235):467-70; Shalon *et al.* (1996) *Genome Res.* 6(7):639-45, USPN 5,807,522, EP 799 897; WO 97/29212; WO 97/27317; EP 785 280; WO 97/02357; USPN 5,593,839; USPN 5,578,832; EP 728 520; USPN 5,599,695; EP 721 016; USPN 5,556,752; WO 95/22058; and USPN 5,631,734.

The probes utilized in the arrays can be of varying types and can include, for example, synthesized probes of relatively short length (e.g., a 20-mer or a 25-mer), cDNA (full length or fragments of gene), amplified DNA, fragments of DNA (generated by restriction enzymes, for example) and reverse transcribed DNA. Both custom and generic arrays can be utilized in detecting differential expression levels. Custom arrays can be prepared using probes that hybridize to particular preselected subsequences of mRNA

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gene sequences or amplification products prepared from them.

Arrays can be used to, for example, examine differential expression of genes and can be used to determine gene function. For example, arrays can be used to detect differential expression, or expression of polymorphic sequences, in TIM genes. Exemplary
 5 uses of arrays are further described in, for example, Pappalarado *et al.* (1998) Sem. Radiation Oncol. 8:217; and Ramsay. (1998) Nature Biotechnol. 16:40. Furthermore, many variations on methods of detection using arrays are well within the skill in the art and within the scope of the present invention. For example, rather than immobilizing the probe to a solid support, the test sample can be immobilized on a solid support which is then
 10 contacted with the probe. Additional discussion regarding the use of microarrays in expression analysis can be found, for example, in Duggan, *et al.*, *Nature Genetics Supplement* 21:10-14 (1999); Bowtell, *Nature Genetics Supplement* 21:25-32 (1999); Brown and Botstein, *Nature Genetics Supplement* 21:33-37 (1999); Cole *et al.*, *Nature Genetics Supplement* 21:38-41 (1999); Debouck and Goodfellow, *Nature Genetics Supplement* 21:48-50 (1999); Bassett, Jr., *et al.*, *Nature Genetics Supplement* 21:51-55 (1999); and Chakravarti, *Nature Genetics Supplement* 21:56-60 (1999).

Pharmacogenetics is the linkage between an individual's genotype and that individual's ability to metabolize or react to a therapeutic agent. Differences in metabolism or target sensitivity can lead to severe toxicity or therapeutic failure by altering the relation
 20 between bioactive dose and blood concentration of the drug. In the past few years, numerous studies have established good relationships between polymorphisms in metabolic enzymes or drug targets, and both response and toxicity. These relationships can be used to individualize therapeutic dose administration.

Genotyping of polymorphic alleles is used to evaluate whether an individual
 25 will respond well to a particular therapeutic regimen. The polymorphic sequences are also used in drug screening assays, to determine the dose and specificity of a candidate therapeutic agent. A candidate TIM polymorphism is screened with a target therapy to determine whether there is an influence on the effectiveness in treating asthma. Drug screening assays are performed as described above. Typically two or more different
 30 sequence polymorphisms are tested for response to a therapy.

The subject gene may be employed for synthesis of a complete TIM protein, or polypeptide fragments thereof, particularly fragments corresponding to functional domains; binding sites; *etc.*; and including fusions of the subject polypeptides to other proteins or parts thereof. For expression, an expression cassette may be employed,
 35 providing for a transcriptional and translational initiation region, which may be inducible or constitutive, where the coding region is operably linked under the transcriptional control of the transcriptional initiation region, and a transcriptional and translational termination

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region. Various transcriptional initiation regions may be employed that are functional in the expression host.

Polypeptides of particular interest that are fragments of the TIM polypeptides include specific domains of the TIM polypeptides, where a domain may comprise, for example, the extracellular domain, or the domains within the extracellular domain: the mucin domain and/or the Ig domain. Domains may also comprise the cytoplasmic domain, e.g. a fragment encompassing the tyrosine kinase phosphorylation motif, RAEDNIY, or the expanded region, SRAEDNIYIVEDRP. Polypeptides encoded by the soluble splice variants are also of interest. The sequence of the Ig domains are as follows: human TIM-1 Ig domain, SEQ ID NO: 17, 19, 21, 23, 25, 27, residues 21-126; human TIM-3 Ig domain, SEQ ID NO: 29 and 31, residues 22-131; human TIM-4 Ig domain, SEQ ID NO: 33 and 35, residues 25-133; mouse TIM-1 Ig domain, SEQ ID NO: 1 and 3, residues 21-129; mouse TIM-2 Ig domain, SEQ ID NO: 7, residues 22-128; mouse TIM-3 Ig domain, BALB/c allele, SEQ ID NO: 9, residues 22-132; mouse TIM-3 Ig domain, DBA/2 allele, SEQ ID No: 11, residues 22-132; mouse TIM-4 Ig domain, SEQ ID NO: 13 and 15, residues 25-135.

Functionally equivalent polypeptides may find use, where the equivalent polypeptide may contain deletions, additions or substitutions of amino acid residues that result in a silent change, thus producing a functionally equivalent differentially expressed on pathway gene product. Amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. "Functionally equivalent", as used herein, refers to a protein capable of exhibiting a substantially similar *in vivo* activity as the polypeptide encoded by a TIM gene.

The polypeptides may be expressed in prokaryotes or eukaryotes in accordance with conventional ways, depending upon the purpose for expression. For large scale production of the protein, a unicellular organism, such as *E. coli*, *B. subtilis*, *S. cerevisiae*, or cells of a higher organism such as vertebrates, particularly mammals, e.g. COS 7 cells, may be used as the expression host cells. In many situations, it may be desirable to express the TIM gene in mammalian cells, where the TIM gene will benefit from native folding and post-translational modifications. Small peptides can also be synthesized in the laboratory, including specific peptide epitopes, domains, and the like, where peptides will usually be at least about 8 amino acids in length, more usually at least about 20 amino acids in length, up to complete domains, and the full length protein. Peptides may comprise polymorphic regions of the protein. Also included are fusion proteins, where all or a fragment of the TIM protein is fused to a heterologous polypeptide, e.g. green fluorescent protein, antibody Fc regions, poly-histidine, and the like.

In mammalian host cells, a number of viral-based expression systems may

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be used, including retrovirus, lentivirus, adenovirus, adeno-associated virus, and the like. In cases where an adenovirus is used as an expression vector, the coding sequence of interest can be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing differentially expressed or pathway gene protein in infected hosts.

Specific initiation signals may also be required for efficient translation of the genes. These signals include the ATG initiation codon and adjacent sequences. In cases where a complete gene, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only a portion of the gene coding sequence is inserted, exogenous translational control signals must be provided. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, *etc.*

With the availability of the polypeptides in large amounts, by employing an expression host, the polypeptides may be isolated and purified in accordance with conventional ways. A lysate may be prepared of the expression host and the lysate purified using HPLC, exclusion chromatography, gel electrophoresis, affinity chromatography, or other purification technique. The purified polypeptide will generally be at least about 80% pure, preferably at least about 90% pure, and may be up to and including 100% pure. Pure is intended to mean free of other proteins, as well as cellular debris.

The polypeptide may be labeled, either directly or indirectly. Any of a variety of suitable labeling systems may be used, including but not limited to, radioisotopes such as ¹²⁵I; enzyme labeling systems that generate a detectable colorimetric signal or light when exposed to substrate; and fluorescent labels. Indirect labeling involves the use of a protein, such as a labeled antibody, that specifically binds to the polypeptide of interest. Such antibodies include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments and fragments produced by a Fab expression library.

SPECIFIC BINDING MEMBERS

The term "specific binding member" or "binding member" as used herein refers to a member of a specific binding pair, *i.e.* two molecules, usually two different molecules, where one of the molecules (*i.e.*, first specific binding member) through chemical or physical means specifically binds to the other molecule (*i.e.*, second specific

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binding member). The complementary members of a specific binding pair are sometimes referred to as a ligand and receptor; or receptor and counter-receptor. For the purposes of the present invention, the two binding members may be known to associate with each other, for example where an assay is directed at detecting compounds that interfere with the association of a known binding pair. Alternatively, candidate compounds suspected of being a binding partner to a compound of interest may be used.

Specific binding pairs of interest include carbohydrates and lectins; complementary nucleotide sequences; peptide ligands and receptor; effector and receptor molecules; hormones and hormone binding protein; enzyme cofactors and enzymes; enzyme inhibitors and enzymes; lipid and lipid-binding protein; etc. The specific binding pairs may include analogs, derivatives and fragments of the original specific binding member. For example, a receptor and ligand pair may include peptide fragments, chemically synthesized peptidomimetics, labeled protein, derivatized protein, etc.

In a preferred embodiment, the specific binding member is an antibody. The term "antibody" or "antibody moiety" is intended to include any polypeptide chain-containing molecular structure with a specific shape that fits to and recognizes an epitope, where one or more non-covalent binding interactions stabilize the complex between the molecular structure and the epitope. Antibodies that bind specifically to one of the TIM proteins are referred to as anti-TIM. The archetypal antibody molecule is the immunoglobulin, and all types of immunoglobulins, IgG, IgM, IgA, IgE, IgD, etc., from all sources, e.g. human, rodent, rabbit, cow, sheep, pig, dog, other mammal, chicken, other avians, etc., are considered to be "antibodies." Antibodies utilized in the present invention may be polyclonal antibodies, although monoclonal antibodies are preferred because they may be reproduced by cell culture or recombinantly, and can be modified to reduce their antigenicity.

Polyclonal antibodies can be raised by a standard protocol by injecting a production animal with an antigenic composition, which may be a polypeptide or a cDNA expressed *in vivo*. See, e.g., Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. When utilizing an entire protein, or a larger section of the protein, antibodies may be raised by immunizing the production animal with the protein and a suitable adjuvant (e.g., Freund's, Freund's complete, oil-in-water emulsions, etc.) When a smaller peptide is utilized, it is advantageous to conjugate the peptide with a larger molecule to make an immunostimulatory conjugate. Commonly utilized conjugate proteins that are commercially available for such use include bovine serum albumin (BSA) and keyhole limpet hemocyanin (KLH). In order to raise antibodies to particular epitopes, such as polymorphic residues, peptides derived from the full sequence may be utilized. The immunogen is injected into the animal host, preferably according to a predetermined

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schedule incorporating one or more booster immunizations, and the animals are bled periodically. Polyclonal antibodies may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Alternatively, for monoclonal antibodies, hybridomas may be formed by
5 isolating the stimulated immune cells, such as those from the spleen of the inoculated animal. These cells are then fused to immortalized cells, such as myeloma cells or transformed cells, which are capable of replicating indefinitely in cell culture, thereby producing an immortal, immunoglobulin-secreting cell line. The immortal cell line utilized is preferably selected to be deficient in enzymes necessary for the utilization of certain
10 nutrients. Many such cell lines (such as myelomas) are known to those skilled in the art, and include, for example: thymidine kinase (TK) or hypoxanthine-guanine phosphoribosyl transferase (HGPRT). These deficiencies allow selection for fused cells according to their ability to grow on, for example, hypoxanthine aminopterinthymidine medium (HAT).

Preferably, the immortal fusion partners utilized are derived from a line that
15 does not secrete immunoglobulin. The resulting fused cells, or hybridomas, are cultured under conditions that allow for the survival of fused, but not unfused, cells and the resulting colonies screened for the production of the desired monoclonal antibodies. Colonies producing such antibodies are cloned, expanded, and grown so as to produce large quantities of antibody, see Kohler and Milstein, 1975 Nature 256:495 (the disclosures of
20 which are hereby incorporated by reference).

Large quantities of monoclonal antibodies from the secreting hybridomas may then be produced by injecting the clones into the peritoneal cavity of mice and harvesting the ascites fluid therefrom. The mice, preferably primed with pristane, or some other tumor-promoter, and immunosuppressed chemically or by irradiation, may be any of
25 various suitable strains known to those in the art. The ascites fluid is harvested from the mice and the monoclonal antibody purified therefrom, for example, by CM Sepharose column or other chromatographic means. Alternatively, the hybridomas may be cultured *in vitro* or as suspension cultures. Batch, continuous culture, or other suitable culture processes may be utilized. Monoclonal antibodies are then recovered from the culture
30 medium or supernatant.

In addition, the antibodies or antigen binding fragments may be produced by genetic engineering. In this technique, as with the standard hybridoma procedure, antibody-producing cells are sensitized to the desired antigen or immunogen. The messenger RNA isolated from the immune spleen cells or hybridomas is used as a
35 template to make cDNA using PCR amplification. A library of vectors, each containing one heavy chain gene and one light chain gene retaining the initial antigen specificity, is produced by insertion of appropriate sections of the amplified immunoglobulin cDNA into

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the expression vectors. A combinatorial library is constructed by combining the heavy chain gene library with the light chain gene library. This results in a library of clones which co-express a heavy and light chain (resembling the Fab fragment or antigen binding fragment of an antibody molecule). The vectors that carry these genes are co-transfected into a host (e.g. bacteria, insect cells, mammalian cells, or other suitable protein production host cell.). When antibody gene synthesis is induced in the transfected host, the heavy and light chain proteins self-assemble to produce active antibodies that can be detected by screening with the antigen or immunogen.

Chimeric antibodies may be made by recombinant means by combining the murine variable light and heavy chain regions (VK and VH), obtained from a murine (or other animal-derived) hybridoma clone, with the human constant light and heavy chain regions, in order to produce an antibody with predominantly human domains. The production of such chimeric antibodies is well known in the art, and may be achieved by standard means (as described, e.g., in U.S. Patent No. 5,624,659, incorporated fully herein by reference). Humanized antibodies are engineered to contain even more human-like immunoglobulin domains, and incorporate only the complementarity-determining regions of the animal-derived antibody. This is accomplished by carefully examining the sequence of the hyper-variable loops of the variable regions of the monoclonal antibody, and fitting them to the structure of the human antibody chains. Although facially complex, the process is straightforward in practice. See, e.g., U.S. Patent No. 6,187,287, incorporated fully herein by reference.

Alternatively, polyclonal or monoclonal antibodies may be produced from animals that have been genetically altered to produce human immunoglobulins. Techniques for generating such animals, and deriving antibodies therefrom, are described in U.S. Patents No. 6,162,963 and 6,150,584, incorporated fully herein by reference.

Alternatively, single chain antibodies (Fv, as described below) can be produced from phage libraries containing human variable regions. See U.S. Patent No. 6,174,708. Intrathecal administration of single-chain immunotoxin, LMB-7 [B3(Fv)-PE38], has been shown to cure of carcinomatous meningitis in a rat model. *Proc Natl. Acad. Sci U S A* 92, 2765-9, all of which are incorporated by reference fully herein.

In addition to entire immunoglobulins (or their recombinant counterparts), immunoglobulin fragments comprising the epitope binding site (e.g., Fab', F(ab')₂, or other fragments) are useful as antibody moieties in the present invention. Such antibody fragments may be generated from whole immunoglobulins by pepsin, papain, or other protease cleavage. "Fragment," or minimal immunoglobulins may be designed utilizing recombinant immunoglobulin techniques. For instance "Fv" immunoglobulins for use in the present invention may be produced by linking a variable light chain region to a variable

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heavy chain region via a peptide linker (e.g., poly-glycine or another sequence which does not form an alpha helix or beta sheet motif).

Fv fragments are heterodimers of the variable heavy chain domain (V_H) and the variable light chain domain (V_L). The heterodimers of heavy and light chain domains that occur in whole IgG, for example, are connected by a disulfide bond. Recombinant Fvs in which V_H and V_L are connected by a peptide linker are typically stable. These are single chain Fvs which have been found to retain specificity and affinity and have been shown to be useful for imaging tumors and to make recombinant immunotoxins for tumor therapy. However, researchers have found that some of the single chain Fvs have a reduced affinity for antigen and the peptide linker can interfere with binding. Improved Fv's have been also been made which comprise stabilizing disulfide bonds between the V_H and V_L regions, as described in U.S. Patent No. 6,147,203, incorporated fully herein by reference. Any of these minimal antibodies may be utilized in the present invention, and those which are humanized to avoid HAMA reactions are preferred for use in embodiments of the invention.

In addition, derivatized immunoglobulins with added chemical linkers, detectable moieties, such as fluorescent dyes, enzymes, substrates, chemiluminescent moieties and the like, or specific binding moieties, such as streptavidin, avidin, or biotin, and the like may be utilized in the methods and compositions of the present invention. For convenience, the term "antibody" or "antibody moiety" will be used throughout to generally refer to molecules which specifically bind to an epitope of the brain tumor protein targets, although the term will encompass all immunoglobulins, derivatives, fragments, recombinant or engineered immunoglobulins, and modified immunoglobulins, as described above.

Candidate antibodies can be tested for activity by any suitable standard means. As a first screen, the antibodies may be tested for binding against the immunogen. As a second screen, antibodies may be screened for cross-reactivity between alleles and between TIM family members, and tested for activity in inhibition of TIM function. For these screens, the candidate antibody may be labeled for detection. Antibodies that alter the biological activity of a TIM protein may be assayed in functional formats.

DIAGNOSIS

Diagnosis of asthma or atopy associated with Tim polymorphisms is performed by protein, DNA or RNA sequence and/or hybridization analysis of any convenient sample from a patient, e.g. biopsy material, blood sample, scrapings from cheek, etc. A nucleic acid sample from a patient having asthma that may be associated with TIM, is analyzed for the presence of a predisposing polymorphism in TIM. A typical patient genotype would have at least one predisposing mutation on at least one chromosome. The presence of a polymorphic TIM sequence that affects the activity or

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expression of the gene product, and confers an increased susceptibility to asthma is considered a predisposing polymorphism. Individuals are screened by analyzing their DNA or mRNA for the presence of a predisposing polymorphism, as compared to an asthma neutral sequence. Specific sequences of interest include any polymorphism that leads to clinical bronchial hyperactivity or is otherwise associated with asthma, including, but not limited to, insertions, substitutions and deletions in the coding region sequence, intron sequences that affect splicing, or promoter or enhancer sequences that affect the activity and expression of the protein. Examples of specific *TIM* polymorphisms are provided in the Examples.

Screening may also be based on the functional or antigenic characteristics of the protein. Immunoassays designed to detect predisposing polymorphisms in *TIM* proteins may be used in screening. Where many diverse mutations lead to a particular disease phenotype, functional protein assays have proven to be effective screening tools.

Biochemical studies may be performed to determine whether a candidate sequence polymorphism in the *TIM* coding region or control regions is associated with disease. For example, a change in the promoter or enhancer sequence that affects expression of *TIM* may result in predisposition to asthma. Expression levels of a candidate variant allele are compared to expression levels of the normal allele by various methods known in the art. Methods for determining promoter or enhancer strength include quantitation of the expressed natural protein; insertion of the variant control element into a vector with a reporter gene such as β -galactosidase, luciferase, chloramphenicol acetyltransferase, etc. that provides for convenient quantitation; and the like. The activity of the encoded *TIM* protein may be determined by comparison with the wild-type protein.

A number of methods are available for analyzing nucleic acids for the presence of a specific sequence. Where large amounts of DNA are available, genomic DNA is used directly. Alternatively, the region of interest is cloned into a suitable vector and grown in sufficient quantity for analysis. Cells that express *TIM* genes, such as trachea cells, may be used as a source of mRNA, which may be assayed directly or reverse transcribed into cDNA for analysis. The nucleic acid may be amplified by conventional techniques, such as the polymerase chain reaction (PCR), to provide sufficient amounts for analysis. The use of the polymerase chain reaction is described in Saiki, *et al.* (1985) *Science* **239**:487, and a review of current techniques may be found in Sambrook, *et al.* *Molecular Cloning: A Laboratory Manual*, CSH Press 1989, pp.14.2-14.33. Amplification may also be used to determine whether a polymorphism is present, by using a primer that is specific for the polymorphism. Alternatively, various methods are known in the art that utilize oligonucleotide ligation as a means of detecting polymorphisms, for examples see Riley *et al.* (1990) *N.A.R.* **18**:2887-2890; and Delahunty *et al.* (1996) *Am. J. Hum. Genet.*

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58:1239-1246.

A detectable label may be included in an amplification reaction. Suitable labels include fluorochromes, *e.g.* fluorescein isothiocyanate (FITC), rhodamine, Texas Red, phycoerythrin, allophycocyanin, 6-carboxyfluorescein (6-FAM), 2',7'-dimethoxy-4',5'-dichloro-6-carboxyfluorescein (JOE), 6-carboxy-X-rhodamine (ROX), 6-carboxy-2',4',7',4,7-hexachlorofluorescein (HEX), 5-carboxyfluorescein (5-FAM) or N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA), radioactive labels, *e.g.* ^{32}P , ^{35}S , ^3H ; *etc.* The label may be a two stage system, where the amplified DNA is conjugated to biotin, haptens, *etc.* having a high affinity binding partner, *e.g.* avidin, specific antibodies, *etc.*, where the binding partner is conjugated to a detectable label. The label may be conjugated to one or both of the primers. Alternatively, the pool of nucleotides used in the amplification is labeled, so as to incorporate the label into the amplification product.

The sample nucleic acid, *e.g.* amplified or cloned fragment, is analyzed by one of a number of methods known in the art. The nucleic acid may be sequenced by dideoxy or other methods, and the sequence of bases compared to a neutral *TIM* sequence. Hybridization with the variant sequence may also be used to determine its presence, by Southern blots, dot blots, *etc.* The hybridization pattern of a control and variant sequence to an array of oligonucleotide probes immobilised on a solid support, as described in US 5,445,934, or in WO95/35505, may also be used as a means of detecting the presence of variant sequences. Single strand conformational polymorphism (SSCP) analysis, denaturing gradient gel electrophoresis (DGGE), mismatch cleavage detection, and heteroduplex analysis in gel matrices are used to detect conformational changes created by DNA sequence variation as alterations in electrophoretic mobility. Alternatively, where a polymorphism creates or destroys a recognition site for a restriction endonuclease (restriction fragment length polymorphism, RFLP), the sample is digested with that endonuclease, and the products size fractionated to determine whether the fragment was digested. Fractionation is performed by gel or capillary electrophoresis, particularly acrylamide or agarose gels.

The hybridization pattern of a control and variant sequence to an array of oligonucleotide probes immobilised on a solid support, as described in US 5,445,934, or in WO95/35505, may be used as a means of detecting the presence of variant sequences. In one embodiment of the invention, an array of oligonucleotides are provided, where discrete positions on the array are complementary to at least a portion of mRNA or genomic DNA of the *TIM* locus. Such an array may comprise a series of oligonucleotides, each of which can specifically hybridize to a nucleic acid, *e.g.* mRNA, cDNA, genomic DNA, *etc.* from the *TIM* locus.

Antibodies specific for *TIM* polymorphisms may be used in screening

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immunoassays. A reduction or increase in neutral TIM and/or presence of asthma associated polymorphisms is indicative that asthma is TIM-associated. A sample is taken from a patient suspected of having TIM-associated asthma. Samples, as used herein, include biological fluids such as tracheal lavage, blood, cerebrospinal fluid, tears, saliva, lymph, dialysis fluid and the like; organ or tissue culture derived fluids; and fluids extracted from physiological tissues. Also included in the term are derivatives and fractions of such fluids. Biopsy samples are of particular interest, *e.g.* trachea scrapings, *etc.* The number of cells in a sample will generally be at least about 10^3 , usually at least 10^4 more usually at least about 10^5 . The cells may be dissociated, in the case of solid tissues, or tissue sections may be analyzed. Alternatively a lysate of the cells may be prepared.

Diagnosis may be performed by a number of methods. The different methods all determine the absence or presence or altered amounts of normal or abnormal TIM in patient cells suspected of having a predisposing polymorphism in TIM. For example, detection may utilize staining of cells or histological sections, performed in accordance with conventional methods. The antibodies of interest are added to the cell sample, and incubated for a period of time sufficient to allow binding to the epitope, usually at least about 10 minutes. The antibody may be labeled with radioisotopes, enzymes, fluorescers, chemiluminescers, or other labels for direct detection. Alternatively, a second stage antibody or reagent is used to amplify the signal. Such reagents are well known in the art. For example, the primary antibody may be conjugated to biotin, with horseradish peroxidase-conjugated avidin added as a second stage reagent. Final detection uses a substrate that undergoes a color change in the presence of the peroxidase. The absence or presence of antibody binding may be determined by various methods, including flow cytometry of dissociated cells, microscopy, radiography, scintillation counting, *etc.*

An alternative method for diagnosis depends on the *in vitro* detection of binding between antibodies and TIM in a lysate. Measuring the concentration of TIM binding in a sample or fraction thereof may be accomplished by a variety of specific assays.

A conventional sandwich type assay may be used. For example, a sandwich assay may first attach TIM-specific antibodies to an insoluble surface or support. The particular manner of binding is not crucial so long as it is compatible with the reagents and overall methods of the invention. They may be bound to the plates covalently or non-covalently, preferably non-covalently.

Other immunoassays are known in the art and may find use as diagnostics. Ouchterlony plates provide a simple determination of antibody binding. Western blots may be performed on protein gels or protein spots on filters, using a detection system specific for TIM as desired, conveniently using a labeling method as described for the sandwich assay.

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The *TIM* genes are useful for analysis of TIM expression, *e.g.* in determining developmental and tissue specific patterns of expression, and for modulating expression *in vitro* and *in vivo*. Vectors useful for introduction of the gene include plasmids and viral vectors. Of particular interest are retroviral-based vectors, *e.g.* Moloney murine leukemia virus and modified human immunodeficiency virus; adenovirus vectors, *etc.* that are maintained transiently or stably in mammalian cells. A wide variety of vectors can be employed for transfection and/or integration of the gene into the genome of the cells. Alternatively, micro-injection may be employed, fusion, or the like for introduction of genes into a suitable host cell. See, for example, Dhawan *et al.* (1991) *Science* **254**:1509-1512 and Smith *et al.* (1990) *Molecular and Cellular Biology* 3268-3271.

The expression vector will have a transcriptional initiation region oriented to produce functional mRNA. The native transcriptional initiation region or an exogenous transcriptional initiation region may be employed. The promoter may be introduced by recombinant methods *in vitro*, or as the result of homologous integration of the sequence into a chromosome. Many strong promoters are known in the art, including the β -actin promoter, SV40 early and late promoters, human cytomegalovirus promoter, retroviral LTRs, methallothionein responsive element (MRE), tetracycline-inducible promoter constructs, *etc.*

Expression vectors generally have convenient restriction sites located near the promoter sequence to provide for the insertion of nucleic acid sequences. Transcription cassettes may be prepared comprising a transcription initiation region, the target gene or fragment thereof, and a transcriptional termination region. The transcription cassettes may be introduced into a variety of vectors, *e.g.* plasmid; retrovirus, *e.g.* lentivirus; adenovirus; and the like, where the vectors are able to transiently or stably be maintained in the cells, usually for a period of at least about one day, more usually for a period of at least about several days to several weeks.

Antisense molecules are used to down-regulate expression of *TIM* in cells. The anti-sense reagent may be antisense oligonucleotides (ODN), particularly synthetic ODN having chemical modifications from native nucleic acids, or nucleic acid constructs that express such anti-sense molecules as RNA. The antisense sequence is complementary to the mRNA of the targeted gene, and inhibits expression of the targeted gene products. Antisense molecules inhibit gene expression through various mechanisms, *e.g.* by reducing the amount of mRNA available for translation, through activation of RNase H, or steric hindrance. One or a combination of antisense molecules may be administered, where a combination may comprise multiple different sequences.

Antisense molecules may be produced by expression of all or a part of the

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target gene sequence in an appropriate vector, where the transcriptional initiation is oriented such that an antisense strand is produced as an RNA molecule. Alternatively, the antisense molecule is a synthetic oligonucleotide. Antisense oligonucleotides will generally be at least about 7, usually at least about 12, more usually at least about 20 nucleotides in length, and not more than about 500, usually not more than about 50, more usually not more than about 35 nucleotides in length, where the length is governed by efficiency of inhibition, specificity, including absence of cross-reactivity, and the like. It has been found that short oligonucleotides, of from 7 to 8 bases in length, can be strong and selective inhibitors of gene expression (see Wagner *et al.* (1996) *Nature Biotechnology* 14:840-844).

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TRANSGENIC ANIMALS

The subject nucleic acids can be used to generate genetically modified non-human animals or site specific gene modifications in cell lines. The term "transgenic" is intended to encompass genetically modified animals having a deletion or other knock-out of *TIM* gene activity, having an exogenous *TIM* gene that is stably transmitted in the host cells, or having an exogenous *TIM* promoter operably linked to a reporter gene. Transgenic animals may be made through homologous recombination, where the *TIM* locus is altered. Alternatively, a nucleic acid construct is randomly integrated into the genome. Vectors for stable integration include plasmids, retroviruses and other animal viruses, YACs, and the like. Of interest are transgenic mammals, *e.g.* cows, pigs, goats, horses, *etc.*, and particularly rodents, *e.g.* rats, mice, *etc.*

A "knock-out" animal is genetically manipulated to substantially reduce, or eliminate endogenous *TIM* function. Different approaches may be used to achieve the "knock-out". A chromosomal deletion of all or part of the native *TIM* homolog may be induced. Deletions of the non-coding regions, particularly the promoter region, 3' regulatory sequences, enhancers, or deletions of gene that activate expression of *TIM* genes. A functional knock-out may also be achieved by the introduction of an anti-sense construct that blocks expression of the native *TIM* genes (for example, see Li and Cohen (1996) *Cell* 85:319-329).

Transgenic animals may be made having exogenous *TIM* genes. The exogenous gene is usually either from a different species than the animal host, or is otherwise altered in its coding or non-coding sequence. The introduced gene may be a wild-type gene, naturally occurring polymorphism, or a genetically manipulated sequence, for example those previously described with deletions, substitutions or insertions in the coding or non-coding regions. The introduced sequence may encode an *TIM* polypeptide, or may utilize the *TIM* promoter operably linked to a reporter gene. Where the introduced gene is a coding sequence, it usually operably linked to a promoter, which may be

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constitutive or inducible, and other regulatory sequences required for expression in the host animal.

Specific constructs of interest, but are not limited to, include anti-sense *TIM*, which will block *TIM* expression, expression of dominant negative *TIM* mutations, and over-expression of a *TIM* gene. A detectable marker, such as *lac Z* may be introduced into the *TIM* locus, where upregulation of *TIM* expression will result in an easily detected change in phenotype.

The modified cells or animals are useful in the study of *TIM* function and regulation. Animals may be used in functional studies, drug screening, *etc.*, *e.g.* to determine the effect of a candidate drug on asthma. A series of small deletions and/or substitutions may be made in the *TIM* gene to determine the role of different exons in DNA binding, transcriptional regulation, *etc.* By providing expression of *TIM* protein in cells in which it is otherwise not normally produced, one can induce changes in cell behavior. These animals are also useful for exploring models of inheritance of asthma, *e.g.* dominant v. recessive; relative effects of different alleles and synergistic effects between *TIM* and other asthma genes elsewhere in the genome.

DNA constructs for homologous recombination will comprise at least a portion of the *TIM* gene with the desired genetic modification, and will include regions of homology to the target locus. DNA constructs for random integration need not include regions of homology to mediate recombination. Conveniently, markers for positive and negative selection are included. Methods for generating cells having targeted gene modifications through homologous recombination are known in the art. For various techniques for transfecting mammalian cells, see Keown *et al.* (1990) Methods in Enzymology **185**:527-537.

Drug screening may be performed in combination with the subject animal models. Many mammalian genes have homologs in yeast and lower animals. The study of such homologs' physiological role and interactions with other proteins can facilitate understanding of biological function. In addition to model systems based on genetic complementation, yeast has been shown to be a powerful tool for studying protein-protein interactions through the two hybrid system described in Chien *et al.* (1991) P.N.A.S. **88**:9578-9582. Two-hybrid system analysis is of particular interest for exploring transcriptional activation by *TIM* proteins.

COMPOUND SCREENING

One can identify ligands or substrates that bind to, modulate or mimic the action of *TIM*. Areas of investigation are the development of treatments for immune disorders, asthma, cancer, ischemia-reperfusion injury, and other diseases that are

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associated with cellular responses to stress. Drug screening identifies agents that provide an inhibition, replacement, or enhancement for TIM function in affected cells. For example, agents that reverse or inhibit TIM function may reduce bronchial reactivity in asthma by reducing levels of Th2 cytokines, and TIM inhibitors may enhance tumor sensitivity to cancer therapy, by potentiating the effects of radiation and chemotherapeutic treatments that induce apoptosis. Of particular interest are screening assays for agents that have a low toxicity for human cells. A wide variety of assays may be used for this purpose, including labeled *in vitro* protein-protein binding assays, protein-DNA binding assays, electrophoretic mobility shift assays, immunoassays for protein binding, and the like. The purified protein may also be used for determination of three-dimensional crystal structure, which can be used for modeling intermolecular interactions, transcriptional regulation, *etc.*

The term "agent" as used herein describes any molecule, *e.g.* protein or pharmaceutical, with the capability of altering or mimicking the physiological function of TIM, such as a signal tyrosine kinase inhibitor, or a peptide inhibitor of an integrin binding site. Generally a plurality of assay mixtures are run in parallel with different agent concentrations to obtain a differential response to the various concentrations. Typically, one of these concentrations serves as a negative control, *i.e.* at zero concentration or below the level of detection.

Candidate agents encompass numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of more than 50 and less than about 2,500 daltons. Candidate agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The candidate agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules including, but not limited to: peptides, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof.

Candidate agents are obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides and oligopeptides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means, and may be used to produce combinatorial libraries. Known pharmacological agents may be subjected to directed or random chemical

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modifications, such as acylation, alkylation, esterification, amidification, *etc.* to produce structural analogs.

Where the screening assay is a binding assay, one or more of the molecules may be joined to a label, where the label can directly or indirectly provide a detectable signal. Various labels include radioisotopes, fluorescers, chemiluminescers, enzymes, specific binding molecules, particles, *e.g.* magnetic particles, and the like. Specific binding molecules include pairs, such as biotin and streptavidin, digoxin and antidigoxin *etc.* For the specific binding members, the complementary member would normally be labeled with a molecule that provides for detection, in accordance with known procedures.

A variety of other reagents may be included in the screening assay. These include reagents like salts, neutral proteins, *e.g.* albumin, detergents, *etc.* that are used to facilitate optimal protein-protein binding and/or reduce non-specific or background interactions. Reagents that improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, *etc.* may be used. The mixture of components are added in any order that provides for the requisite binding. Incubations are performed at any suitable temperature, typically between 4 and 40°C. Incubation periods are selected for optimum activity, but may also be optimized to facilitate rapid high-throughput screening. Typically between 0.1 and 1 hours will be sufficient.

Other assays of interest detect agents that mimic TIM function. For example, candidate agents are added to a cell that lacks functional TIM, and screened for the ability to reproduce TIM in a functional assay.

THERAPEUTIC METHODS

Agents that modulate activity of TIM genes or proteins provide a point of therapeutic or prophylactic intervention, particularly agents that inhibit or upregulate activity of the polypeptide, or expression of the gene. Numerous agents are useful in modulating this activity, including agents that directly modulate expression, *e.g.* expression vectors, antisense specific for the targeted polypeptide; and agents that act on the protein, *e.g.* specific antibodies and analogs thereof, small organic molecules that block catalytic activity, *etc.*

Methods can be designed to selectively deliver nucleic acids to certain cells. Examples of such cells include T cells, *etc.* Certain treatment methods are designed to selectively express an expression vector to cells of interest. One technique for achieving selective expression in nerve cells is to operably link the coding sequence to a promoter that is primarily active in immune system cells, *e.g.* IL-2 promoter, T cell antigen receptor promoter, and the like. Alternatively, or in addition, the nucleic acid can be administered with an agent that targets the nucleic acid to the cells of interest. For instance, the nucleic

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acid can be administered with an antibody that specifically binds to a cell-surface antigen. When liposomes are utilized, substrates that bind to a cell-surface membrane protein associated with endocytosis can be attached to the liposome to target the liposome to nerve cells and to facilitate uptake.

- 5 Antisense molecules can be used to down-regulate expression in cells. The antisense reagent may be antisense oligonucleotides (ODN), particularly synthetic ODN having chemical modifications from native nucleic acids, or nucleic acid constructs that express such antisense molecules as RNA. The antisense sequence is complementary to the mRNA of the targeted gene, and inhibits expression of the targeted gene products.
- 10 Antisense molecules inhibit gene expression through various mechanisms, e.g. by reducing the amount of mRNA available for translation, through activation of RNase H, or steric hindrance. One or a combination of antisense molecules may be administered, where a combination may comprise multiple different sequences.

- Antisense molecules may be produced by expression of all or a part of the target gene sequence in an appropriate vector, where the transcriptional initiation is oriented such that an antisense strand is produced as an RNA molecule. Alternatively, the antisense molecule is a synthetic oligonucleotide. Antisense oligonucleotides will generally be at least about 7, usually at least about 12, more usually at least about 20 nucleotides in length, and not more than about 500, usually not more than about 50, more usually not
- 20 more than about 35 nucleotides in length, where the length is governed by efficiency of inhibition, specificity, including absence of cross-reactivity, and the like. It has been found that short oligonucleotides, of from 7 to 8 bases in length, can be strong and selective inhibitors of gene expression (see Wagner *et al.* (1996) *Nature Biotechnology* 14:840-844).

- A specific region or regions of the endogenous sense strand mRNA sequence is chosen to be complemented by the antisense sequence. Selection of a specific sequence for the oligonucleotide may use an empirical method, where several candidate sequences are assayed for inhibition of expression of the target gene *in vitro* or in an animal model. A combination of sequences may also be used, where several regions of the mRNA sequence are selected for antisense complementation.

- 30 Antisense oligonucleotides may be chemically synthesized by methods known in the art (see Wagner *et al.* (1993) *supra.* and Milligan *et al.*, *supra.*) Preferred oligonucleotides are chemically modified from the native phosphodiester structure, in order to increase their intracellular stability and binding affinity. A number of such modifications have been described in the literature, which alter the chemistry of the backbone, sugars or
- 35 heterocyclic bases.

Among useful changes in the backbone chemistry are phosphorothioates; phosphorodithioates, where both of the non-bridging oxygens are substituted with sulfur;

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phosphoroamidites; alkyl phosphotriesters and boranophosphates. Achiral phosphate derivatives include 3'-O'-5'-S-phosphorothioate, 3'-S-5'-O-phosphorothioate, 3'-CH₂-5'-O-phosphonate and 3'-NH-5'-O-phosphoroamidate. Peptide nucleic acids replace the entire ribose phosphodiester backbone with a peptide linkage. Sugar modifications are also used to enhance stability and affinity. The alpha.-anomer of deoxyribose may be used, where the base is inverted with respect to the natural .beta.-anomer. The 2'-OH of the ribose sugar may be altered to form 2'-O-methyl or 2'-O-allyl sugars, which provides resistance to degradation without comprising affinity. Modification of the heterocyclic bases must maintain proper base pairing. Some useful substitutions include deoxyuridine for deoxythymidine; 5-methyl-2'-deoxycytidine and 5-bromo-2'-deoxycytidine for deoxycytidine. 5-propynyl-2'-deoxyuridine and 5-propynyl-2'-deoxycytidine have been shown to increase affinity and biological activity when substituted for deoxythymidine and deoxycytidine, respectively.

The compounds having the desired pharmacological activity may be administered in a physiologically acceptable carrier to a host for treatment of asthma attributable to a defect in TIM function. The compounds may also be used to enhance TIM function. The therapeutic agents may be administered in a variety of ways, orally, topically, parenterally *e.g.* subcutaneously, intraperitoneally, by viral infection, intravascularly, *etc.* Inhaled treatments are of particular interest. Depending upon the manner of introduction, the compounds may be formulated in a variety of ways. The concentration of therapeutically active compound in the formulation may vary from about 0.1-100 wt.%.

The pharmaceutical compositions can be prepared in various forms, such as granules, tablets, pills, suppositories, capsules, suspensions, salves, lotions and the like. Pharmaceutical grade organic or inorganic carriers and/or diluents suitable for oral and topical use can be used to make up compositions containing the therapeutically-active compounds. Diluents known to the art include aqueous media, vegetable and animal oils and fats. Stabilizing agents, wetting and emulsifying agents, salts for varying the osmotic pressure or buffers for securing an adequate pH value, and skin penetration enhancers can be used as auxiliary agents.

It is to be understood that this invention is not limited to the particular methodology, protocols, cell lines, animal species or genera, and reagents described, as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

As used herein the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a

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cell" includes a plurality of such cells and reference to "the array" includes reference to one or more arrays and equivalents thereof known to those skilled in the art, and so forth. All technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs unless clearly indicated otherwise.

All publications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing, for example, the cell lines, constructs, and methodologies that are described in the publications which might be used in connection with the presently described invention. The publications discussed above and throughout the text are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention.

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the subject invention, and are not intended to limit the scope of what is regarded as the invention. Efforts have been made to ensure accuracy with respect to the numbers used (*e.g.* amounts, temperature, concentrations, *etc.*) but some experimental errors and deviations should be allowed for. Unless otherwise indicated, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees centigrade; and pressure is at or near atmospheric.

EXPERIMENTAL

To analyze the human 5q23-35 region for asthma susceptibility genes, we utilized a mouse model, which offers several potential advantages. Environmental variation can be controlled, multiple phenotypes can be tested simultaneously, and inbred strains can be sensitized with allergen to develop airway hyperreactivity (AHR), a cardinal feature of human asthma. We utilized congenic inbred mouse strains that differed only by a small chromosomal region homologous to human chromosome 5q, thereby allowing this region to be studied in the absence of genetic variation outside the region. Positional cloning revealed a novel gene family encoding T cell membrane proteins (*Tim*), TIM-1, TIM-2, TIM-3, TIM-4, TIM-5, TIM-6, and TIM-7, in which major sequence variants of TIM-1, TIM-3, and TIM-4, cosegregate completely with *Tapr*.

IL-4 production and airway hyperreactivity are reduced in HBA mice. We examined congenic mice produced on a BALB/c genomic background with discrete genomic intervals inherited from individual DBA/2 chromosomes. BALB/c mice develop Th2 biased, atopy-resembling immune responses with enhanced AHR, while DBA/2 mice

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develop reduced IL-4 responses that protect against the development of AHR. By screening several of these congenic strains for reduced Th2 responsiveness, we identified one congenic line, C.D2 Es-Hba (HBA), which contained a segment of chromosome 11 inherited from DBA/2 mice, homologous to human 5q23-35. Fig. 1a shows that lymph node cells from immunized control BALB/c mice, as expected, produced high levels of IL-4, confirming the proclivity of BALB/c mice to develop Th2-biased immune responses. In contrast, lymph node cells from HBA mice produced significantly lower levels of IL-4, similar to that observed in DBA/2 mice. In addition, HBA mice produced significantly less IL-13 and IL-10, and somewhat lower levels of IL-5 compared to BALB/c mice, whereas production of IFN- γ was increased, as shown in Fig. 1b. These results indicated that the DBA/2-derived region of HBA chromosome 11, which has large regions of conserved synteny with human 5q23-35, contains a gene that reduces antigen-specific IL-4, IL-13, and IL-10 production, enhances IFN- γ production, and converts the BALB/c cytokine phenotype into a DBA/2 cytokine phenotype.

The HBA mice were examined for the capacity to develop antigen-induced airway hyperreactivity (AHR), which is associated with Th2-biased immune responses. Upon sensitization and challenge with allergen, control BALB/c mice developed high AHR, whereas similarly immunized HBA congenic mice, like DBA/2 mice, expressed normal airway reactivity in response to methacholine (Fig. 1c). Collectively, these results strongly suggested that genetic variation in a single locus on chromosome 11 regulated both Th2 cytokine production and AHR; therefore, we tentatively refer to the relevant genetic determinant(s) in HBA mice as a single locus, *T cell and Airway Phenotype Regulator (Tapr)*.

We also examined (BALB/c x HBA) F1 mice, which like the BALB/c mice, produced high levels of IL-4, IL-13, and IL-10 (Fig. 1a and 1b) and developed elevated antigen-induced AHR (Fig. 1c). These results indicate that a DBA/2 allele on chromosome 11, in isolation of other genes that regulate IL-4 synthesis, reduced IL-4 production and AHR in a recessive manner. In contrast, (BALB/c x DBA/2) F1 mice produced low levels of IL-4 and had normal airway responsiveness on immunization (Fig. 1), indicating that loci from other regions of the DBA genome also modulated IL-4 production and antigen-induced AHR, and that the DBA/2 alleles, in aggregate, functioned in a dominant manner to limit IL-4 production and AHR. These results underscore the multigenic, complex nature of atopic traits and demonstrate the potential advantages of using a congenic strain to isolate and characterize a single locus without interference from multiple epistatic genes that also influence the asthmatic phenotype.

Genetic Mapping of Tapr, the locus which controls AHR and IL-4 responsiveness. Previously, the congenic region in the HBA mice was delineated with 36

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genome-wide markers, including two chromosome 11 markers, hemoglobin- $\alpha 2$ (*hba- $\alpha 2$*) and esterase-3 (*es-3*) loci. The HBA genome, outside of chromosome 11, was inherited from BALB/c. A more precise analysis with 25 simple sequence length polymorphism (SSLP) markers known to be polymorphic between DBA/2 and BALB/c mice showed that

5 HBA mice inherited two segments of chromosome 11 from DBA/2 (Fig. 2, left column). The proximal region contained a 20 cM segment with homology to chromosome 5q23-35, which afforded the possibility that a genetic locus implicated in human asthma linkage studies could be identified in a mouse model of asthma.

To map at higher resolution the T_{H2} -AHR controlling locus, *Tapr*, (BALB/c x

10 HBA) F1 mice were backcrossed to HBA mice to produce N2 animals. With this backcross approach, the set of alleles contributed by the HBA parent is pre-determined, and the set of alleles contributed by the F1 parent can be assessed by genotyping. Thus, recombination events that produce informative haplotypes within the congenic region can be detected in the N2 mice and used to assess the linkage of *Tapr* to loci in the congenic interval.

15 Because of the recessive nature of *Tapr*, we tested N2 mice from these backcrosses to identify the minimum homozygous region of HBA-derived genes sufficient to confer the HBA *Tapr* phenotype. More than 2,000 N2 animals were generated and genotyped. Using SSLP markers, we selected those N2 mice with informative recombination events, and the N2 mice were phenotyped for the capacity to produce IL-4 in response to immunization with

20 keyhole limpet hemocyanin (KLH). In this primary analysis, we determined that the relevant locus resided within the proximal congenic region, between *D11Mit135* and *D11Mit260*. In order to map *Tapr* at higher resolution, 22 additional markers were identified and utilized to provide 0.1-1 cM resolution in the area of interest.

To accurately compare the results of IL-4 cytokine analyses performed over several

25 months time, an IL-4 index for each experiment was generated for each N2 mouse, $\left(\frac{B-x}{B-H} \right)$, where B=IL-4 production by cells from BALB/c mice, H=IL-4 production by cells from HBA mice, and x=IL-4 production by cells from the N2 mouse being assessed. High concentrations of IL-4 (BALB/c-like) are represented by index values near 0, and low concentrations of IL-4 (HBA-like) are represented by index values near 1.0. The "B and "H" values were established with 3-5 control mice for each group of 3-6 N2 mice carrying

30 informative recombinations that we tested. The index values fall within a bimodal distribution (Fig. 3a), in which the phenotype index associated with N2 mice that had nonrecombinant HBA genotypes was significantly higher ($P<0.0001$, in a paired Student's t-test) than the phenotype index associated with N2 mice that had nonrecombinant (BALB/c x

35 HBA)F1 genotypes.

For the mice with unique genotypes, we used several methods to ensure the

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adequacy of single measurements of cytokine production and AHR, since this is critical in linkage analysis. First, at the same time that we tested each of the N2 mice carrying recombinations of interest, we also tested "non-recombinant" siblings of each "recombinant N2" that were strictly HBA or F1 (BALB x HBA) in genotype. Furthermore, we bred additional N3 mice by crossing some of the N2 mice carrying recombinations of interest back to HBA mice, in order to have more individual mice with that particular N2 genotype. All values were the average of the values for the individual mice tested with a given genotype. In this way, we are confident of the measures of cytokine production and AHR, and that we have overcome assay variations due to variables inherent in biological systems.

Because the IL-4 values associated with the N2 mice that inherited recombinant haplotypes segregated in a bimodal distribution (Fig. 3a), were able to demonstrate that the genetic locus that controls high IL-4 responses is located between markers *D11Mit271* and *D11Mit22* (Fig. 3b). Moreover, high levels of IL-4 production were observed in all mice with a BALB/c allele present at *Kim1sscp*, and low levels of IL-4 production were observed in all mice with homozygous HBA genotypes at *Kim1sscp*. Thus, *Tapr* was nonrecombinant with *Kim1sscp*, an intronic marker within a mouse homologue of *Rattus norvegicus* Kidney Injury Molecule (*Kim-1*). In contrast, *Tapr* segregated from all other markers with at least one recombination. The fact that *Tapr* and *Kim1sscp* segregated together, indicated that the *Tapr* locus is located very close to or is indistinguishable from *Kim1sscp*. Based on the frequency recombinant haplotypes between *D11Mit271* and *D11Mit22*, we calculate a recombination frequency, 0.0039, which indicates that the *Tapr* locus maps to a small, 0.3-0.5 cM, region. We also calculated a recombination frequency of 0.08 between *Tapr* and IL-4. Therefore, *Tapr* is located 5-10 cM away from the IL-4 cytokine cluster but is within the a region of the mouse genome that has highly conserved synteny with the 5q23-35 region that has been linked to human atopy and asthma.

Using an analogous approach, we examined the segregation of allergen-induced AHR phenotypes in mice with informative recombinant haplotypes. With indexed AHR values, N2 mice clearly exhibit parental phenotypes, which produced a bimodal distribution in a histogram of AHR index values in a group of sensitized N2 mice (Fig. 3c). By analyzing the segregation of AHR phenotypes associated with more than 1,000 N2 mice, we demonstrated that the genetic locus which controls AHR responses is also located between markers *D11Mit271* and *D11Mit22* (Fig. 3d) and that the AHR phenotype was nonrecombinant with *Kim1sscp*. Thus, we demonstrate that both IL-4 responsiveness and AHR cosegregate with the *Tapr* locus, which suggests that the same locus regulates both IL-4 expression and AHR (Fig. 3).

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These findings further demonstrate that the *Tapr* locus is more than 5 cM centromeric to the IL-4 cytokine cluster and the cytokine genes in the cluster previously thought to be 'candidate' atopy or asthma susceptibility genes. Our mapping results also establish that *Tapr* is genetically separable from both the IL-12p40 gene and the region of mouse chromosome 11 that includes the T_H1 -IL12 regulatory locus, *Tpm*.

Mouse and human homologues anchor Tapr to human 5q33. In order to construct a composite map around the *Tapr* locus, we integrated available information from the Mouse Genome Database (MGD) linkage, backcross, and radiation hybrid maps and identified a region of conserved synteny in maps of the human genome. Current radiation hybrid maps place the markers that are near *D11Mit271* and *D11Mit22*, including several expressed sequence tags (ESTs) that have extensive homology to known genes or unigene clusters, onto a physical map of the mouse genome. We further examined these markers and their associated ESTs for previously unidentified similarity to known gene clusters. We assembled these markers onto a scaffold for comparison to the human genome. Using this approach, we found significant similarity between particular radiation hybrid markers and the following human genes: *KIAA0171*, *Adam-19*, *Sox-30*, *Pir-121*, *Crsp9* (*Crsp33*), and *hHAVcr-1* (*hHAVcr-1*). Fig. 4 demonstrates that once we anchored these genes to a physical map of the mouse genome between our flanking markers, we were able to locate those genes in the Human Genome Browser.

The high degree of conservation between the mouse and human genomes in this region indicates linkage of the *Tapr* locus to human 5q33.2. As shown Fig. 4, we identified all known genes and ESTs in this region of the human map. Genes of particular interest near human *hHAV-cr* and the mouse homologue of *Kim-1*, include IL-2 inducible T cell kinase (*Itk*) and a coregulator of the SP-1 transcription factor (*Crsp9*), both known to be involved in T cell differentiation. We sequenced coding regions from these candidate genes and found no polymorphisms in either ITK or CRSP-9.

Localization of a Family of Novel T cell Surface Proteins to the Tapr Region. Because the mouse homolog of rat *Kim1* is located within the 0.4 cM region and is tightly linked with *Tapr*, we examined publicly available databases and found clusters of ESTs with some sequence similarity that provided only partial coverage and contained large segments of variation. The closest human homolog of *Kim-1* is the human hepatitis A virus cellular receptor, *hHAV-cr*, and tBLAST searches of the human genome suggested that two additional homologs of *Kim-1*, perhaps members of a gene family, also are located on human chromosome 5 and mouse chromosome 11.

Using cDNA from conA-stimulated splenocytes, we identified and cloned two

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mouse orthologues of *Kim1*, which we term *Tim1* and *Tim2*, that map to the *Tapr* region, as shown in Fig. 5A. TIM-3 is a third, more distantly related, orthologue of KIM-1.

All three members of this gene family are expressed by stimulated T cells, and all three forms map to the *Tapr* region of mouse chromosome 11/ human chromosome 5 where they encode cell surface glycoproteins with common structural motifs, including an immunoglobulin (Ig) domain, mucin domain, and intracellular tail with phosphorylation sites. Because the cellular functions of these proteins is unknown, we refer to the genes as members of a *T cell, Immunoglobulin domain, Mucin domain (Tim)* gene family. Mouse *Tim1* is the mouse homologue of rat *Kim1* and the *HAVcr-1* identified in African green monkeys and humans. *Tim2* is a previously unknown gene that had not been identified in any organism prior to this study.

The mouse *Tim1* gene encodes a 305 amino acid membrane protein, that has 78% overall identity with rat KIM-1 and 35% identity with human HAVcr-1. A gapped multiple sequence alignment with mouse TIM-1, rat KIM-1, human HAVcr-1 and African green monkey HAVcr-1, shown in Fig. 5B, demonstrates the degree of homology between the TIM-1/KIM-1/HAVcr-1 proteins in these species. The cytoplasmic region of TIM-1 contains two tyrosine residues and includes a highly conserved tyrosine kinase phosphorylation motif, RAEDNIY, which is integral to the predicted Itk and EGFR kinase site of TIM-1, SRAEDNIYVEDRP. The mucin domain of TIM-1 has multiple sites for O-linked glycosylation, and there two sites for N-linked glycosylation found in the immunoglobulin domain.

TIM-2, a similar 305 amino acid membrane protein, has 64% identity to mouse TIM-1, 60% identity to rat KIM-1, and 32% identity to hHAVcr-1 (Figure 5A, B). Like TIM-1, TIM-2 has two extracellular N-linked glycosylation sites and a serine, threonine-rich mucin domain with many O-linked glycosylation sites. TIM-2 also has an intracellular tyrosine kinase phosphorylation motif, RTRCEDQVY.

Tim3 encodes a 281 amino acid membrane protein that has a similar, integral membrane glycoprotein structure with multiple extracellular glycosylation sites and an intracellular tyrosine phosphorylation motif. Although the mucin domain is not as prominent in TIM-3 as it is in TIM-1 and TIM-2 (Figure 5A), TIM-3 expressed on T cells likely interacts with a ligand on APCs and alters APC activation. TIM-3 does have four sites for N-linked and five sites for O-linked glycosylation, suggesting that TIM-3, like TIM-1 and TIM-2, is heavily glycosylated and might interact with a ligand present on other cells, such as antigen presenting cells.

Tim4 encodes a 344 amino acid protein in mice, and a 378 amino acid protein in humans. The predicted TIM-4 also shares the general membrane glycoprotein structural motifs of the other TIM proteins, a with an IgV-like domain with highly conserved

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cysteine residues, a threonine-rich mucin-like domain, and a short intracellular tail. However, TIM-4 lacks the phosphotyrosine motif present in the other TIM proteins, and therefore may modulate the function of the other TIM proteins.

Each of the TIM Ig domain shares an predicted integrin-binding motif that is similar to the SVVYGLR motif found in osteopontin, an transmembrane protein like the TIMs that is implicated in the regulation of cell adhesion, survival, and oncogenesis, as well as in the regulation of helper T cell differentiation. This integrin binding motif demonstrates alpha(9) and alpha(4) specificity.

Comparison of the sequences of the BALB/c and HBA/DBA coding regions for the three *Tim* genes revealed major polymorphisms in TIM-1, TIM-3, and TIM-4, but not TIM-2. In TIM-1, these polymorphisms encode three amino acid differences and a fifteen amino acid deletion in HBA/DBA. Seven predicted amino acid differences were identified in TIM-3 (Fig. 5c). Genomic sequences confirm that these polymorphisms, including the deletion, are true polymorphisms, not splicing variants. By further sequencing genomic segments of TIM-1 and TIM-3 in other mouse strains, we found that C57/BL6, a strain similar to DBA/2 with respect to its tendency to develop reduced T_H2 and AHR responses, also has the HBA/DBA allele of *Tim1* and *Tim3*. The polymorphisms in TIM-1 and TIM-4 are located in the signal and mucin-like domains, while the polymorphisms identified in TIM-3 are clustered in the Ig domain (Fig. 5c). In glycoproteins with Ig and mucin domains, variants in either domain may affect receptor-ligand interactions, as shown for MAdCAM-1. Although the predicted cleavage sites of TIM-1 and TIM-4 are unaltered by the polymorphism in the signal sequence, it is possible that the polymorphism may affect the efficiency of cleavage and/or trafficking of the receptor to the cell surface. These *Tim* sequences and polymorphisms are important for immune responses, and for HAV viral pathogenesis in humans.

Analysis of genomic DNA samples from our N2 backcross (Fig. 3) demonstrated that the TIM-1 and TIM-3 polymorphisms cosegregate completely with *Tapr*. While these observations do not distinguish the extent to which changes in TIM-1, TIM-3, or both, are responsible for changes in AHR and T_H2 -mediated inflammation, we suggest that polymorphisms in human TIM-1(hHAVcr-1) and/or TIM-3 underlie the strong association between asthma susceptibility and human chromosome 5q. This idea is supported by the fact that major variants in coding regions of human *Tim1* are evident on examination of human genome and EST databases. Comparison of these human cDNA variants with the previously described variants of monkey HAVcr-1 and the mouse variants identified here demonstrates that there is extensive variation in the predicted protein sequences of TIM-1 (Fig 5b,c). This high degree of variation distinguishes TIM-1 and its family members from

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many other candidate genes, such as the cytokines and the cytokine receptors that have been most closely studied as asthma susceptibility candidate genes. In addition, the association between *Tim1* and asthma susceptibility is further supported by reports of significant linkage of mite-sensitive childhood asthma to D5S820 (mean LOD score = 4.8),
 5 a marker which is approximately 0.5 megabases from *Tim1* and *Tim3* (Fig. 4).

In addition to the above genetic polymorphisms, there are several expression polymorphisms in the TIM genes that arise due to alternate splicing. Alternate splicing of TIM-1, TIM-2 and TIM-4 mRNA produces several TIM variants, some of which are secreted, soluble forms of the TIM receptors. These splice variants, along with TIM splice
 10 variants that have alternate 5' untranslated regions, may contribute to the cell-specific and condition-specific expression patterns of the TIM proteins.

T cells confer the Tapr effect. To better understand the function of the *Tapr* locus we determined whether allelic variation of *Tapr* affected the function of T cells or of antigen presenting cells (APC). For these experiments, we generated ovalbumin (OVA)-
 15 specific T cell receptor (TCR) transgenic mice (Tg) with the HBA background (HBA DO11.10), which we compared to TCR-Tg mice with the BALB/c background (BALB/c DO11.10). Purified CD4+ T cells from either of these strains were cocultured with OVA and dendritic cells (DCs) derived from either BALB/c or HBA bone marrow, and the cytokines
 20 produced were evaluated. Irradiated spleen cells were not used as APCs for this experiment, because it was found that irradiated spleen cells and other tissues express high levels of the TIM genes.

BALB/c DO11.10 T cells produced higher levels of IL-4 and IL-13 than did HBA DO11.10 T cells, in a manner that was independent of the source of the antigen
 25 presenting cells (Fig. 6A). In addition, the source of the CD4 T cells determined the amount of IL-4/IL-13 produced at each antigen concentration, regardless of the source of the APC during either the primary or secondary stimulation. Equivalent levels of IL-12 were detected in culture supernatants for each combination of cell types, further demonstrating that BALB/c and HBA DC function were comparable. Furthermore, BALB DO11.10 and
 30 HBA DO11.10 T cells produced equivalent levels of IL-2 and demonstrated comparable levels of proliferation in response to OVA during the secondary cultures, indicating that HBA and BALB/c T cells are similarly activated, although the levels of Th2 cytokines they produce are quite distinct.

We show in Figure 6B that within the first twelve hours of primary culture in our DO11.10/DC system, we find that mRNA for TIM-1 is expressed by both BALB/c and
 35 HBA CD4+ T cells. Within four days of primary stimulation, we find significant levels of IL-13 in supernatants of the BALB/c DO11.10 and detect none in the HBA DO11.10

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supernatants. This differentiation is detectable in mRNA levels at 36 hours (Fig. 6B). Between twelve and thirty six hours, expression of IL-13 mRNA is reduced in HBA CD4 T cells, while IL-13 expression is maintained in the BALB/c CD4 T cells. Thus, during the primary response to antigen, BALB/c CD4 T cells develop a stronger Th2 response than do HBA CD4 T cells. Our findings demonstrate that *Tapr* regulates helper T cell differentiation during primary antigen specific responses, and we detect TIM-1 expression in CD4 T cells during the earliest stages of these responses.

Following differentiation into mature Th1 and Th2 subsets, helper T cells demonstrate committed TIM expression by RT-PCR, such that Th1 cells express TIM-3, while Th2 cells preferentially express TIM-1. All T cell populations demonstrate weak TIM-4 expression. While the Itk signal through TIM-1 is likely to promote Th2 differentiation, the EGFR signal through the TIM proteins is likely to enhance cell survival in effector and especially memory T cell populations. Since Itk is expressed only in T cell and mast cells, the Itk kinase activity on TIM-1 is restricted to immune cells, particularly those involved in asthma and allergy. However, other protein tyrosine kinases, such as EGFR, are involved in the function of TIM proteins expressed by other tissues, including ischemic epithelial cells, irradiated spleen cells, and tumor cells.

In these studies, we mapped *Tapr*, a locus that regulates the development of Th2 cytokine production and antigen-induced AHR, a cardinal feature of asthma. We localized *Tapr* using an interval specific congenic mouse (HBA) that carried a chromosomal segment homologous to human chromosome 5q, a region of the human genome that has been repeatedly linked to atopy and asthma. This region has also been repeatedly linked to 5q- syndrome associated with myelodysplasia and neoplastic cytogenic abnormalities. Using this congenic mouse strategy that converted a complex trait into a simpler, possibly single gene, trait, we narrowed the interval of *Tapr* to 0.4 cM interval, sequenced several candidate genes in this region, and positionally cloned the TIM gene family.

The TIM gene family has not been previously described. We identified and cloned the full cDNA sequence and discovered significant polymorphisms in the TIM-1 proteins of BALB/c compared to HBA mice. We found that the BALB/c sequences for TIM-1 and TIM-3 are associated with susceptibility to AHR and allergic T cell responses, whereas the HBA sequences are associated with protection against these responses. TIM-3 is preferentially expressed by differentiated T_H1 . The association of polymorphic *Tim3* variants with *Tapr* suggests that TIM-3 might regulate T_H1 and T_H2 cell function. However, the variations in *Tim3* might also be attributed to a haplotype tightly linked to *Tim4* or *Tim1*.

We believe that TIM-1 plays a very important role in the regulation of the immune system (particularly with respect to asthma and allergic disease) and in the the

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regulation of epithelial and hematopoietic cell survival in response to stress (hypoxia, nutritional deficiency, irradiation, chemotherapy, etc.) for several reasons. First, *Tim1*, like *Tim3*, is expressed in CD4 T cells during primary antigen stimulation, when it is most likely that the *Tapr* effect occurs. T cells play a critical role in the development of AHR and in the pathogenesis of asthma, our results suggest that *Tapr* affects asthma by enhancing early CD4 commitment to Th2 responses by controlling the production of IL-13 and subsequent T cell differentiation. Second, HAV infection in humans during infancy or childhood is inversely associated with the development of asthma and allergy. We suggest that the HAV interaction with TIM-1/HAVER-1 may alter the T cell cytokine production may able to reverse or prevent the biased Th1/Th2 balance in individuals otherwise prone to atopy and asthma. SLAM, a measles virus receptor, is an example of another T cell surface glycoprotein that regulates the Th1/Th2 balance in a manner that may be altered by viral interaction. Because some viral receptors, such as SLAM for the measles virus or CD4, CCR5, and CXCR4 for HIV, are receptors of the host's own immune system, even when an infection does not succeed, virus-receptor mediated signal transduction can lead to the release of cytokines and the development of disease.

Third, the polymorphisms in TIM-1 are associated with the different types of helper T cell responses that we observe. Therefore, the variants of TIM-1 may themselves contribute to the genetic Th1/Th2 predisposition that occurs in the absence of any known environmental cause of immune deviation. The HAV receptor in primates is known to be highly variable, and we propose that polymorphic alleles of human TIM-1/hHAVER-1, like those we have identified in mice, may be associated with variations in Th2 bias and asthma susceptibility. Mutations in the genes for cell surface molecules that serve as viral receptors and that alter susceptibility to infection are not uncommon, and therefore significant genetic variation in TIM-1 and other members of the TIM gene family is far more likely to be observed than variation in other genes such as those for cytokines. It is unclear why asthma susceptibility alleles might be prevalent in the human gene pool, but the association of *Tapr* with HAVER provides an interesting explanation for the persistence of asthma susceptibility alleles. During human evolution certain alleles of the *Tim* gene family may have conferred resistance to atopic diseases and other immune disorders, but selection of those resistance alleles may have been counterbalanced by selection of alternate alleles that confer resistance to viral pathogenesis.

In summary, our studies represent the first successful utilization of a congenic mouse strategy to locate a strong candidate asthma susceptibility gene and overcome the inherent difficulties in the examination of this complex genetic trait. We identified a previously unknown gene family that exists in a region homologous to human

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chromosome 5q, and which plays a major role in Th cell development and in asthma susceptibility. While prior studies in humans identified several candidate genes on human chromosome 5q, the *Tim1* gene product identified in our study also provides an explanation for the inverse relationship between HAV infection and reduced asthma susceptibility.

5 Subpopulations of CD4⁺ T cells (Th) produce distinct patterns of cytokines, and this has led to the concept of functional heterogeneity among Th cells. Type 1 Th cells (Th1) produce interleukin 2 (IL-2) and/or interferon γ , elicit delayed type hypersensitivity (DTH) responses and activate macrophages. Type 2 Th cells (Th2), on the other hand, produce IL-4, IL-5 and IL-10 and are especially important for IgE production and eosinophilic inflammation, and may suppress cell mediated immunity. Th2 cells are
10 believed to play a pivotal role in the pathogenesis of atopy. Several factors determine whether a T helper cell will differentiate into Th1 versus Th2 during a particular immune response. These include, but are not necessarily restricted to, the cytokine milieu, the strength of the TCR signal and/or antigen density, and the costimulatory pathways. CD4⁺ T
15 helper cell differentiation into Th1 or Th2 subsets has profound effects on the outcome of atopy, autoimmune diseases, infectious diseases, and graft rejection.

The specific features of immune responses that protect nonatopic individuals from the development of allergic diseases and which could inhibit allergic responses in atopic individuals are poorly understood. Because Th1 cells cross regulate Th2 cells in
20 some systems, allergen-specific Th1 cells have been assumed to regulate allergic disease and asthma. Th1 cells inhibit the development and proliferation of Th2 cells, and IgE production is reciprocally regulated by IL-4 and IFN- γ . This suggests that protection from allergy is due to the development of inhibitory allergen-specific Th1 cells. Allergen-specific T cell clones derived from the peripheral blood of nonallergic individuals have been shown
25 to produce Th1 cytokines. These observations have also supported the hygiene hypothesis of asthma, which suggests that the prevalence of infections, particularly those that induce Th1 responses, are reduced in westernized societies by improved public health measures and the use of vaccines and antibiotics. As a result, Th2 responses and atopy develop more intensely and rapidly in the absence of Th1 mediated responses.

30 The TIM genes identified herein are also candidate oncogenes. Transfection of cell lines with TIM genes confers resistance to cell death, and the predicted EGFR kinase motif described in TIM-1 provides a probable mechanism by which this cell survival is controlled. Furthermore, TIM-1 demonstrates a significant degree of sequence identity (approximately 20%) and structural similarity (a transmembrane glycoprotein with
35 an IgV domain, mucin/syndecan domain, transmembrane domain, and intracellular domain with similar phosphotyrosine motifs) with TOSO, a protein that protects cells from Fas-mediated apoptosis. Like the TIM genes, TOSO is a likely oncogene, which maps to a

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region of the genome with frequent changes in hematologic malignancies and solid tumors.

METHODS

- Animals. Congenic lines, including C.D2 Es-HBA were generated by
- 5 introgressively backcrossing DBA/2N onto a BALB/cAnPt background. BALB/cBy, DBA/2J, and (BALB/c x DBA/2) F1 mice (CByD2F1/J) were obtained from the Jackson Laboratory (Bar Harbor, ME), while BALB/cAn and DBA/2N were obtained from Taconic Labs. (BALB/c x HBA) F1 mice were produced with a cross between BALB/cByJ and HBA. N2
- 10 mice were generated by backcrossing (BALB/c x HBA) F1 to HBA. In our analysis of recombinant N2 animals, recombinant mice were tested along with non-recombinant siblings, whenever possible. In order to examine individual N2 genotypes in multiple assays, we preserved selected recombinant haplotypes by backcrossing selected N2 mice to HBA to generate N3 mice, which were genotyped to chose mice carrying the
- 15 recombinant N2 haplotype. DO11.10 mice, which are transgenic for TCR recognizing OVA peptide 323-339 (pOVA³²³⁻³³⁹) and backcrossed to BALB/c(43), were kindly provided by Dr. Dennis Loh and were bred in our facilities. HBA DO11.10 mice were produced by backcrossing DO11.10 to HBA. DO11.10 mice were selected by FACS analysis for the TCR-Tg and genotyped to select for HBA alleles between *D11Mit135* and *D11Mit168*. The Stanford University Committee on Animal Welfare approved all animal protocols.
- 20 Genotyping. Additional markers around the *Tapr* locus were identified by testing all available "D11Mit-" markers present between *D11Mit140* and *D11Mit269* and all radiation hybrid markers near *D11Mit271* and *D11Mit22* for any polymorphisms between DBA/2 and BALB/c. MIT MapPair primers were obtained from Research Genetics (Huntsville, AL), and all other primers were synthesized in the Protein and Nucleic Acid
- 25 Facility (Stanford, CA). PCR was performed as previously described, and SSCP polymorphisms were resolved with 4-5% Metaphor agarose (BioWhittaker, Walkersville, MD). Products analyzed for SSCP were amplified with ³²P-dCTP and separated on denaturing acrylamide gels at 40W and 4°C, with a Sequi-Gen GT System (Bio-Rad, Hercules, CA).
- 30 Immunization protocols. Mice studied in cytokine production assays were primed with KLH (Calbiochem, La Jolla, CA) in complete Freund's adjuvant (CFA) (DeKruyff et al. *J Immunol* **149**, 3468-76 (1992)). For measurement of airway hyperreactivity, mice were immunized with OVA intraperitoneally (i.p., 50 µg) complexed with aluminum
- 35 potassium sulfate (alum) on day 0, and intranasally (i.n. 50 µg OVA in 50 µl of PBS) after light anesthesia on days 7, 8 and 9. Control mice received i.p. injections of alum alone and intranasal PBS. Airway hyperreactivity to inhaled methacholine was measured 24 hours after the last intranasal dose of OVA (day 10).

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Measurement of Airway Responsiveness. Airway responses were assessed by methacholine-induced airflow obstruction from conscious mice placed in a whole body plethysmograph (Buxco Electronics Inc., Troy, NY), as described previously (Hansen et al. *J Clin Invest* **103**, 175-83 (1999)).

5 *Cell Culture.* Lymph node cells from mice primed with KLH were prepared as described previously (Yeung et al. *J Immunol* **161**, 4146-52 (1998)). Transgenic DO11.10 CD4 T cells were positively selected using MACS columns following incubation with anti-CD4 magnetic beads (Miltenyi Biotech, Germany). 2×10^4 cells/well were cocultured in 96-well round bottom plates with 250 μ g/ml OVA and 1×10^4 bone marrow-
10 derived dendritic cells/well. After seven days, the DO11.10 T cells were washed and restimulated with fresh antigen presenting cells and antigen at the concentration indicated. Antigen concentration for the primary DO11.10 cultures was titrated during the restimulation. Bone marrow-derived dendritic cells were generated as previously described with some modifications; 5×10^5 bone marrow cells were cultured in 9-cm diameter tissue
15 culture dishes with 10 ml culture medium containing 20-25 U/ml GM-CSF. Loosely adherent cells were transferred onto a second dish on the sixth day of culture; within four days, these transferred cells were used as a source of dendritic cells.

Cytokine ELISA. ELISAs were performed as previously described in Macaulay et al. *J Immunol* **160**, 1694-700 (1998); and Macaulay et al. *J Immunol* **158**,
20 4171-9 (1997).

Monoclonal Antibodies. Monoclonal antibodies for ELISA and FACS analysis were purified from ascites fluid by ammonium sulfate precipitation and ion-exchange chromatography. Anti-clonotypic antibody KJ1-26.1, was generously provided by Dr. Philippa Marrack, National Jewish Medical Center, and the antibody was FITC-
25 conjugated according to standard protocols prior to FACS.

Example 2

Identification of Human Tim Sequences

The positional cloning of the TIM gene family within a locus that confers
30 protection against the development of Th2 responses and allergen-induced airway hyperreactivity provides an opportunity to greatly improve our understanding of the regulation of Th2 driven responses and atopic diseases. In addition, TIM-3 is specifically expressed on murine Th1 cells and anti-TIM-3 mAb leads to increased severity of experimental autoimmune encephalomyelitis (EAE). This emphasizes the importance of
35 the gene family in T helper subset regulation.

The human Tim cDNAs, which are the orthologues of murine Tim-3 and Tim-4 were cloned by PCR. The human orthologue of TIM-1 was cloned as HAVcr-1, the

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cellular receptor for hepatitis A virus. The TIM family genes are immediately adjacent to each other on human chromosome 5, in the order TIM-4, TIM-1, TIM-3, with no intervening genes. There are TIM pseudogenes on chromosomes 12 and 19. The gene family members are only moderately related. The protein sequences and relationship among the
5 Tim gene family are shown in Figure 7.

The cytoplasmic domains of TIM gene family members are the most conserved domain between mouse and human orthologues, e.g., 77% identity between the human and mouse TIM-3 cytoplasmic domains. In contrast, the whole TIM-3 is only 63% identical between human and mouse. Each TIM gene contains a distinct predicted tyrosine
10 signaling motif. The cytoplasmic region of TIM-1 contains two tyrosine residues and includes a highly conserved tyrosine kinase phosphorylation motif, RAEDNIY. The expanded region, SRAEDNIYVEDRP, contains a predicted site for Itk and EGF receptor phosphorylation. Itk is known to phosphorylate phospholipase C- γ (PLC- γ), and thereby
15 trigger a cascade of signaling events that are involved in T cell activation and helper T cell differentiation. Furthermore, Itk signaling affects Th1/Th2 differentiation, and Itk^{-/-} mice do not develop strong Th2 responses. EGF receptor kinase activity is associated with cell survival and resistance to cell death. Similarly, TIM-3 contains distinct, conserved tyrosine phosphorylation and SH2 binding motifs in the cytoplasmic domain. This suggests that the
20 interaction of a TIM with its ligand will engage an intracellular signaling pathway and that each TIM will be distinct in this signaling.

The extracellular IgV domain of the TIM proteins also contains a predicted integrin-binding motif that is similar to the SVVYGLR motif of osteopontin that is involved in adhesion via $\alpha(9)\beta(1)$, $\alpha(4)\beta(1)$, and $\alpha(4)\beta(7)$ integrins. TIM-1
25 transfected pre-B cells of the 300.19 line demonstrate a high degree of adhesion and increased survival in cell culture, as compared to non-transfected 300.19 cells. TIM-1 and TIM-2 transfected CHO cells also demonstrate enhanced survival compared to untransfected CHO cells. These results demonstrate that the TIM proteins regulate cell adhesion and death

Genetic polymorphisms in the human Tim1 and Tim3 genes. SNPs or
30 nucleotide polymorphisms and deletions/insertions present in the human *Tim1* gene are identified. Because SNPs are extremely common in the genome, occurring every 300-600 base pairs, only the coding region of *Tim1* was analyzed. Moreover, genetic variations that are common are also likely to be important. Initially cDNA is sequenced from T cells taken
35 from 30-40 individuals (60-80 chromosomes). Power calculations show that surveying target sequences in coding regions of 60 chromosomes will easily detect SNPs with a population frequency of greater than 1%, and having a more than 90% chance of detecting alleles with a population frequency of 5% or greater. Therefore, screening 30-40

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individuals for sequence variations captures most of the common, functionally relevant, non-conservative, DNA variation present in a population.

Since DNA variants/SNPs in close physical proximity often show strong dependency relationships (i.e., linkage disequilibrium), it is determined if a group of DNA variants (SNP haplotypes) are inherited together, and determined if screening for only a portion of these SNPs will be sufficient for identifying the haplotype. Analysis of large regions of various chromosomes indicate that discrete haplotype blocks (of tens to hundreds of kilobases) are generally present, each with limited diversity punctuated by apparent sites of recombination. To find haplotypes, cDNA is sequenced and searched for combinations of sequence variations that are seen repeatedly in multiple individuals.

Peripheral blood mononuclear cells (PBMC) were from 38 donors, and were stimulated *in vitro* with PHA (7.5 µg/ml) for 24 and 72 hours, or with Concanavalin A (2 µg/ml) for 24 hours. PMA (20 ng/ml) and Ionomycin (1 µM) were added during the last six hours of stimulation. The cells were then harvested and the total RNA was extracted using Trizol reagent (Invitrogen). To obtain cDNA templates for sequencing, RNA was reverse transcribed using Superscript II reverse transcriptase (Invitrogen), according to the manufacturer's protocol. The cDNA were used to PCR amplify the full length of *Tim* cDNA using Herculase Hot Start[™] high fidelity polymerase (Stratagene). The PCR primers were: (SEQ ID NO:37) GTGTCTGACAGTGGCGTA (forward), (SEQ ID NO:38) TTTGCCAGGCAGAACCA (forward), CCACCAAGGTCACGACT (reverse), (SEQ ID NO:39) ATGCCACGGACTAAGACC (reverse). The PCR products were purified with Qiagen QIAquick gel extraction reagents, and sequenced using four internal sequencing primers for *Tim1* and two internal sequencing primers for *Tim3*.

The full length *Tim1* RT-PCR product was cloned in these individuals by taking total RNA from activated T cells and transcribing it with Superscript II and oligo dT. *Tim1* cDNA was amplified with Expand high fidelity polymerase (Roche) to generate a 1 kb product spanning the *Tim1* coding region, which was purified with a PCR Purification kit (Invitrogen). This purified product was then cloned into the TOPO pEF6 vector (Invitrogen), followed by transformation of TOP10 competent bacteria. Bacterial colonies were grown on LB plates with ampicillin selection. Single colonies were picked and plasmid preps generated using Qiagen mini prep kits. Restriction mapping using Hind III digestion was used to select plasmids containing inserts in the correct orientation. These plasmids were then sequenced with three different primers, forward (T7), internal and reverse (BGH), and the sequences aligned in SeqMan program with NCBI human TIM reference sequence.

After sequencing *Tim1* from the chromosomes from 35 individuals (70 chromosomes) several polymorphisms in *Tim1* were identified, which are shown in Figure 8. These polymorphisms are numbered 1-7 (left column). The full sequence of human

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TIM-1, which is listed in the NCBI database (NM_012206), is provided in Figure 8 as a reference point. This sequence is present in less than 20% of the chromosomes that were sequenced, due to the existence of multiple, prevalent sequence polymorphisms in the coding region. 6 additional sequence variations were identified, shown in Figure 8, and all of the polymorphisms were observed in the mucin, extracellular domain, as was true for mice, although the specific variations were distinct from those seen in mice. Importantly, there is a limited degree of association between these variants, in various combinations. The most pronounced variations are the insertion labeled polymorphism 1, 157insMTTTP, which was observed in 65% of the chromosomes, and the deletion in polymorphism 5, 187ΔThr, was observed in 65% of the chromosomes. Polymorphism 4 was observed in 40% of the chromosomes, and the other polymorphisms were each observed in ≤5% of the chromosomes. Notably, most of these variations (2-6) are located within exon 3, the first mucin-encoding exon, and all of the variants occur at the genomic level and are not splice variants.

Based on this sequence analysis of mRNA, a more rapid method for analyzing the genomic DNA from the larger number of patients/controls has been developed. To screen individuals for the variations seen in sequences shown in Figure 8, the DNA is initially tested for simple sequence length polymorphisms (SSLP) in a 150 PCR product, which can detect the major insertion, polymorphism 1, and the deletion, polymorphism 5.

In addition, to genotype the other polymorphisms (2-4, 6, and 7) and identify novel polymorphisms, a relatively simple assay using single strand conformational polymorphism (SSCP) analysis of PCR products has been developed. Under well-optimized conditions, SSCP analysis detects more than 90% of single nucleotide substitutions and all length polymorphisms. For this analysis, PCR primers have been identified that amplify each exon of the *Tim* genes, and variants can be distinguished using standard non-denaturing SSCP gel electrophoresis methods (Figure 9). Non-denaturing polyacrylamide gel electrophoresis is used with an ABI 377 DNA sequence for high resolution SSCP analysis of each exon. Fluorescent end-labeled primers are synthesized and purified. Novel SSCP patterns that are detected during the high-throughput genotyping process will identify novel variants. Using this method, the genotype of patients and controls is rapidly analyzed.

The *Tim3* gene was analyzed using essentially the same methodologies. mRNA from activated T cells is sequenced to identify *Tim3* polymorphisms, as well as long range haplotypes between the *Tim1* and *Tim3*. After sequencing *Tim3* cDNA representing 60 chromosomes, it has been found that *Tim3* is polymorphic, as it is in the mouse genome. However, only one polymorphism, Leu140Arg, is prevalent, found in

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approximately 12% of the chromosomes represented.

Example 3

Expression of Tim Sequences

5 Murine TIM-3 protein is expressed on Th1 clones but not on naive T cells or Th2 cells. Using TCR transgenic T cells, TIM-3 protein was not expressed on Th1 cells after one or two rounds of Th1-directed differentiation but was expressed after the third and further rounds of Th1 stimulation. TIM-3 mRNA expression was detected somewhat earlier. In order to determine if TIM-3 gene expression was the same in human, TIM-3 and TIM-1
10 mRNA expression in human Th1 cells was examined using tetanus toxoid specific T cells generated by stimulation with antigen in the presence of IL-12 and anti IL-4 mAb. Given the association of TIM-1 with asthma, TIM-1 and TIM-3 mRNA expression in human Th2 cells was examined. Th2 cell lines were generated from allergic donors by *in vitro* stimulation with allergen, IL-4, and anti IL-12 mAb. RNA was analyzed by PCR for TIM
15 gene expression.

TIM-3 was generally expressed after Th1 differentiation whereas TIM-1 was lost. Conversely, TIM-3 was not expressed in any of the Th2 but TIM-1 was expressed in all Th2 cells. Both TIM-1 and TIM-3 are expressed in monocyte-depleted, unstimulated peripheral blood mononuclear cells from the donors used to derive the Th1 and Th2 cell
20 lines, presumably because this mixed population contains both Th1 and Th2 memory cells. These results suggest a reciprocal relationship with TIM-1 being expressed in Th2 and TIM-3 in Th1. This reciprocal relationship between TIM-1 and TIM-3 has also been observed in the mouse.

In human tissues, a 4.4 kb TIM-1 mRNA was very strongly expressed in
25 kidney and testis. The 4.4-kb mRNA was present in almost all tissues, though it was faint in most. A 5.5-kb band was observed in colon and liver. A 7.5-kb band was observed in spleen, thymus, and peripheral blood leukocytes, and smaller than 4.4-kb bands were observed in some organs. These results suggest that hTIM-1 is expressed at some level in most human tissues and that a message of 7.5-kb may code for hTIM-1 in tissues of
30 immunological interest. However, expression of Kim-1 (Kidney Injury Molecule-1), the rat homologue of TIM-1, increases in kidney upon ischemic injury. Since the MTN blots used in the expression analysis were prepared from mRNA extracted from cadavers, the increased expression of TIM-1 in kidney was re-analyzed. TIM-1 was not found to be overexpressed in kidney RNA obtained from normal kidney biopsies. Therefore, it is likely
35 that the high levels of expression of TIM-1 observed in kidney and testis were due to an up-regulation in the expression of TIM-1 resulting from tissue injury. The injured kidney may express proteins that direct incoming inflammatory cells towards a more protective Th2

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response rather than a destructive Th1 response.

Example 4

TIM Ligands and Antibodies

5 *Generation of Antibodies.* Generation of monoclonal antibodies against mouse TIM-1 allows examination of the cell surface expression of TIM-1 in different tissues, cell lines and mouse strains. Both alleles of mouse TIM-1 have been cloned into a vector for high protein expression (Invitrogen, pEF6-TOPO). Rats have been immunized and boosted with both Tim1 cDNA constructs to rapidly generate antibodies against cell surface
10 molecules. This method with cDNA vaccination favors the production of mAb against cell surface epitopes since the Tim1 cDNA will be taken up by APC, which will express the TIM-1 as a cell surface molecule. In order to generate mAb that would bind equally well to both the BALB/c and the HBA TIM-1 (by binding to conserved domains of TIM-1 such as the Immunoglobulin domain of TIM-1), both the BALB/c and HBA Tim1 cDNA (pEF6-mTIMbalb
15 and pEF6-mTIMhba) were injected into each rat.

Further boosting of the Tim1 cDNA-immunized rats was done with CHO cells stably transfected with the pEF6-mTIM-1-GFP expression constructs. CHO transfectants expressing high levels of mouse TIM-1 were sorted by FACS, and injected into the rats. Another mTIM-1 expressing cell was generated by stably transfecting the pre-B cell line
20 300.19 with the pEF6-mTIM-1 expression constructs. This line is used to screen the rat serum and the hybridomas following fusion for anti-TIM-1 antibody by flow cytometry. Rats have been generated which have high polyclonal titers against anti-TIM-1, as detected by the binding of rat serum (and a secondary FITC-goat anti-rat Ig) to stable pEF6-mTIM1-transfected 300.19 cells, as compared with control serum from unimmunized rats. This
25 staining is specific for TIM-1 since there is no reactivity with nontransfected cells or cells transfected with TIM-2.

The rat spleen is removed and the splenocytes fused with a myeloma cell line (SP/2) to produce hybridomas. Hybridoma supernatants are screened using the TIM-1 transfected 300.19 cell lines to identify hybridoma clones that produce monoclonal anti-
30 TIM-1. Specificity of the mAb for TIM-1 (and not other TIM proteins) is confirmed using TIM-2 transfected cells and mTIM-3 transfected cells or TIM-3 Ig fusion protein.

Antibody Staining. Th1 and Th2 cell lines were generated from both BALB/c and HBA DO11.10 spleen cells. RT-PCR for TIM-1 mRNA expression demonstrated that
35 TIM-1 is expressed in Th2 lines, but not in Th1 lines, following two rounds of restimulation with antigen under standard polarizing conditions. DO11.10 T cells following two rounds of stimulation with antigen/APC under Th2 polarizing conditions were stained with the

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polyclonal rat anti-TIM-1 antiserum. These Th2 cells expressed high levels of TIM-1.

These experiments showing preferential expression of TIM-1 in Th2 lines are quantified and confirmed using anti-Tim-1 mAbs and Northern blots. DO11.10 cells from BALB and HBA are cultured with antigen and APC, and restimulated for 1, 2, and 3 weeks under standard polarizing conditions (anti-IL-12 plus IL-4 or anti-IL-4 plus IL-12). After each week of stimulation, cells are stained with anti-TIM-1 mAb. By harvesting stimulated cells at various time points the kinetics of TIM-1 expression on T cells undergoing differentiation to Th1 or Th2 subset is determined. To determine if Tim-1 surface expression changes following T cell activation, we will also compare TIM-1 expression on resting and activated T cells one week after each round of antigen stimulation, by stimulating some cells with PMA and ionomycin. Activated cells are stained for intracellular cytokine expression to verify the Th subset differentiation of the T cells. Alternatively, quantitative RT-PCR or northern blots using mRNA harvested from T cells activated with PMA plus ionomycin, following each round of stimulation, are used to determine relative levels of mRNA production.

TIM-1-Ig fusion proteins BALB/c TIM-1-mIgG2a has been prepared, which is a fusion protein between the TIM-1 polypeptide and the Fc region of mouse immunoglobulin. The vector has been engineered to contain a mutation in murine IgG2a Fc that minimizes binding to Fc receptors. The TIM-1 fusion protein is utilized in characterization of TIM-1 function. The TIM-1 Ig fusion protein is expected to block TIM-1 function by binding to the TIM-1 ligand and interrupt TIM-1/TIM-1-ligand interactions.

Purified D1muc-Fc fusion protein containing the cys-rich immunoglobulin domain and 2/3 of the mucin-like region of TIM-1 fused to the hinge and Fc fragment of human IgG1 (IgVmuc-hlg) was run on a gel. This protein was expressed in CHO cells, and the IgVmuc-hlg protein was purified from CHO supernatants with protein-A agarose columns. Purified IgVmuc-hlg fusion protein neutralizes about 2 logs of HAV infectivity. In addition, treatment of HAV with IgVmuc-hlg produced a major shift in the sedimentation of the HAV particles, indicating that IgVmuc-hlg induced uncoating of the viral genome, whereas a fusion protein containing only the Ig-like region without the mucin domain (IgV-hlg) did not. This HAV neutralization system and the IgVmuc-hlg fusion protein will be used to analyze the function of TIM-1/HAV receptor alleles.

Based upon the *in vivo* effect of anti-TIM-3 mAb on macrophage expansion and activation, it was hypothesized that the TIM-3 ligand would be expressed on cells of the myeloid lineage. Dendritic cells (DC) were prepared from blood monocytes according to established protocols with 1000 U/ml IL-4 and 800 U/ml GM-CSF. DC were matured by replating the cells for 2 days in IL-4 (1000 U/ml) and GM-CSF (800 U/ml) supplemented

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with IL-1 β (10 ng/ml), TNF- α (10 ng/ml), IL-6 (1000 U/ml), and PGE₂ (1 μ g/ml). Mature DCs stained positively with hTIM-3-Ig, though there was variability among donors, suggesting that mature DC express a ligand for the IgV domain of TIM-3. Bone marrow derived endothelial cells stained very weakly and B cell lines did not stain with TIM-3-Ig.

5

Although the intracytoplasmic tail of Tim1/huhavcr-1 is relatively short, it contains a sequence that is highly conserved between mouse, rat, human and monkey (RAEDNIYI), and which may be phosphorylated, and signal through interaction with other signal transduction molecules. The most likely candidate molecule that can bind the
10 RAEDNIYI motif in T cells is the tyrosine kinase Itk. The interleukin-2 inducible tyrosine kinase, Itk is a nonreceptor protein tyrosine kinase of the Tec family that participates in the intracellular signaling events leading to T cell activation. Tec family members contain the conserved SH3, SH2, and catalytic domains common to many kinase families, but they are distinguished by unique sequences outside of this region. It is known that Itk phosphorylate
15 phospholipase C- γ (PLC- γ), and triggers a cascade of signaling events that are involved in T cell activation and helper T cell differentiation. In the absence of Itk signaling, Th2 cells do not develop. These results suggest that TIM-1/huhavcr-1 may signal through Itk, thereby altering the cytokine development in CD4 T cells.

20

Example 5

TIM Knockout Mice

A knockout construct is used in the generation of a *Tim1* deficient mouse, which enables analysis of development of immune responses in the absence of TIM-1 molecules. In another approach, TIM-1-Ig fusion proteins or anti-TIM-1 monoclonal
25 antibody are used to block the function of TIM-1 in *Tim1*^{+/+} (wild type) mice. The *Tim1* knockout mice and the anti-TIM-1 mAb approaches are complementary for the evaluation of the role of TIM-1 in T cell differentiation and in asthma pathogenesis.

The HBA mouse genomic sequence of *Tim1* has a deletion of exon 4, compared to the BALB/c sequence, due to an integrated L1 retroviral element at exon 4. If
30 truncation of one exon reduces TIM-1 function, then total deletion of TIM-1 function by generation of *Tim1* knockout mice, should severely limit the capacity of such mice to generate Th2 responses. TIM-1 KO mice are generated by deleting the TIM-1 exons 1 and 2, using cre-lox technology and BALB/c ES cells, which should eliminate cell surface expression of TIM-1, thereby demonstrating the importance of TIM-1 function in the
35 development of Th2 cells and AHR.

To create an appropriate targeting construct, specific BACs containing members of the TIM gene family were identified by screening a C57/Bl6 BAC library (RPCI-

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23) using high density filter sets. These BAC clones were used to build a 500 kb contig and physical map that covers the approximately 350 kb genomic region encompassing the *Tim* gene family. One specific BAC, RPCI-23-222F8, that contains the complete *Tim1* gene was chosen to generate a TIM targeting construct. The targeting construct deletes a 4 kb region encompassing the promoter region, signal exon, and IgV exon (exons 1 and 2), via homologous recombination with 5' and 3' arms flanking this region. This targeting vector can be used in either C57Bl/6 or BALB/c ES cells, since the homologous arms of the targeting construct are homologous to both BALB/c and HBA (C57Bl/6) DNA. The construct is introduced into ES cells, which are screened for the targeted allele by PCR and by Southern blots.

The targeting vector, pLOX, contains three loxP sites, and by expressing cre recombinase in the targeted ES cells, recombination will generate three forms of the targeted region, of which two alleles, A and B, are used to create *Tim1* KO mice. Selected ES cell clones are introduced into blastocysts to generate chimeric mice and bred for germline transmission of the knockout. Tail DNAs are analyzed for transmission of the knockout. Allele A is used initially, in which cre/lox recombination has used the outermost loxP sites to delete the TK and neomycin selection cassettes in addition to TIM-1 exons 1 and 2. The removal of the entire neo and TK cassette from the TIM-1 genomic targeting region prevents confounding artifacts that can arise secondary to the transcription of neo in close proximity to other genes of the same family. Alternatively, if allele A produces a lethal or confounding phenotype, mice are generated with targeted allele B, in which the neo and TK cassette has been deleted and the TIM-1 region is flanked by lox-p sites. Use of this conditional targeting approach should allow T cell-specific deletion of Tim-1 in mice expressing cre-recombinase under the control of a T cell specific promoter.

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WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising a mammalian TIM gene sequence.
- 5 2. An isolated nucleic acid molecule according to Claim 1, wherein said mammalian TIM gene sequence is a human sequence selected from the group consisting of alleles 2, 3, 4, 5, and 6 of TIM-1, TIM-3, and TIM-4.
3. An isolated nucleic acid according to Claim 1, wherein said
10 mammalian TIM gene sequence is a mouse sequence selected from the group consisting of TIM-1, TIM-2, TIM-3 and TIM-4.
4. An isolated nucleic acid comprising at least 20 nucleotides of
contiguous sequence as set forth in any one of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18,
15 20, 22, 24, 26, 28, 30, 32, 34 or 36.
5. An isolated nucleic acid comprising a sequence as set forth in any
one of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34 or 36.
- 20 6. An isolated nucleic acid molecule according to Claim 1, comprising a probe for detection of a TIM gene polymorphism.
7. An array of oligonucleotides comprising:
two or more probes according to Claim 6.
- 25 8. A cell comprising a nucleic acid composition according to claim 1.
9. A purified polypeptide composition comprising at least 50 weight % of
the protein present as the product of the nucleic acid of Claim 1 or a fragment thereof.
- 30 10. The purified polypeptide according to Claim 9, wherein said polypeptide comprises a sequence as set forth in SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33 and 35.
- 35 11. An antibody specific for a mammalian TIM polypeptide.
12. A method for detecting a predisposition to asthma in an individual,

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the method comprising:

analyzing said individual for the presence of at least one predisposing TIM polymorphism; wherein the presence of said predisposing polymorphism is indicative of an increased susceptibility to asthma.

5

13. The method according to Claim 12, wherein said analyzing step comprises detection of specific binding between genomic DNA or mRNA of said individual with a probe or probes according to Claim 6.

10

14. The method according to Claim 12, wherein said analyzing step comprises detection of specific binding between cells or protein from said individual and the antibody according to Claim 11.

15. A non-human transgenic animal model for TIM gene function comprising a knockout of a TIM gene.

16. A method of screening for biologically active agents that modulate TIM function, the method comprising:

combining a candidate biologically active agent with any one of:

20

(a) a mammalian TIM polypeptide;

(b) a cell comprising a nucleic acid encoding a mammalian TIM polypeptide;

or

(c) a non-human transgenic animal model for TIM gene function; and determining the effect of said agent on TIM function.

25

17. The method according to Claim 16, wherein said biologically active agent downregulates or upregulates TIM expression.

18. The method according to Claim 16, wherein said biologically active agent inhibits or increases activity of said TIM polypeptide.

30

19. A method for the diagnosis or staging of immune dysfunction in an individual, the method comprising:

analyzing said individual for the presence of at least one predisposing TIM polymorphism; wherein the presence of said predisposing polymorphism is indicative of an increased susceptibility to said immune dysfunction.

35

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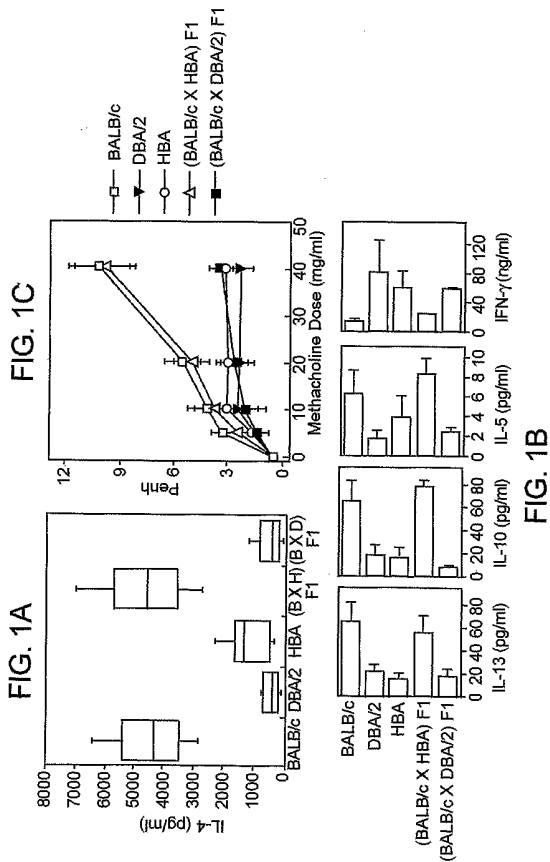
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20. A method for the diagnosis or staging of cancer in an individual, the method comprising:
analyzing said individual for the presence of at least one predisposing TIM polymorphism; wherein the presence of said predisposing polymorphism is indicative of an increased resistance to cancer therapy.
21. A method for the diagnosis or staging of cancer in an individual, the method comprising:
analyzing said individual for the presence of at least one predisposing TIM-1 expression profile; wherein the expression profile of TIM-1 indicative of an increased resistance to cancer therapy.
22. A method for the diagnosis or staging of cancer in an individual, the method comprising:
analyzing said individual for the presence of at least one predisposing TIM-4 expression profile; wherein the expression profile of TIM-4 indicative of an increased resistance to cancer therapy.
23. A method for the treatment of an immunological disorder in an individual, the method comprising:
administering to said individual an agent that modulates function of a TIM polypeptide.
24. The method according to Claim 23, wherein said TIM polypeptide is TIM-1 or TIM-4.
25. The method according to Claim 24, wherein said agent is an antibody or antibody fragment specific for TIM-1.
26. The method according to Claim 24, wherein said agent is a compound that inhibits TIM-1 function.
27. The method according to Claim 24, wherein said immunological disorder is asthma.
28. The method according to Claim 24, wherein said immunological disorder is an allergy.

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29. The method according to Claim 24, wherein said immunological disorder is eczema.
- 5 30. The method according to Claim 24, wherein said immunological disorder is an autoimmune disease.
31. The method according to Claim 23, wherein said agent is an antibody or antibody fragment specific for TIM-4.
- 10 32. The method according to Claim 30, wherein said agent is a compound that inhibits the function of TIM-4.
- 15 33. A method for the treatment of malignancy in an individual, the method comprising: administering to said individual an agent that modulates function of a TIM polypeptide.
34. The method according to Claim 33, wherein said TIM polypeptide is TIM-1.
- 20 35. The method according to Claim 34, wherein said agent is an antibody or antibody fragment specific for TIM-1.
36. The method according to Claim 35, wherein said agent is a compound that inhibits the function of TIM-1 and potentiates the effect of chemotherapeutic agents or radiation therapy.
- 25 37. The method according to Claim 33, wherein said TIM polypeptide is TIM-4.
- 30 38. The method according to Claim 37, wherein said agent is an antibody or antibody fragment specific for TIM-4.
- 35 39. The method according to Claim 38, wherein said agent is a compound that inhibits the function of TIM-4 and potentiates the effect of chemotherapeutic agents or radiation therapy.



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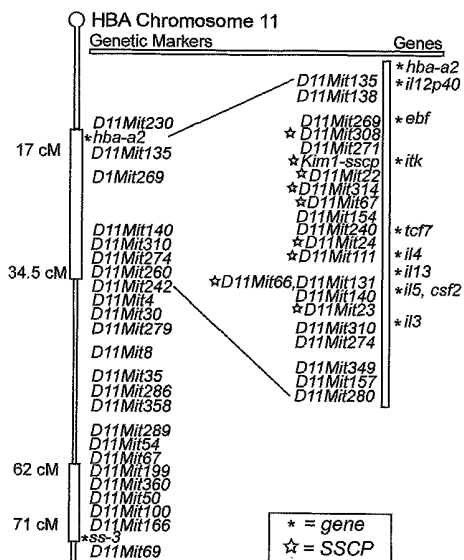
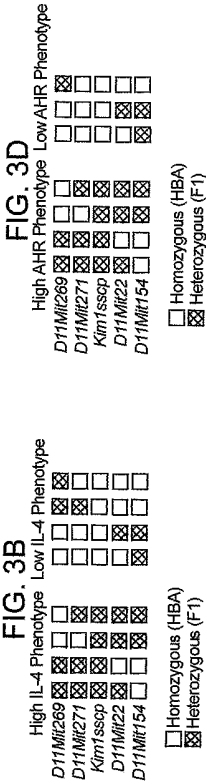
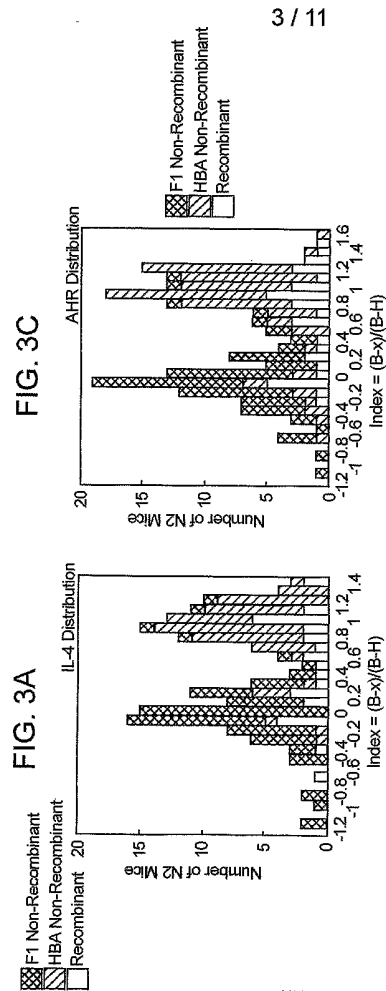


FIG. 2

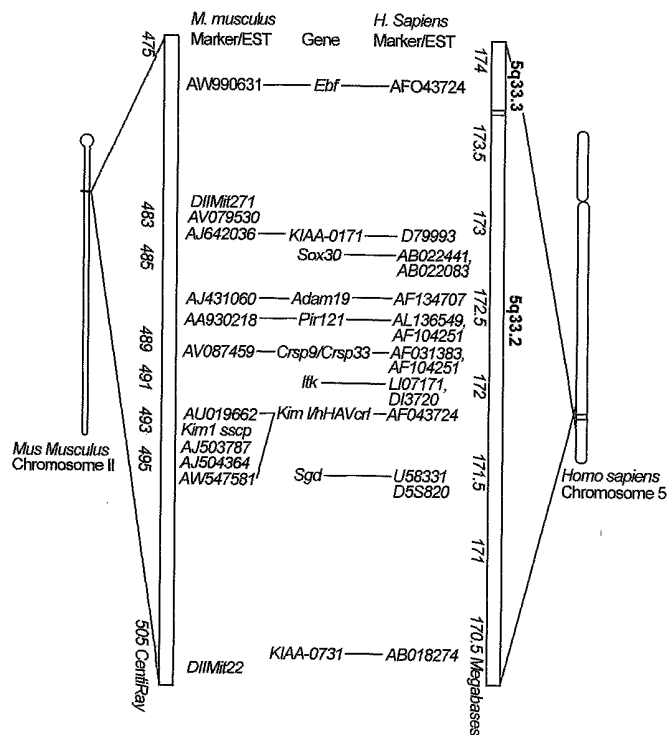


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FIG. 4



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FIG. 5A

Mouse TIM-1	MAQVPSISLILKQVSVKGVGHEVPLKVSPLKLTTCRKCQCCSCQCMINWVCHVLSKSRNLKCEICSEVSLITSEVSE	100
Rat TIM-1	MAQVPSISLILKQVSVKGVGHEVPLKVSPLKLTTCRKCQCCSCQCMINWVCHVLSKSRNLKCEICSEVSLITSEVSE	100
Human HAVcr-1	M-HQVWLSLILKQVSVKGVGHEVPLKVSPLKLTTCRKCQCCSCQCMINWVCHVLSKSRNLKCEICSEVSLITSEVSE	156
Monkey HAVcr-1	M-HQVWLSLILKQVSVKGVGHEVPLKVSPLKLTTCRKCQCCSCQCMINWVCHVLSKSRNLKCEICSEVSLITSEVSE	97
Mouse TIM-1	STSGVCKRVEIQGNNDQVLESHVPEPE	149
Rat TIM-1	STSGVCKRVEIQGNNDQVLESHVPEPE	148
Human HAVcr-1	STSGVCKRVEIQGNNDQVLESHVPEPE	156
Monkey HAVcr-1	STSGVCKRVEIQGNNDQVLESHVPEPE	197
Mouse TIM-1	PTI	180
Rat TIM-1	PTI	178
Human HAVcr-1	PTI	218
Monkey HAVcr-1	PTI	297
Mouse TIM-1	PTI	275
Rat TIM-1	PTI	273
Human HAVcr-1	PTI	317
Monkey HAVcr-1	PTI	396

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[illegible]

TIM-1 Variants

HBA
BALB/c

A2-11/TIM-3 Variants

SAGLTALIIIGVILIKWYSCKKKLSLSLTLANLPPGGLANAGAVRIRSEENITYTTEENVYEVENSNEYCYVNSQQPS
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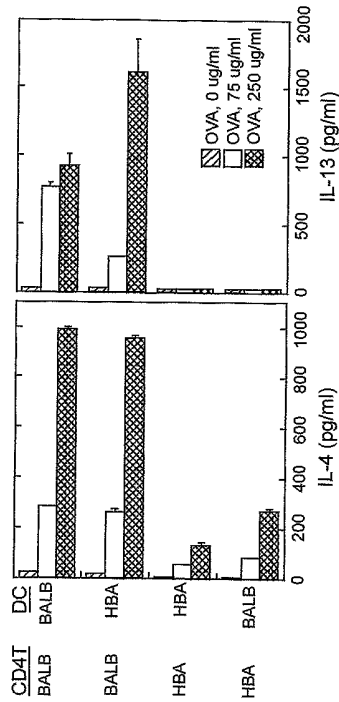


FIG. 6

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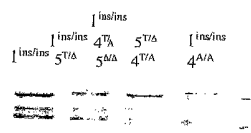
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FIG. 9



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SEQUENCE LISTING

<110> McIntire, Jennifer Jones
 Umetsu, Dale T.
 Dekruyff, Rosemarie
 Kuchroo, Vijay
 Freeman, Gordon J.

<120> T Cell Regulatory Genes and Methods of
 Use Thereof

<130> STAN-235WO

<140> Unassigned

<141> 2002-07-01

<150> 60/302,344

<151> 2001-06-29

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<212> PRT

<213> M. musculus

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<221> VARIANT

<222> (1)...(305)

<223> TIM-1 BALB/c allele

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 20 25 30
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 35 40 45
 Cys Trp Gly Arg Gly Gln Cys Pro Ser Ser Ala Cys Gln Asn Thr Leu
 50 55 60
 Ile Trp Thr Asn Gly His Arg Val Thr Tyr Gln Lys Ser Ser Arg Tyr
 65 70 75 80
 Asn Leu Lys Gly His Ile Ser Glu Gly Asp Val Ser Leu Thr Ile Glu
 85 90 95
 Asn Ser Val Glu Ser Asp Ser Gly Leu Tyr Cys Cys Arg Val Glu Ile
 100 105 110
 Pro Gly Trp Phe Asn Asp Gln Lys Val Thr Phe Ser Leu Gln Val Lys
 115 120 125
 Pro Glu Ile Pro Thr Arg Pro Pro Thr Arg Pro Thr Thr Arg Pro
 130 135 140
 Thr Ala Thr Gly Arg Pro Thr Thr Ile Ser Thr Arg Ser Thr His Val
 145 150 155 160
 Pro Thr Ser Ile Arg Val Ser Thr Ser Thr Pro Pro Thr Ser Thr His
 165 170 175
 Thr Trp Thr His Lys Pro Glu Pro Thr Thr Phe Cys Pro His Glu Thr
 180 185 190
 Thr Ala Glu Val Thr Gly Ile Pro Ser His Thr Pro Thr Asp Trp Asn
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      245      250      255
Ala Ile Thr Arg Tyr Ile Leu Met Lys Arg Lys Ser Ala Ser Leu Ser
      260      265      270
Val Val Ala Phe Arg Val Ser Lys Ile Glu Ala Leu Gln Asn Ala Ala
      275      280      285
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Pro
305

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caaaatcacac ttatttggac caatggacat cgtgtcacct atcagaagag cagtctgtac 240
saacttaagg ggcatatttc agaaggagat gtgtccttga cyatagagaa ctctgttgag 300
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gtgacatttt cattgcaagt taaaccagag attcccacac gtccctccac aagaccacaa 420
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<213> Mus musculus

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<223> TIM-1, C.D2 ES-HEA and DBA/2J allele

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Val Thr Leu Pro Cys Thr Tyr Ser Thr Tyr Arg Gly Ile Thr Thr Thr
35      40      45
Cys Trp Gly Arg Gly Gln Cys Pro Ser Ser Ala Cys Gln Asn Thr Leu
50      55      60
Ile Trp Thr Asn Gly His Arg Val Thr Tyr Gln Lys Ser Ser Arg Tyr
65      70      75      80
Asn Leu Lys Gly His Ile Ser Glu Gly Asp Val Ser Leu Thr Ile Glu
85      90      95
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Thr Ala Thr Gly Arg Pro Thr Thr Ile Ser Thr Arg Ser Thr His Val
      145      150      155      160
Pro Thr Ser Thr Arg Val Ser Thr Ser Thr Pro Pro Thr Ser Thr His
      165      170      175
Thr Trp Thr His Lys Pro Asp Trp Asn Gly Thr Val Thr Ser Ser Gly
      180      185      190
Asp Thr Trp Ser Asn His Thr Glu Ala Ile Pro Pro Gly Lys Pro Gln
      195      200      205
Lys Asn Pro Thr Lys Gly Phe Tyr Val Gly Ile Cys Ile Ala Ala Leu
      210      215      220
Leu Leu Leu Leu Val Ser Thr Val Ala Ile Thr Arg Tyr Ile Leu
      225      230      235      240
Met Lys Arg Lys Ser Ala Ser Leu Ser Val Val Ala Phe Arg Val Ser
      245      250      255
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Tyr Gln Leu Lys Gly Asn Ile Ser Glu Gly Asn Val Ser Leu Thr Ile
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Glu Asn Thr Val Val Gly Asp Gly Gly Pro Tyr Cys Cys Val Val Glu
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Thr Thr Ile Ser Thr Arg Ser Thr His Val Pro Thr Ser Thr Arg Val
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Glu Ala Thr Thr Phe Tyr Pro Asp Gln Thr Thr Ala Glu Val Thr Glu
  180                               185                               190
Thr Leu Pro Ser Thr Pro Ala Asp Trp His Asn Thr Val Thr Ser Ser
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Gln Lys Asn Leu Asn Lys Gly Phe Tyr Val Gly Ile Ser Ile Ala Ala
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Ser
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<213> Mus musculus

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tcacctgtgc acacttccat gtaattatct gacacacctt ggtggaatcg ttcttatgtg 180
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ttccacagat ccaccaacaa ggcccacagc tacaggaaga ccacaacta ttccaacaag 480
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 35 40 45
 Met Cys Trp Gly Leu Gly Glu Cys Arg His Ser Tyr Cys Ile Arg Ser
 50 55 60
 Leu Ile Trp Thr Asn Gly Tyr Thr Val Thr His Gln Arg Asn Ser Arg
 65 70 75 80
 Tyr Gln Leu Lys Gly Asn Ile Ser Glu Gly Asn Val Ser Leu Thr Ile
 85 90 95
 Glu Asn Thr Val Val Gly Asp Gly Gly Pro Tyr Cys Cys Val Val Glu
 100 105 110
 Ile Pro Gly Ala Phe His Phe Val Asp Tyr Met Leu Glu Val Lys Pro
 115 120 125
 Glu Ile Ser Thr Ser Pro Pro Thr Arg Pro Thr Ala Thr Gly Arg Pro
 130 135 140
 Thr Thr Ile Ser Thr Arg Ser Thr His Val Pro Thr Ser Thr Arg Val
 145 150 155 160
 Ser Thr Ser Thr Ser Pro Thr Pro Ala His Thr Glu Thr Tyr Lys Pro
 165 170 175
 Glu Ala Thr Thr Phe Tyr Pro Asp Gln Thr Thr Ala Glu Val Thr Glu
 180 185 190
 Thr Leu Pro Ser Thr Pro Ala Asp Trp His Asn Thr Val Thr Ser Ser
 195 200 205
 Asp Asp Pro Trp Asp Asp Asn Thr Glu Val Ile Pro Pro Gln Lys Pro
 210 215 220
 Gln Lys Asn Leu Asn Lys Gly Phe Tyr Val Gly Ile Ser Ile Ala Ala
 225 230 235 240
 Leu Leu Ile Leu Met Leu Leu Ser Thr Met Val Ile Thr Arg Tyr Val
 245 250 255
 Val Met Lys Arg Lys Ser Glu Ser Leu Ser Phe Val Ala Phe Pro Ile
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 Ser
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 tcacctgtc acattccat gtatttatt gacacactt ggtggaatcg ttctatgtg 180
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agtggagata cctggagcgt tccattttgt ggaactatag ttggaagtta aaccagaagt 420
ttccacaggt ccaccaacaa ggcaccacgc tacaggaaga cccacaacta tttcaacaag 480
atccacacac gtaccaacat caaccagagt ctctacctct actctctcaa caccagcaca 540
cacagagacc tacaaacag aggcactac atttatcca gatcagacta cagctgaggt 600
gacagaaacc ttaccctcta ctctgcaga ctggcataac actgtgarat cctcagatga 660
cccttgggat gataacactg aagtaatccc tccacagaag ccacagaaaa acctgaataa 720
gggcttctat gttggcatct ccattgcagc cctgctgata ttgatgcttc tgagcaccat 780
ggttatcacc aggtacgtgg ttatgaaaag gaagtcagaa tctctgagct tcyttgcctt 840
ccctatctct aagattggag cttcccccaa aaaagtggtc gaacggacca gatgtgaaga 900
ccaggtctac attattgaag acactcctta ccccgaaaga gagtccatgt gcctctac 958

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<210> 9
 <211> 281
 <212> PRT
 <213> Mus musculus

<220>
 <221> VARIANT
 <222> (1)...(281)
 <223> TIM-3 BALB/c allele

<400> 9
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 1 5 10 15
 Leu Leu Ala Arg Ser Leu Glu Asp Gly Tyr Lys Val Glu Val Gly Lys
 20 25 30
 Asn Ala Tyr Leu Pro Cys Ser Tyr Thr Leu Pro Thr Ser Gly Thr Leu
 35 40 45
 Val Pro Met Cys Trp Gly Lys Gly Phe Cys Pro Trp Ser Gln Cys Thr
 50 55 60
 Asn Glu Leu Leu Arg Thr Asp Glu Arg Asn Val Thr Tyr Gln Lys Ser
 65 70 75 80
 Ser Arg Tyr Gln Leu Lys Gly Asp Leu Asn Lys Gly Asp Val Ser Leu
 85 90 95
 Ile Ile Lys Asn Val Thr Leu Asp Asp His Gly Thr Tyr Cys Cys Arg
 100 105 110
 Ile Gln Phe Pro Gly Leu Met Asn Asp Lys Lys Leu Glu Leu Lys Leu
 115 120 125
 Asp Ile Lys Ala Ala Lys Val Thr Pro Ala Gln Thr Ala His Gly Asp
 130 135 140
 Ser Thr Thr Ala Ser Pro Arg Thr Leu Thr Thr Glu Arg Asn Gly Ser
 145 150 155 160
 Glu Thr Gln Thr Leu Val Thr Leu His Asn Asn Asn Gly Thr Lys Ile
 165 170 175
 Ser Thr Trp Ala Asp Glu Ile Lys Asp Ser Gly Glu Thr Ile Arg Thr
 180 185 190
 Ala Ile His Ile Gly Val Gly Val Ser Ala Gly Leu Thr Leu Ala Leu
 195 200 205
 Ile Ile Gly Val Leu Ile Leu Lys Trp Tyr Ser Cys Lys Lys Lys Lys
 210 215 220
 Leu Ser Ser Leu Ser Leu Ile Thr Leu Ala Asn Leu Pro Pro Gly Gly
 225 230 235 240
 Leu Ala Asn Ala Gly Ala Val Arg Ile Arg Ser Glu Glu Asn Ile Tyr
 245 250 255
 Thr Ile Glu Glu Asn Val Tyr Glu Val Glu Asn Ser Asn Glu Tyr Tyr
 260 265 270
 Cys Tyr Val Asn Ser Gln Gln Pro Ser
 275 280

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<210> 10
 <211> 2725
 <212> DNA
 <213> Mus musculus

<400> 10
 ttttaaccga ggaagctaaag ctatccctac acagagctgt ccttggattt cccctgcoaa 60
 gtaactcatgt ttcoaggtct taacctcaac tgtgtcctgc tgetgctgca actactactt 120
 gcaaggtcat tgggaagatgg ttataaggtt gaggttggtt aaatgccta tctgcoctgc 180
 agttacactc taacctacatc tgggacactt gtgctatgt gctggggcaa gggattctgt 240
 ccttggtoac agtgtaccaa tgagttgctc agaactgatg aaagaaatgt gaatatcag 300
 aaatccagca gataccagct aaagggcgat ctcaacaaag gagatgtgtc tctgatcata 360
 aagaatgtga ctctggatga ccatgggagc taotgtgtga ggatacagtt cctgggtctt 420
 atgaatgata aaaaattaga actgaaatta gacatcaaa gaggcaaggt cactccagct 480
 cagactgccc atggggactc tactacagct tctccaaaga cctcaaccac ggagagaaat 540
 ggttcagaga cacagacact ggtgacctc cataataca atgggaacaa atttccaca 600
 ggggtgatg aaattaagga ctctggagaa acgatacaga ctgctatcca cattggagtg 660
 ggaagtctctg ctgggttgac cctggcactt atcattggtg tcttaactct taaatgggat 720
 tctgttaaga aaaagaagtt atcgagtttg agcttatta cactggccaa cttgctcca 780
 ggagggttgg caaatgcagg agcagtcagg attcgctctg aggaatatat ctacaccatc 840
 gaggagaacg tatatgaagt ggagaattca aatgagtact actgctactg caacagccag 900
 cagccatcct caccgctctc ggactgccac tttaaaggc tgccttcatt tctgactttt 960
 ggtatttccc tttttgaaaa ctatgtgata tgtcacttgg caactcattt ggaggttctg 1020
 accacagcca ctgagaaaag agttccagtt ttctggggat aattaactca caaggggatt 1080
 gactgttaac tcatgttaca ttgaatgctt cacttttctc cctgagtttc agggatcgga 1140
 tctccactc cagagacttc aatcatgctt gttgaagctc actcgtgctt tcatacata 1200
 ggaatgggta gtgtgatgtc tttagacat agaggtttgt ggtatatccg caaagctcct 1260
 gaacaggtag ggggaataaa gggctaagat aggaaggtgc ggttcttctt tgatgttgaa 1320
 aatctaaga agtttgtagc ttctctagag attcttgacc ttgaagattt aagaaaaagc 1380
 caagtggtcat atgcttaaca cgatataact tgggaacctt aggcaggagg gtgataagtt 1440
 caaggtcagc cagggctatg ctggtaagac tgtctcaaaa tccaaagacg aaataaaca 1500
 tagagacagc aggaagcttg agatgaggtc cggacagtga ggtgcatttt gtacaagcac 1560
 gaggaaatcta tatttgatcg tagacccac atgaaaaagc taggcctggt agagcatgct 1620
 ttagactcca agagatggag aggtaaaggc acaacagatc cccggggctt gcgtgcagtc 1680
 agcttagcct aggtgctgag ttccaagtc acaagagtc ctgtctcaaa gtaagatgga 1740
 ctgagtatct ggcgaatgtc catgggggtt tctctctgct ctcaagagac acatgcacat 1800
 gaacctgcac acacacacac acacacacac acacacacac acacatgaaa 1860
 tgaaggttct ctctgtgctt gctacctctc tataacatgt atctctacag gactctctc 1920
 tgctctgtt aagacatgag tgggagcatg gcagagcagt ccagtaatta attccagcac 1980
 tcagaaggct ggaagcagaag cgtggagagt tcaggagcac tgtgcccaac actgccagac 2040
 tcttcttaca caagaaaaag gttacccgca agcagcctgc tgtctgtaaa aggaacct 2100
 gcgaaggcca aactttgact gttgtgtgct caaggggaac tgactcagac aacttctcca 2160
 ttctgtggag aaactggagc tgtttctgac agagaacaaa ccggtgactg ggacatacga 2220
 aggcagagct cttgcagcaa tcttatagtt cagcaaaata ttcttggga ggacagtcgt 2280
 caccnaattg atttccaagc cgttggaact cagtttcttc tgggttacag ctgctgccc 2340
 agtgcctctg atctgtgctg gctccatct ataacagaat caaattaaat agaccagag 2400
 tgaataattt aagttagcag aaagtagctt ttgttcaaa attttttgc attggggagc 2460
 aactgtgtac atcagaggac atctgttagt gaggaaccca aaacctgtgg taccgttttt 2520
 tcatgtatga attttgtgtt ttagggttct tctagctagc tgtggagtc ctggtttctt 2580
 taggtgggta tggaaaggag accatcaac aaatccatt agagataaca gctctcatg 2640
 agaagggaaa actaatctca aatgttttaa agtaataaaa ctgtactggc aaagtacttt 2700
 gagcatattt aaaaaaaaaa aaaaa 2725

<210> 11
 <211> 281
 <212> PRT
 <213> Mus musculus

<220>
 <221> VARIANT
 <222> (1)...(281)
 <223> TIM-3, C.D2 ES-HBA and DBA/2J allele

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<400> 11
Met Phe Ser Gly Leu Thr Leu Asn Cys Val Leu Leu Leu Gln Leu
1 5 10 15
Leu Leu Ala Arg Ser Leu Glu Asn Ala Tyr Val Phe Glu Val Gly Lys
20 25 30
Asn Ala Tyr Leu Pro Cys Ser Tyr Thr Leu Ser Thr Pro Gly Ala Leu
35 40 45
Val Pro Met Cys Trp Gly Lys Gly Phe Cys Pro Trp Ser Gln Cys Thr
50 55 60
Asn Glu Leu Leu Arg Thr Asp Glu Arg Asn Val Thr Tyr Gln Lys Ser
65 70 75 80
Ser Arg Tyr Gln Leu Lys Gly Asp Leu Asn Lys Gly Asp Val Ser Leu
85 90 95
Ile Ile Lys Asn Val Thr Leu Asp Asp His Gly Thr Tyr Cys Cys Arg
100 105 110
Ile Gln Phe Pro Gly Leu Met Asn Asp Lys Lys Leu Glu Leu Lys Leu
115 120 125
Asp Ile Lys Ala Ala Lys Val Thr Pro Ala Gln Thr Ala His Gly Asp
130 135 140
Ser Thr Thr Ala Ser Pro Arg Thr Leu Thr Thr Glu Arg Asn Gly Ser
145 150 155 160
Glu Thr Gln Thr Leu Val Thr Leu His Asn Asn Asn Gly Thr Lys Ile
165 170 175
Ser Thr Trp Ala Asp Glu Ile Lys Asp Ser Gly Glu Thr Ile Arg Thr
180 185 190
Ala Ile His Ile Gly Val Gly Val Ser Ala Gly Leu Thr Leu Ala Leu
195 200 205
Ile Ile Gly Val Leu Ile Leu Lys Trp Tyr Ser Cys Lys Lys Lys
210 215 220
Leu Ser Ser Leu Ser Leu Ile Thr Leu Ala Asn Leu Pro Pro Gly Gly
225 230 235 240
Leu Ala Asn Ala Gly Ala Val Arg Ile Arg Ser Glu Glu Asn Ile Tyr
245 250 255
Thr Ile Glu Glu Asn Val Tyr Glu Val Glu Asn Ser Asn Glu Tyr Tyr
260 265 270
Cys Tyr Val Asn Ser Gln Gln Pro Ser
275 280

<210> 12
<211> 862
<212> DNA
<213> Mus musculus

<400> 12
ccccccca gtactcatgt ttccaggtct taccctcaac tgtgtctctgc tgetgtctga 60
actactactt gcaaggtoat tggaaaatgc ttatgtgttt gaggttggtt agaatgccta 120
ctgtccctgc agttacactc tatctacacc tggggcactt gtgcctatgt gctggggcaa 180
gggattctgt ccttgggtcac agtgtaccac cgagttgctc agaactgatg aaagaatgt 240
gacatctcag aaatccagca gataccagct aaagggcgat ctcaacaag gagacgtgtc 300
tctgatcata aagaatgtga ctctggatga ccattgggacc tactgtctga ggatcacgtt 360
ccctgtctct atgaatgata aaaaattaga actgaaatta gacatcaaa gaggcaaggt 420
cactccagct cagactgcc atggggactc tactacagct tctccaagaa cctaaccac 480
ggagagaaat ggttcagaga cacagacact ggtgacctc cataataaca atggaacaaa 540
aatccaca tgggtgatg aaattaagga ctctggagaa acgatcagaa ctgctatcca 600
cattggagtg ggaagtctct ctgggttgac cctggcactt atcattgggt tcttaacct 660
taaatggtat tctgttaaga aaaagaagtt atcgagtttg agccttatta cactggccaa 720
ctgtctcca ggaaggttg caaatgcagg agcagtcagg attcgctctg aggaaaatat 780
ctacacacac gaagagancg tataatgaat ggagaattca aatgagtact actgttaagt 840
caacagccag cagccatcct ga 862

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<210> 13
 <211> 345
 <212> PRT
 <213> Mus musculus

<220>
 <221> VARIANT
 <222> (1)...(345)
 <223> TIM-4, BALB/c allele

<400> 13
 Met Ser Lys Gly Leu Leu Leu Leu Trp Leu Val Thr Glu Leu Trp Trp
 1 5 10 15
 Leu Tyr Leu Ser Lys Ser Pro Ala Ala Ser Glu Asp Thr Ile Ile Gly
 20 25 30
 Phe Leu Gly Gln Pro Val Thr Leu Pro Cys His Tyr Leu Ser Trp Ser
 35 40 45
 Gln Ser Arg Asn Ser Met Cys Trp Gly Lys Gly Ser Cys Pro Asn Ser
 50 55 60
 Lys Cys Asn Ala Glu Leu Leu Arg Thr Asp Gly Thr Arg Ile Ile Ser
 65 70 75 80
 Arg Lys Ser Thr Lys Tyr Thr Leu Leu Gly Lys Val Gln Phe Gly Glu
 85 90 95
 Val Ser Leu Thr Ile Ser Asn Thr Asn Arg Gly Asp Ser Gly Val Tyr
 100 105 110
 Cys Cys Arg Ile Glu Val Pro Gly Trp Phe Asn Asp Val Lys Lys Asn
 115 120 125
 Val Arg Leu Glu Leu Arg Arg Ala Thr Thr Thr Lys Lys Pro Thr Thr
 130 135 140
 Thr Thr Arg Pro Thr Thr Thr Pro Tyr Val Thr Thr Thr Pro Glu
 145 150 155 160
 Leu Leu Pro Thr Thr Val Met Thr Thr Ser Val Leu Pro Thr Thr Thr
 165 170 175
 Pro Pro Gln Thr Leu Ala Thr Thr Ala Phe Ser Thr Ala Val Thr Thr
 180 185 190
 Cys Pro Ser Thr Thr Pro Gly Ser Phe Ser Gln Glu Thr Thr Lys Gly
 195 200 205
 Ser Ala Ile Thr Thr Glu Ser Glu Thr Leu Pro Ala Ser Asn His Ser
 210 215 220
 Gln Arg Ser Met Met Thr Ile Ser Thr Asp Ile Ala Val Leu Arg Pro
 225 230 235 240
 Thr Gly Ser Asn Pro Gly Ile Leu Pro Ser Thr Ser Gln Leu Thr Thr
 245 250 255
 Gln Lys Thr Thr Leu Thr Thr Ser Glu Ser Leu Gln Lys Thr Thr Lys
 260 265 270
 Ser His Gln Ile Asn Ser Arg Gln Thr Ile Leu Ile Ile Ala Cys Cys
 275 280 285
 Val Gly Phe Val Leu Met Val Leu Leu Phe Leu Ala Phe Leu Leu Arg
 290 295 300
 Gly Lys Val Thr Gly Ala Asn Cys Leu Gln Arg His Lys Arg Pro Asp
 305 310 315 320
 Asn Thr Glu Val Ser Asp Ser Phe Leu Asn Asp Ile Ser His Gly Arg
 325 330 335
 Asp Asp Glu Asp Gly Ile Phe Thr Leu
 340 345

<210> 14
 <211> 1032
 <212> DNA
 <213> Mus musculus

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<400> 14
atgtccaaagg ggctttctct cctctggtgt gtgacggagc tctggtgget ttatctgaca 60
ccagctgcct cagaggatag aataataggg tttttgggcc agccggtgac ttgctctgt 120
cattacctct cgtggtccca gagccgcaac agtatgtgct ggggcaaaag ttcatgtccc 180
aatccaaagt gcaatgcaga gctttccagt acagatggaa caagaatcat ctccaggaag 240
tcaacaaaat ataacatttt ggggaaggtc cagtttggtg aagtgtcctt gaccatctca 300
aacaccaatc gaggtgacag tggggtgtac tgctgccgta tagaggtgcc tggctggttc 360
aatgatgtca agaagaatgt gcgcttgag ctgaggagag ccacaacaac caaaaaacca 420
acacaacaac ccggcacaac caccaccctt tatgtaacca ccaccacccc agagctgctt 480
ccacaacaac tcatgaccac atctgttctt ccaaccacca caccacccc gacactagcc 540
accactgctt tcaatgacag agtgaccacg tggccctcaa caacacotgg ctcttctca 600
caagaacaac caaaggggtc ggcacacact acagacatag aaactctgcc tgcattccat 660
cactctcaaa gaagcatgat gaccatctca acagacatag cagtactcag gccacagggc 720
tctaaccctg ggattctccc atccacttca cagctgacga cacagaaaac aacattaaca 780
acaagtgaat ctttgacaga gacaactaaa tcaacacaga tcaacagcag acagaccatc 840
ttgatcattg cctgctgtgt gggatttgtg ctaatgggtt tattgtttct ggcgtttctc 900
cttcgaggga aagtcacagg agccaactgt ttgcagagac acaagaggcc agacaacact 960
gaagatagtg acagcgtcct caatgacatg tcacacggga gggatgatga agacgggac 1020
ttcactctct ga 1032

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<210> 15

<211> 345

<212> PRT

<213> Mus musculus

<220>

<221> VARIANT

<222> (1)...(345)

<223> C.D2 ES-HBA and DBA/2J allele

<400> 15

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Met Ser Lys Gly Leu Leu Leu Trp Leu Val Met Glu Leu Trp Trp
1 5 10 15
Leu Tyr Leu Ser Lys Ser Pro Ala Ala Ser Glu Asp Thr Ile Ile Gly
20 25 30
Phe Leu Gly Gln Pro Val Thr Leu Pro Cys His Tyr Leu Ser Trp Ser
35 40 45
Gln Ser Arg Asn Ser Met Cys Trp Gly Lys Gly Ser Cys Pro Asn Ser
50 55 60
Lys Cys Asn Ala Glu Leu Leu Arg Thr Asp Gly Thr Arg Ile Ile Ser
65 70 75 80
Arg Lys Ser Thr Lys Tyr Thr Leu Leu Gly Lys Val Gln Phe Gly Glu
85 90 95
Val Ser Leu Thr Ile Ser Asn Thr Asn Arg Gly Asp Ser Gly Val Tyr
100 105 110
Cys Cys Arg Ile Glu Val Pro Gly Trp Phe Asn Asp Val Lys Lys Asn
115 120 125
Val Arg Leu Glu Leu Arg Arg Ala Thr Thr Thr Lys Lys Pro Thr Thr
130 135 140
Thr Thr Arg Pro Thr Thr Thr Pro Tyr Val Thr Thr Thr Thr Pro Glu
145 150 155 160
Leu Leu Pro Thr Thr Val Met Thr Thr Ser Val Leu Pro Thr Thr Thr
165 170 175
Pro Pro Gln Thr Leu Ala Thr Thr Ala Phe Ser Thr Ala Val Thr Thr
180 185 190
Cys Pro Ser Thr Thr Pro Gly Ser Phe Ser Gln Glu Thr Thr Lys Gly
195 200 205
Ser Ala Phe Thr Thr Glu Ser Glu Thr Leu Pro Ala Ser Asn His Ser
210 215 220
Gln Arg Ser Met Met Thr Ile Ser Thr Asp Ile Ala Val Leu Arg Pro
225 230 235 240
Thr Gly Ser Asn Pro Gly Ile Leu Pro Ser Thr Ser Gln Leu Thr Thr

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245 250 255
Gln Lys Thr Thr Leu Thr Thr Ser Glu Ser Leu Gln Lys Thr Thr Lys
260 265 270
Ser His Gln Ile Asn Ser Arg Gln Thr Ile Leu Ile Ile Ala Cys Cys
275 280 285
Val Gly Phe Val Leu Met Val Leu Leu Phe Leu Ala Phe Leu Leu Arg
290 295 300
Gly Lys Val Thr Gly Ala Asn Cys Leu Gln Arg His Lys Arg Pro Asp
305 310 315 320
Asn Thr Glu Val Ser Asp Ser Phe Leu Asn Asp Ile Ser His Gly Arg
325 330 335
Asp Asp Glu Asp Gly Ile Phe Thr Leu
340 345

<210> 16
<211> 1032
<212> DNA
<213> Mus musculus

<400> 16
atgtccaagg ggcttctcct cctctggctg gtgatggagc tctgggtggct ttatctgaca 60
ccagctgcct cagaggatac aataataggg tttttgggcc agccggtgac ttgtccttgt 120
cattacctct cgtggtccca gagccgcaac agtatgtgct ggggcaaagg ttcatgtccc 180
aattccaagt gcaatgcaga gcttctccgt acagatggaa caagaatcat ctccaggaag 240
tcacaaaaat atacactttt ggggaaggtc cagtttgggt aagtgtcctt gaccatctca 300
aacaccaatc gaggtgacag tgggtgtgtac tgctgccgta tagaggtgcc tggctggttc 360
aatgatgtca agagaatgt ggccttggag ctgaggagag ccacaacaac caaaaaacca 420
acaacaacca ccgggcaac caaccacctt catytaaaca ccaccacccc agagctgtct 480
ccaacaacag tcatgacac atctgttctt ccaaccacca caccaccccc gacactagcc 540
accactgcct tcatgacagc agtgaccacg tgccctctca caaaccttgg ctctctctca 600
caagaaccca caaagggtc cgccttccct acagaaatca aaactctgac tgcattccat 660
cactctcaaa gaagcatgat gaccatatct acagacatag ccgtactcag gccacacggc 720
tctaaccctg ggattctccc atccacttca cagctacaga cacagaaaac aacattaaca 780
acaagtgagt ctttgcagaa gacaactaaa tcacatcaga tcaacagcag acagaccatc 840
ttgatcattg cctgtctgtg gggatttgtg ctaatgggtt tattgtttct ggcgttttct 900
cttcgaggga aagtcacagg agccaactgt ttgcagagac acaagaggcc agacaacact 960
gaagatagtg acagcgtcct caatgacatg tcacacggga gggatgatga agacggggtc 1020
ttcactctct ga 1032

<210> 17
<211> 359
<212> PRT
<213> H. sapiens

<220>
<221> VARIANT
<222> (1)...(360)
<223> TIM-1 allele 1

<400> 17
Met His Pro Gln Val Val Ile Leu Ser Leu Ile Leu His Leu Ala Asp
1 5 10 15
Ser Val Ala Gly Ser Val Lys Val Gly Gly Glu Ala Gly Pro Ser Val
20 25 30
Thr Leu Pro Cys His Tyr Ser Gly Ala Val Thr Ser Met Cys Trp Asn
35 40 45
Arg Gly Ser Cys Ser Leu Phe Thr Cys Gln Asn Gly Ile Val Trp Thr
50 55 60
Asn Gly Thr His Val Thr Tyr Arg Lys Asp Thr Arg Tyr Lys Leu Leu
65 70 75 80
Gly Asp Leu Ser Arg Arg Asp Val Ser Leu Thr Ile Glu Asn Thr Ala

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      85          90          95
Val Ser Asp Ser Gly Val Tyr Cys Arg Val Glu His Arg Gly Trp
      100          105          110
Phe Asn Asp Met Lys Ile Thr Val Ser Leu Glu Ile Val Pro Pro Lys
      115          120          125
Val Thr Thr Thr Pro Ile Val Thr Thr Val Pro Thr Val Thr Thr Val
      130          135          140
Arg Thr Ser Thr Thr Val Pro Thr Thr Thr Val Pro Thr Thr Thr
      145          150          155          160
Val Pro Thr Thr Thr Met Ser Ile Pro Thr Thr Thr Thr Val Pro Thr Thr
      165          170          175
Met Thr Val Ser Thr Thr Thr Ser Val Pro Thr Thr Thr Ser Ile Pro
      180          185          190
Thr Thr Thr Ser Val Pro Val Thr Thr Thr Val Ser Thr Phe Val Pro
      195          200          205
Pro Met Pro Leu Pro Arg Gln Asn His Glu Pro Val Ala Thr Ser Pro
      210          215          220
Ser Ser Pro Gln Pro Ala Glu Thr His Pro Thr Thr Leu Gln Gly Ala
      225          230          235          240
Ile Arg Arg Glu Pro Thr Ser Ser Pro Leu Tyr Ser Tyr Thr Thr Asp
      245          250          255
Gly Asn Asp Thr Val Thr Glu Ser Ser Asp Gly Leu Trp Asn Asn Asn
      260          265          270
Gln Thr Gln Leu Phe Leu Glu His Ser Ser Leu Leu Thr Ala Asn Thr Thr
      275          280          285
Lys Gly Ile Tyr Ala Gly Val Cys Ile Ser Val Leu Val Leu Leu Ala
      290          295          300
Leu Leu Gly Val Ile Ile Ala Lys Lys Tyr Phe Phe Lys Lys Glu Val
      305          310          315          320
Gln Gln Leu Ser Val Ser Phe Ser Ser Leu Gln Ile Lys Ala Leu Gln
      325          330          335
Asn Ala Val Glu Lys Glu Val Gln Ala Glu Asp Asn Ile Tyr Ile Glu
      340          345          350
Asn Ser Leu Tyr Ala Thr Asp
      355

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<210> 18
<211> 1080
<212> DNA
<213> H. sapiens

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<400> 18
atgcatctctc aagtgggtcat cttaagcctc atctctacatc tggcagattc tgtagctggt 60
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gctgtcacat caatgtgtcg gaatagaagg tcatgtcttc tattacatg ccaaaatggc 180
attgtctgga ccaatgggac ccacgtcaccc tatcgggaag acacacgcta caagctattg 240
ggggaccttt caagaaggga tgtctctttg accatagaaa atacagctgt gcttgacct 300
ggcgatatatt gttgcctgtt tgagtcacct ggggtggttca atgacatgaa aatcacctga 360
tcattggaga ttgtgccacc caaggtccagc actactccaa ttgtcacacc tgttccaacc 420
gtcacgactg ttgcaacgag caccactgtt ccaacgacaa cgactgttcc aacgacaact 480
gttccaacaa caatgagcat tccaacgaca acgactgttc cgacgacaat gactgtttca 540
acgacaacga gcgttccaac gacaacgagc attccaacaa caacacagtgt tccagtggca 600
acaacggtct ctacctttgt tcttccaatg cctttgccca ggcagaacca tgaaccagta 660
gccacttcac catcttcacc tcagccagca gaaacccacc ctacgacact gcaggagaca 720
ataaggagag aaccaccacg ctccaccatg tacttttaca caacagatgg gaatgacacc 780
gtgacagagt cttcagatgg cctttggaat aacaatcaaa ctcaactgtt cctagaacat 840
agctactctga cggccaatac cactaaagga atctatgctg gactctgtat ttctgtcttg 900
gtgctctctg ctcttttggg tgtcatcatt gccaaaaagt atttcttcaa aaaggaggtt 960
caacaactaa gtgtttcatt tagcagcett caaattaaag ctttgcaaaa tgcagttgaa 1020
aaggaagtcc aagcagaaga caatatctac atigagaata gtctttatgc cacggactaa 1080

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<210> 19
 <211> 359
 <212> PRT
 <213> H. sapiens

 <220>
 <221> VARIANT
 <222> (1)...(359)
 <223> TIM-1, allele 2

 <400> 19
 Met His Pro Gln Val Val Ile Leu Ser Leu Ile Leu His Leu Ala Asp
 1 5 10 15
 Ser Val Ala Gly Ser Val Lys Val Gly Gly Glu Ala Gly Pro Ser Val
 20 25 30
 Thr Leu Pro Cys His Tyr Ser Gly Ala Val Thr Ser Met Cys Trp Asn
 35 40 45
 Arg Gly Ser Cys Ser Leu Phe Thr Cys Gln Asn Gly Ile Val Trp Thr
 50 55 60
 Asn Gly Thr His Val Thr Tyr Arg Lys Asp Thr Arg Tyr Lys Leu Leu
 65 70 75 80
 Gly Asp Leu Ser Arg Asp Val Ser Leu Thr Ile Glu Asn Thr Ala
 85 90 95
 Val Ser Asp Ser Gly Val Tyr Cys Cys Arg Val Glu His Arg Gly Trp
 100 105 110
 Phe Asn Asp Met Lys Ile Thr Val Ser Leu Glu Ile Val Pro Pro Lys
 115 120 125
 Val Thr Thr Thr Pro Ile Val Thr Thr Val Pro Thr Val Thr Val
 130 135 140
 Arg Thr Ser Thr Thr Val Pro Thr Thr Thr Thr Val Pro Thr Thr Thr
 145 150 155 160
 Val Pro Thr Thr Met Ser Ile Pro Thr Thr Thr Thr Val Pro Thr Thr
 165 170 175
 Met Thr Val Ser Thr Thr Thr Ser Val Pro Thr Thr Thr Ser Ile Pro
 180 185 190
 Thr Thr Thr Ser Val Pro Val Thr Thr Ala Val Ser Thr Phe Val Pro
 195 200 205
 Pro Met Pro Leu Pro Arg Gln Asn His Glu Pro Val Ala Thr Ser Pro
 210 215 220
 Ser Ser Pro Gln Pro Ala Glu Thr His Pro Thr Thr Leu Gln Gly Ala
 225 230 235 240
 Ile Arg Arg Glu Pro Thr Ser Ser Pro Leu Tyr Ser Tyr Thr Thr Asp
 245 250 255
 Gly Asn Asp Thr Val Thr Glu Ser Ser Asp Gly Leu Trp Asn Asn Asn
 260 265 270
 Gln Thr Gln Leu Phe Leu Glu His Ser Leu Leu Thr Ala Asn Thr Thr
 275 280 285
 Lys Gly Ile Tyr Ala Gly Val Cys Ile Ser Val Leu Val Leu Leu Ala
 290 295 300
 Leu Leu Gly Val Ile Ile Ala Lys Lys Tyr Phe Phe Lys Lys Glu Val
 305 310 315 320
 Gln Gln Leu Ser Val Ser Phe Ser Ser Leu Gln Ile Lys Ala Leu Gln
 325 330 335
 Asn Ala Val Glu Lys Glu Val Gln Ala Glu Asp Asn Ile Tyr Ile Glu
 340 345 350
 Asn Ser Leu Tyr Ala Thr Asp
 355

 <210> 20
 <211> 1080

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<212> DNA
<213> H. sapiens

<400> 20
atgcacccctc aagtggtcat cttaagcctc atccctacac tggcagattc ttagctggt 60
tctgtaaagg ttggtggaga ggcagggtcca tctgtcacac taccctgcc ctacagtga 120
gctgtcacat caatgtgctg gaatagagcc tcatgtttct tattcacatg ccaaaatggc 180
attgtctgga ccaatgggaa ccacgtcacc tatcggaagg acacagctga taagctattg 240
ggggaccttt caagaaggga tgtctctttg accatagaaa atacagctgt gtctgacagt 300
ggcgtatatt gttgccgtgt tgagcaccgt ggggtggtta atgacatgaa aatcaccgtg 360
tcattggaga ttgtgccacc caaggtcacg actactccaa ttgtcacaa tgttccaacc 420
gtcacgactg ttggaacgag caccactgtt ccaacgacaa cgactgttcc aacgacaact 480
gttccaacaa caatgagcat tccaacgaca acgactgttc cgacgacaat gactgttca 540
acgacaacga ggtgtccaac gacaacgagc attccaacaa caacaagtgt tccagtga 600
acagcggtct ctacttttgt tctctccatg cctttgccaa ggcagaacca tgaaccagta 660
gccacttcac catcttoacc ttagccagca gaaacccacc ctacgacact gcaaggagca 720
ataagagag aaccocagg ctcaccattg tactcttaca caacagatgg gaatgacacc 780
gtcacagagt cttcagatgg cctttggaat aacaatcaaa ctcaactgtt cctagaacat 840
agtctactga cgcccaatc cactaaagga atctatgctg gactgtgtat tctgtcttg 900
gtgtctcttg ctcttttggg tgtcatcatt gccaaaaagt atttcttcaa aaagagagtt 960
caacactaa gtgtttcatt tagcagcatt caaattaag ctttgcaaaa tgcagttgaa 1020
aagggaagtcc aagcagaaga caatatctac attgagaata gtctttatgc caggactaa 1080

<210> 21
<211> 365
<212> PRT
<213> H. sapiens

<220>
<221> VARIANT
<222> (1)...(365)
<223> TIM-1, allele 3

<400> 21
Met His Pro Gln Val Val Ile Leu Ser Leu Ile Leu His Leu Ala Asp
1 5 10 15
Ser Val Ala Gly Ser Val Lys Val Gly Gly Glu Ala Gly Pro Ser Val
20 25 30
Thr Leu Pro Cys His Tyr Ser Gly Ala Val Thr Ser Met Cys Trp Asn
35 40 45
Arg Gly Ser Cys Ser Leu Phe Thr Cys Gln Asn Gly Ile Val Trp Thr
50 55 60
Asn Gly Thr His Val Thr Tyr Arg Lys Asp Thr Arg Tyr Lys Leu Leu
65 70 75 80
Gly Asp Leu Ser Arg Arg Asp Val Ser Leu Thr Ile Glu Asn Thr Ala
85 90 95
Val Ser Asp Ser Gly Val Tyr Cys Cys Arg Val Glu His Arg Gly Trp
100 105 110
Phe Asn Asp Met Lys Ile Thr Val Ser Leu Glu Ile Val Pro Pro Lys
115 120 125
Val Thr Thr Thr Pro Ile Val Thr Thr Val Pro Thr Val Thr Thr Val
130 135 140
Arg Thr Ser Thr Thr Val Pro Thr Thr Thr Thr Val Pro Met Thr Thr
145 150 155 160
Thr Val Pro Thr Thr Thr Val Pro Thr Thr Met Ser Ile Pro Thr Thr
165 170 175
Thr Thr Val Pro Thr Thr Met Thr Val Ser Thr Thr Thr Ser Val Pro
180 185 190
Thr Thr Thr Ser Ile Pro Thr Thr Thr Ser Val Pro Val Thr Thr Ala
195 200 205
Val Ser Thr Phe Val Pro Pro Met Pro Leu Pro Arg Gln Asn His Glu

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210          215          220
Pro Val Ala Thr Ser Pro Ser Ser Pro Gln Pro Ala Glu Thr His Pro
225          230          235          240
Thr Thr Leu Gln Gly Ala Ile Arg Arg Glu Pro Thr Ser Ser Pro Leu
245          250          255
Tyr Ser Tyr Thr Thr Asp Gly Asn Asp Thr Val Thr Glu Ser Ser Asp
260          265          270
Gly Leu Trp Asn Asn Asn Gln Thr Gln Leu Phe Leu Glu His Ser Leu
275          280          285
Leu Thr Ala Asn Thr Thr Lys Gly Ile Tyr Ala Gly Val Cys Ile Ser
290          295          300
Val Leu Val Leu Leu Ala Leu Leu Gly Val Ile Ile Ala Lys Lys Tyr
305          310          315          320
Phe Phe Lys Lys Glu Val Gln Gln Leu Ser Val Ser Phe Ser Ser Leu
325          330          335
Gln Ile Lys Ala Leu Gln Asn Ala Val Glu Lys Glu Val Gln Ala Glu
340          345          350
Asp Asn Ile Tyr Ile Glu Asn Ser Leu Tyr Ala Thr Asp
355          360          365

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<210> 22
<211> 1098
<212> DNA
<213> H. sapiens

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```

<400> 22
atgcatctctc aagtggtcat cttaaagcttc atoctacatc tggcagattc tgtagctggt 60
tctgtaaagg ttggtgaga ggcaggtcca tctgtcacac taccctgcca ctacagtgga 120
gctgtcacat caatgtgctg gaatagagcc tcatgtcttc tattcacatg ccaaaatggc 180
attgtctgga ccaatggaac ccacgtcacc tatcggaaag acacacgcta taagotattg 240
ggggaccttt caagaaggga tgtctctttg accatagaaa atacagctgt gtctgacast 300
ggcgatatatt gttgccgtgt tgagcacccgt ggggtggttca atgacatgaa aatcacogta 360
tcatgggaga ttgtgccacc caaggtccag actactccaa ttgtcacacs tgttccaacc 420
gtcacgactg ttccaacgag caccactgtt ccaacgacaa cgactgttcc satgacaacg 480
actgttccaa cgacaactgt tccaacaaca atgagcattc caacgacaac gactgttccg 540
acgacaatga ctgtttcaac gacaacgagc gttccaacga caacgagcat tccaacaaca 600
acaagtgttc cagtgcacac arogtgtctc acctttgttc ctccaatgcc tttgccacgg 660
cagaacctatg aaccagtgc cacttcacca tcttcacctc agccagcaga aacccacct 720
acgacactgc agggagcaat aaggagagaa cccaccagct caccattgta ctcttacaca 780
acagatggga atgacacctg gacagagtct tccagatggc tttggaataa caatcaact 840
caactgttcc tagaacatag tctactgacg gccaatacca cttaaaggaat ctatgctgga 900
gtctgtattt ctgtcttggg gcttcttggc cttttgggtg tcatcattgc caaaaagtat 960
ttcttcaaaa aggaggttca acaactaagt gtttcattta gcagccttca aattaaagct 1020
ttgcacaaatg cagttgnaaa ggaagtccaa gcagaagaca atatctacat tgagaatagt 1080
ctttatgcca cggactaa
1098

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<210> 23
<211> 359
<212> PRT
<213> H. sapiens

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<220>
<221> VARIANT
<222> (1)...(359)
<223> TIM-1, allele 4

```

```

<400> 23
Met His Pro Gln Val Val Ile Leu Ser Leu Ile Leu His Leu Ala Asp
1          5          10          15
Ser Val Ala Gly Ser Val Lys Val Gly Gly Glu Ala Gly Pro Ser Val
20          25          30

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Thr Leu Pro Cys His Tyr Ser Gly Ala Val Thr Ser Met Cys Trp Asn
 35 40 45
 Arg Gly Ser Cys Ser Leu Phe Thr Cys Gln Asn Gly Ile Val Trp Thr
 50 55 60
 Asn Gly Thr His Val Thr Tyr Arg Lys Asp Thr Arg Tyr Lys Leu Leu
 65 70 75 80
 Gly Asp Leu Ser Arg Arg Asp Val Ser Leu Thr Ile Glu Asn Thr Ala
 85 90 95
 Val Ser Asp Ser Gly Val Tyr Cys Cys Arg Val Glu His Arg Gly Trp
 100 105 110
 Phe Asn Asp Met Lys Ile Thr Val Ser Leu Glu Ile Val Pro Pro Lys
 115 120 125
 Val Thr Thr Thr Pro Ile Val Thr Thr Val Pro Thr Val Thr Thr Val
 130 135 140
 Arg Thr Ser Thr Thr Val Pro Thr Thr Thr Thr Val Pro Thr Thr Thr
 145 150 155 160
 Val Pro Thr Thr Met Ser Ile Pro Thr Thr Thr Val Pro Thr Thr
 165 170 175
 Met Thr Val Ser Thr Thr Thr Ser Val Pro Thr Thr Ser Ile Pro
 180 185 190
 Thr Thr Thr Ser Val Pro Val Thr Thr Ser Val Ser Thr Phe Val Pro
 195 200 205
 Pro Met Pro Leu Pro Arg Gln Asn His Glu Pro Val Ala Thr Ser Pro
 210 215 220
 Ser Ser Pro Gln Pro Ala Glu Thr His Pro Thr Thr Leu Gln Gly Thr
 225 230 235 240
 Ile Arg Arg Glu Pro Thr Ser Ser Pro Leu Tyr Ser Tyr Thr Thr Asp
 245 250 255
 Gly Asn Asp Thr Val Thr Glu Ser Ser Asp Gly Leu Trp Ser Asn Asn
 260 265 270
 Gln Thr Gln Leu Phe Leu Glu His Ser Leu Leu Thr Ala Asn Thr Thr
 275 280 285
 Lys Gly Ile Tyr Ala Gly Val Cys Ile Ser Val Leu Val Leu Leu Ala
 290 295 300
 Leu Leu Gly Val Ile Ile Ala Lys Lys Tyr Phe Phe Lys Lys Glu Val
 305 310 315 320
 Gln Gln Leu Ser Val Ser Phe Ser Ser Leu Gln Ile Lys Ala Leu Gln
 325 330 335
 Asn Ala Val Glu Lys Glu Val Gln Ala Glu Asp Asn Ile Tyr Ile Glu
 340 345 350
 Asn Ser Leu Tyr Ala Thr Asp
 355

<210> 24

<211> 1079

<212> DNA

<213> H. sapiens

<400> 24

atgcacccctc aagtgggtcat cttaagcctc atoctacatc tggcagattc ttagctgggt 60
 tctgtaaagg ttggtggaga ggcagggtcca tctgtcacac taccctgcc a ctacagtggg 120
 gctgtcacat caatgtgctg gaatagaggc tcatgttctc tattcacatg ccaaaatggc 180
 attgtcttga ccaatggaac ccacgtcacc tatcggaagg acacacgcta taagctattg 240
 ggggaccttt caagaaggga tgtctctttg accatagaaa atacagctgt gctgtacagt 300
 ggcgtatatt gttgccctgt tgagcacctg ggggtggttc atgacatgaa aatcacctga 360
 tcatgggaga ttgtgcacac caaggtcacg actactccaa ttgtcacaa tgttccaaac 420
 gtccagactg ttctgaacgag caccactgtt ccaacyacaa cgaactgttc aacgacaact 480
 gtccacaaca caatgagcat tccaacgaca acgagctgtt cgaacyacaa tgaactgttc 540
 aacgacaacg agcggttccaa cgaacagag cattccaaac acacaaagtg ttccagtac 600
 aacatgtctc tacctttgtt cctccaatgc ctttgcccag gcagaacctg gaacagtag 660
 ccacttcacc atcttcacct cagccagcag aaacccacc taccagactg cagggagcaa 720

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taaggagaga acccaccagc tcaccattgt actcttacac aacagatggg aatgacacg 780
 tgacagagtc ttcagatggc ctttggartt acaatcaaac tcaactgttc ctagaacata 840
 gtctactgac ggccaatacc actaaaggaa tctatgctgy agtctgtatt tctgtcttgg 900
 tgcttcttgc tcttttgggt gtcacatctg ccaaaaagta tttcttcaaa aaggaggttc 960
 aacaactaag tgtttcatit agcagccttc aaattaaagc ttgcacaaat gcagttgaaa 1020
 aggaagtcca agcagaagac aatatctaca ttgagaatag tctttatgcc acggactaa 1079

<210> 25
 <211> 364
 <212> PR1
 <213> H. sapiens

<220>
 <221> VARIANT
 <222> (1)...(364)
 <223> TIM-1 allele 5

<400> 25
 Met His Pro Gln Val Val Ile Leu Ser Leu Ile Leu His Leu Ala Asp
 1 5 10 15
 Ser Val Ala Gly Ser Val Lys Val Gly Gly Glu Ala Gly Pro Ser Val
 20 25 30
 Thr Leu Pro Cys His Tyr Ser Gly Ala Val Thr Ser Met Cys Trp Asn
 35 40 45
 Arg Gly Ser Cys Ser Leu Phe Thr Cys Gln Asn Gly Ile Val Trp Thr
 50 55 60
 Asn Gly Thr His Val Thr Tyr Arg Lys Asp Thr Arg Tyr Lys Leu Leu
 65 70 75 80
 Gly Asp Leu Ser Arg Asp Val Ser Leu Thr Ile Glu Asn Thr Ala
 85 90 95
 Val Ser Asp Ser Gly Val Tyr Cys Cys Arg Val Glu His Arg Gly Trp
 100 105 110
 Phe Asn Asp Met Lys Ile Thr Val Ser Leu Glu Ile Val Pro Pro Lys
 115 120 125
 Val Thr Thr Thr Pro Ile Val Thr Thr Val Pro Thr Val Thr Thr Val
 130 135 140
 Arg Thr Ser Thr Thr Val Pro Thr Thr Thr Thr Val Pro Met Thr Thr
 145 150 155 160
 Thr Val Pro Thr Thr Thr Val Pro Thr Thr Met Ser Ile Pro Thr Thr
 165 170 175
 Thr Thr Val Pro Thr Thr Met Thr Val Ser Thr Thr Thr Ser Val Pro
 180 185 190
 Thr Thr Thr Ser Ile Pro Thr Thr Ser Val Pro Val Thr Thr Thr Val
 195 200 205
 Ser Thr Phe Val Pro Pro Met Pro Leu Pro Arg Gln Asn His Glu Pro
 210 215 220
 Val Ala Thr Ser Pro Ser Ser Pro Gln Pro Ala Glu Thr His Pro Thr
 225 230 235 240
 Thr Leu Gln Gly Ala Ile Arg Arg Glu Pro Thr Ser Ser Pro Leu Tyr
 245 250 255
 Ser Tyr Thr Thr Asp Gly Asn Asp Thr Val Thr Glu Ser Ser Asp Gly
 260 265 270
 Leu Trp Asn Asn Asn Gln Thr Gln Leu Phe Leu Glu His Ser Leu Leu
 275 280 285
 Thr Ala Asn Thr Thr Lys Gly Ile Tyr Ala Gly Val Cys Ile Ser Val
 290 295 300
 Leu Val Leu Leu Ala Leu Leu Gly Val Ile Ile Ala Lys Lys Tyr Phe
 305 310 315 320
 Phe Lys Lys Glu Val Gln Gln Leu Ser Val Ser Phe Ser Ser Leu Gln
 325 330 335
 Ile Lys Ala Leu Gln Asn Ala Val Glu Lys Glu Val Gln Ala Glu Asp
 340 345 350

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Asn Ile Tyr Ile Glu Asn Ser Leu Tyr Ala Thr Asp
 355 360

<210> 26
 <211> 1095
 <212> DNA
 <213> H. sapiens

<400> 26
 atgcatcttc aagtggtcat cttaaagctc atctacatc tggcagattc ttagctggt 60
 tctgtaaaag ttggtggaga ggcagggtcca tctgtcacac taccctgcca ctacagtgg 120
 gctgtcacat caatgtgctg gaataagagg tcatgttctc tattccatg ccaaaatgg 180
 attgtctgga ccaatgggac ccacgtaccc tatcggaaag acacacgcta taagctattg 240
 ggggaccttt caagaaggga tgtctctttg accatagaaa atacagctgt gtctgacagt 300
 gggttatatt gttgccgtgt tgagcacagt ggttggttca atgacatgaa atcaccgtta 360
 tcattggaga ttgtgccacc caaggtcacg actactccaa ttgtcacaa tgttccacc 420
 gtacgactg ttggaacgag caccactgtt ccaacgacaa cgaactgttc aatgacaacg 480
 actgttccaa cgacaactgt tccaacaaca atgacattc caacgacaac gactgtccg 540
 acgacaatga ctgtttcaac gacaacgagc gttccaacga caacgagcat tccaacaaca 600
 agtgttccag tgacaacaac ggtctctacc tttgttctc caatgccttt gccacggcag 660
 aacatgaac cagtaccac ttcaccatct tcacctcagc cagcagaaac caccctcag 720
 acactgcagg gagcaataag gagagaaccc accagctcac cattgtactc ttacacaaca 780
 gatgggaatg acaccgtgac agagtcttca gatggccttt ggaataacaa tcaaaactaa 840
 ctgttctctag aacatagctc actgacggcc aataccacta aaggaatcta tgctggagtc 900
 tgaattctcg tcttggtgct tcttgctctt ttgggtgtca tcattgcca aaagtatttc 960
 ttcaaaaagg aggttcacaa actaagtgtt tcatttagca gccttcaaat taaagctttg 1020
 caaaatgcag ttgaaaagga agtccaagca gaagacaata tctacattga gaatagcttt 1080
 tatgcccagg actaa 1095

<210> 27
 <211> 364
 <212> PRT
 <213> H. sapiens

<220>
 <221> VARIANT
 <222> (1)...(364)
 <223> TIM-1, allele 6

<400> 27
 Met His Pro Gln Val Val Ile Leu Ser Leu Ile Leu His Leu Ala Asp
 1 5 10 15
 Ser Val Ala Gly Ser Val Lys Val Gly Glu Ala Gly Pro Ser Val
 20 25 30
 Thr Leu Pro Cys His Tyr Ser Gly Ala Val Thr Ser Met Cys Trp Asn
 35 40 45
 Arg Gly Ser Cys Ser Leu Phe Thr Cys Gln Asn Gly Ile Val Trp Thr
 50 55 60
 Asn Gly Thr His Val Thr Tyr Arg Lys Asp Thr Arg Tyr Lys Leu Leu
 65 70 75 80
 Gly Asp Leu Ser Arg Arg Asp Val Ser Leu Thr Ile Glu Asn Thr Ala
 85 90 95
 Val Ser Asp Ser Gly Val Tyr Cys Cys Arg Val Glu His Arg Gly Trp
 100 105 110
 Phe Asn Asp Met Lys Ile Thr Val Ser Leu Gly Ile Val Pro Pro Lys
 115 120 125
 Val Thr Thr Thr Pro Ile Val Thr Thr Val Pro Thr Val Thr Val
 130 135 140
 Arg Thr Ser Thr Thr Val Pro Thr Thr Thr Val Pro Met Thr Thr
 145 150 155 160
 Thr Val Pro Thr Thr Thr Val Pro Thr Thr Met Ser Ile Pro Thr Thr

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      165      170      175
Thr Thr Val Pro Thr Thr Met Thr Val Ser Thr Thr Thr Ser Val Pro
      180      185      190
Thr Thr Thr Ser Ile Pro Thr Thr Ser Val Pro Val Thr Thr Thr Val
      195      200      205
Ser Thr Phe Val Pro Pro Met Pro Leu Pro Arg Gln Asn His Glu Pro
      210      215      220
Val Ala Thr Ser Pro Ser Ser Pro Gln Pro Ala Glu Thr His Pro Thr
      225      230      235      240
Thr Leu Gln Gly Ala Ile Arg Arg Glu Pro Thr Ser Ser Pro Leu Tyr
      245      250      255
Ser Tyr Thr Thr Asp Gly Asp Asp Thr Val Thr Glu Ser Ser Asp Gly
      260      265      270
Leu Trp Asn Asn Asn Gln Thr Gln Leu Phe Leu Glu His Ser Leu Leu
      275      280      285
Thr Ala Asn Thr Thr Lys Gly Ile Tyr Ala Gly Val Cys Ile Ser Val
      290      295      300
Leu Val Leu Leu Ala Leu Leu Gly Val Ile Ile Ala Lys Lys Tyr Phe
      305      310      315      320
Phe Lys Lys Glu Val Gln Gln Leu Ser Val Ser Phe Ser Ser Leu Gln
      325      330      335
Ile Lys Ala Leu Gln Asn Ala Val Glu Lys Glu Val Gln Ala Glu Asp
      340      345      350
Asn Ile Tyr Ile Glu Asn Ser Leu Tyr Ala Thr Asp
      355      360

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<210> 28

<211> 1099

<212> DNA

<213> H. sapiens

<400> 28

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atgcaccttc aagtggtcat cttaaagctc atcctacatc tggcagattc tgtagctggt 60
tctgtaaagg ttggtggaga ggcaggtcca tctgtcacac taccctgcca ctacagtggg 120
gctgtcacat caatgtgctg gaatagaggg tcatgttttc tattcacatg ccaaaatggc 180
attgtctgga ccaatggaac ccacgtcacc tatcggaagg acacacgcta taagctattg 240
ggggaacctt caagaaggga tgtctctttg accatagaaa atacagctgt gctcgacagt 300
ggcgtatatt gttgcgtgtg tgagcaccgt ggggtggttc atgacatgaa aatcaccgta 360
tcattggaga ttgtgccacc caaggtcacc actactccaa ttgtcacaa tggtccaacc 420
gtacgactcg ttggaacgag caccactgtt ccaacgacaa cgactgttcc aatgacaacc 480
gaactgtcca acgacaactg ttccaaccaac aatgagcatt ccaacgacaa cgactgttcc 540
gacgacaatg actgtttcaa cgaacaacgag cgttccaaac acaacgagca ttccaaccaac 600
aacaagtgtt ccagtgaaca caacggtctc tactttgtt cctccaatgc ctttgcacag 660
gcagaacctt gaaccagtag ccacttcacc atcttcacct cagccagcag aaaccacccc 720
tacgacactg caggagagcaa taaggagaga acccaccagc tcaccatgtg actcttaaac 780
aacagatggg gatgacaccg tgacagagtc ttacatgggc ctttgggaata acaatcaaac 840
tcaactgttc ctagaacata gtctactgac ggccaatacc actaaaggaa tctatgctgg 900
agtctgtatt tctgtcttgg tgcttcttgc tcttttgggt gtcatcattg ccaaaaagta 960
tttcttcaaa aaggaggttc aacaactaag tgttttcatt agcagccttc aaattaaagc 1020
tttgcaaaat gcagtggaaa aggaagtcca agcagaagac aatatctaca ttgagaatag 1080
tctttatgcc acggactaa

```

<210> 29

<211> 301

<212> PRT

<213> H. sapiens

<220>

<221> VARIANT

<222> (1)...(301)

<223> TIM-3, allele 1

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<400> 29
Met Phe Ser His Leu Pro Phe Asp Cys Val Leu Leu Leu Leu Leu
1      5      10      15
Leu Leu Thr Arg Ser Ser Glu Val Glu Tyr Arg Ala Glu Val Gly Gln
20      25      30
Asn Ala Tyr Leu Pro Cys Phe Tyr Thr Pro Ala Ala Pro Gly Asn Leu
35      40      45
Val Pro Val Cys Trp Gly Lys Gly Ala Cys Pro Val Phe Glu Cys Gly
50      55      60
Asn Val Val Leu Arg Thr Asp Glu Arg Asp Val Asn Tyr Trp Thr Ser
65      70      75
Arg Tyr Trp Leu Asn Gly Asp Phe Arg Lys Gly Asp Val Ser Leu Thr
80      85      90
Ile Glu Asn Val Thr Leu Ala Asp Ser Gly Ile Tyr Cys Cys Arg Ile
95      100      105
Gln Ile Pro Gly Ile Met Asn Asp Glu Lys Phe Asn Leu Lys Leu Val
110      115      120
Ile Lys Pro Ala Lys Val Thr Pro Ala Pro Thr Arg Gln Arg Asp Phe
125      130      135
Thr Ala Ala Phe Pro Arg Met Leu Thr Thr Arg Gly His Gly Pro Ala
140      145      150
Glu Thr Gln Thr Leu Gly Ser Leu Pro Asp Ile Asn Leu Thr Gln Ile
155      160      165
Ser Thr Leu Ala Asn Glu Leu Arg Asp Ser Arg Leu Ala Asn Asp Leu
170      175      180
Arg Asp Ser Gly Ala Thr Ile Arg Ile Gly Ile Tyr Ile Gly Ala Gly
185      190      195
Ile Cys Ala Gly Leu Ala Leu Ala Leu Ile Phe Gly Ala Leu Ile Phe
200      205      210
Lys Trp Tyr Ser His Ser Lys Glu Lys Ile Gln Asn Leu Ser Leu Ile
215      220      225
Ser Leu Ala Asn Leu Pro Pro Ser Gly Leu Ala Asn Ala Val Ala Glu
230      235      240
Gly Ile Arg Ser Glu Glu Asn Ile Tyr Thr Ile Glu Glu Asn Val Tyr
245      250      255
Glu Val Glu Glu Pro Asn Glu Tyr Tyr Cys Tyr Val Ser Ser Arg Gln
260      265      270
Gln Pro Ser Gln Pro Leu Gly Cys Arg Phe Ala Met Pro
275      280      285
290      295      300

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<210> 30
<211> 1116
<212> DNA
<213> H. sapiens

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<400> 30
ggagaggttaa aactgtgcct aacagaggtg tcctctgact ttctctctgc aagctccatg 60
ttttcacatc ttccctttga ctgtgtcctg ctgtgtcctg tgctactact tacaaggctc 120
tcagaagtgg aatacagagc ggaggtcggt cagaatgcct atctgccttg cttctacacc 180
ccagccgccc cagggaaacct cgtgcccgtc tgctggggca aaggagcctg tcctgtgttt 240
gaatgtggca acgtggtgct caggactgat gaaagggatg tgaattattg gacatccaga 300
tactggctaa atggggattt ccgcaaggga gatgtgtccc tgaccataga gaatgtgact 360
ctagcagaca gtgggatcta ctgctgcctg atccaaatcc caggcataat gaatgtgaa 420
aaatttaacc tgaagtgggt catcaaacca gcccaaggtca cccctgcacc gactctgcag 480
agagacttca ctgcagcctt tccaaggatg cttaacacac ggggacatgg cccagcagag 540
acacagacac tggggagcct cctgatata aatcaaacac aaataccac attgycat 600
gagttacggg actctagatt gccaatgac ttacgggact ctggagcaac catcagaata 660
ggcatctaca tcggagcagg gatctgtgct ggcctgctc tggctcttat cttcggcgt 720
ttaatttca aatggtattc tcatagcaaa gagaagatac agaatttag cctcatctct 780
ttggccaacc tcctccctc aggtatggca aatgcagtag cagaggggat tcgctcagaa 840

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gaaacatct ataccattga agagaacgta tatgaagtg aggagcccaa ttagtattat 900
tgctatgtca gcagcaggca gcaaccctca caacctttgg gttgtcgctt tgcattgcca 960
tagatccaac caccttattt ttgagcttgg tgttttgtct ttttcagaaa ctatgagctg 1020
tgtcacctga ctggttttgg aggtttctgtc cactgctatg gacgagagtt ttccattttt 1080
cagaagataa tgactcacat gggaattgaa ctggga 1116

```

<210> 31
 <211> 301
 <212> PRT
 <213> H. sapiens

<220>
 <221> VARIANT
 <222> (1)...(301)
 <223> TIM-3, allele 2

```

<400> 31
Met Phe Ser His Leu Pro Phe Asp Cys Val Leu Leu Leu Leu Leu
1      5      10      15
Leu Leu Thr Arg Ser Ser Glu Val Glu Tyr Arg Ala Glu Val Gly Gln
20      25      30
Asn Ala Tyr Leu Pro Cys Phe Tyr Thr Pro Ala Ala Pro Gly Asn Leu
35      40      45
Val Pro Val Cys Trp Gly Lys Gly Ala Cys Pro Val Phe Glu Cys Gly
50      55      60
Asn Val Val Leu Arg Thr Asp Glu Arg Asp Val Asn Tyr Trp Thr Ser
65      70      75      80
Arg Tyr Trp Leu Asn Gly Asp Phe Arg Lys Gly Asp Val Ser Leu Thr
85      90      95
Ile Glu Asn Val Thr Leu Ala Asp Ser Gly Ile Tyr Cys Cys Arg Ile
100     105     110
Gln Ile Pro Gly Ile Met Asn Asp Glu Lys Phe Asn Leu Lys Leu Val
115     120     125
Ile Lys Pro Ala Lys Val Thr Pro Ala Pro Thr Leu Gln Arg Asp Phe
130     135     140
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【国際調査報告】

INTERNATIONAL SEARCH REPORT		International application No.															
A. CLASSIFICATION OF SUBJECT MATTER IPC(7) : A61K 39/00, 39/395, 67/00, C12Q 1/68; G01N 22/53; C12N 15/00, 15/64; C07K 16/00; C07H 21/04 US CL : 424/130.1, 184.1, 185.1; 436/6, 7.1, 91.42; 530/387.1; 536.3.5; 800/9, 21 According to International Patent Classification (IPC) or to both national classification and IPC		PCT/US02/20890															
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 424/130.1, 184.1, 185.1; 436/6, 7.1, 91.42; 530/387.1; 536/23.5; 800/9, 21 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base used, where practicable, search terms used) GenBank, EST, C. nucleotide sequence, STN, MEDLINE, BIOSIS, EMBASE, CAPLUS, SCISEARCH																	
C. DOCUMENTS CONSIDERED TO BE RELEVANT <table border="1"> <thead> <tr> <th>Category *</th> <th>Citation of document, with indication, where appropriate, of the relevant passages</th> <th>Relevant to claim No.</th> </tr> </thead> <tbody> <tr> <td>X</td> <td>US 5,721,351 A (LEVINSON) 24 February 1998 (24.02.1998), column 87, especially reference SEQ ID NO: 8, column 61, line 50, column 34, line 8, columns 44-48, column 49, lines 36-32, and column 51, lines 22-27.</td> <td>1, 3-4, 6-9, 11-14, 16-18, 23-32</td> </tr> <tr> <td>X</td> <td>FEIGELSTOCK, D. et al. The Human Homolog of HAVcr-1 Codes for a Hepatitis A Virus Cellular Receptor. J. Virology. August 1998, Vol. 72, No. 8, pages 6621-28, especially page 6624, Figure 2.</td> <td>1, 4-6 and 19-22</td> </tr> <tr> <td>Y</td> <td>CARNINCI, P. et al. Normalization and Subtraction of Cap-Trapper-Selected cDNAs to Prepare Full-Length cDNA Libraries for Rapid Discovery of New Genes. Genome Res. 2000, Vol. 10, No. 10, pages 1617-30, especially page 1619-1621.</td> <td>4 and 7</td> </tr> <tr> <td>X</td> <td>ICHIMURA, T. et al. Kidney Injury Molecule-1 (KIM-1), a Putative Epithelial Cell Adhesion Molecule Containing a Novel Immunoglobulin Domain, Is Up-regulated in Renal Cells After Injury. J. Biol. Chem. 13 February 1998, Vol. 273, No. 7, pages 4135-4142, especially page 4138, and page 4136, column 2.</td> <td>1, 4 and 11</td> </tr> </tbody> </table>			Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	X	US 5,721,351 A (LEVINSON) 24 February 1998 (24.02.1998), column 87, especially reference SEQ ID NO: 8, column 61, line 50, column 34, line 8, columns 44-48, column 49, lines 36-32, and column 51, lines 22-27.	1, 3-4, 6-9, 11-14, 16-18, 23-32	X	FEIGELSTOCK, D. et al. The Human Homolog of HAVcr-1 Codes for a Hepatitis A Virus Cellular Receptor. J. Virology. August 1998, Vol. 72, No. 8, pages 6621-28, especially page 6624, Figure 2.	1, 4-6 and 19-22	Y	CARNINCI, P. et al. Normalization and Subtraction of Cap-Trapper-Selected cDNAs to Prepare Full-Length cDNA Libraries for Rapid Discovery of New Genes. Genome Res. 2000, Vol. 10, No. 10, pages 1617-30, especially page 1619-1621.	4 and 7	X	ICHIMURA, T. et al. Kidney Injury Molecule-1 (KIM-1), a Putative Epithelial Cell Adhesion Molecule Containing a Novel Immunoglobulin Domain, Is Up-regulated in Renal Cells After Injury. J. Biol. Chem. 13 February 1998, Vol. 273, No. 7, pages 4135-4142, especially page 4138, and page 4136, column 2.	1, 4 and 11
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☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.																	
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INTERNATIONAL SEARCH REPORT		PCT/US02/2085
C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	XA. J.Q. et al. Immune Response and Airway Reactivity in Wild and IL-4 Knockout Mice Exposed to Latex Allergens. Int. Arch. Aller. Immunol. January 1999, Vol 118, No. 1, pages 23-29, see entire document.	15
Y	US 5,986,059 A (SHANAFELT et al) 16 November, 1999 (16.11.1999), column 4, line 15-19, column 15, lines 1-5.	33-39

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(51)Int.Cl. ⁷	F I	テーマコード (参考)
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(72)発明者 マクインタイア, ジェニファー ジョーンズ
アメリカ合衆国 カリフォルニア 9 4 3 0 4, パロ アルト, クラーク ウェイ 1 1 0 6

(72)発明者 デクルイフ, ローズマリー エイチ.
アメリカ合衆国 カリフォルニア 9 4 3 0 5, スタンフォード, アラーディス ウェイ 8 2 5

(72)発明者 ウメツ, デール ティー.
アメリカ合衆国 カリフォルニア 9 4 3 0 5, スタンフォード, アラーディス ウェイ 8 2 5

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(72)発明者 ククロウ, ヴィジェイ
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申请号	JP2003509084	申请日	2002-07-01
[标]申请(专利权)人(译)	斯坦福大学		
申请(专利权)人(译)	在利兰·斯坦福初级大学董事会		
[标]发明人	マクインタイアジェニファージョーンズ デクルイフローズマリイエイチ ウメツデールティー フリーマンゴードンジェイ ククローヴィジエイ		
发明人	マクインタイア, ジェニファージョーンズ デクルイフ, ローズマリイ エイチ. ウメツ, デール ティー. フリーマン, ゴードン ジェイ. ククロー, ヴィジエイ		
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F-TERM分类号	2G045/AA35 2G045/CB01 2G045/FB02 4B024/AA01 4B024/AA11 4B024/BA80 4B024/CA04 4B024 /CA05 4B024/CA09 4B024/CA11 4B024/DA02 4B024/HA14 4B029/AA07 4B029/FA12 4B063/QA06 4B063/QA13 4B063/QA18 4B063/QA19 4B063/QQ08 4B063/QQ43 4B063/QQ79 4B063/QR32 4B063 /QR48 4B063/QR55 4B063/QR62 4B063/QR77 4B063/QS25 4B063/QS34 4B065/AA91X 4B065 /AA91Y 4B065/AA93X 4B065/AA93Y 4B065/AB01 4B065/CA24 4B065/CA44 4B065/CA46 4C084 /AA17 4C084/AA20 4C084/MA02 4C084/NA14 4C084/ZA59 4C084/ZA89 4C084/ZB02 4C084/ZB07 4C084/ZB08 4C084/ZB11 4C084/ZB13 4C084/ZB26 4C084/ZC02 4C084/ZC41 4C084/ZC75 4C085 /AA13 4C085/AA14 4C085/BB11 4C085/BB18 4C085/CC02 4C085/CC07 4C085/CC08 4C085/CC21 4C085/CC31 4C085/DD23 4C085/DD62 4C085/DD63 4C085/EE01 4C085/EE03 4H045/AA10 4H045 /AA11 4H045/AA20 4H045/AA30 4H045/BA10 4H045/CA40 4H045/DA50 4H045/DA75 4H045/EA20 4H045/EA50 4H045/FA74		
代理人(译)	夏木森下		
优先权	60/302344 2001-06-29 US		
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摘要(译)

提供了与免疫功能和细胞存活的调节有关的遗传基因座和相应的蛋白质家族。 这些基因编码具有保守的IgV和粘蛋白结构域的细胞表面分子。 包含TIM家族的基因座与免疫功能障碍 (包括哮喘) 遗传相关。 此外, TIM基因家族位于人类5号染色体区域内, 该区域在恶性肿瘤和骨髓增生异常综合征中通常被删除。 基因序列中的多态性与气道高反应性和过敏性炎症的发展以及IL-4和IL-13的T细胞产生有关。 这些蛋白质包括人类甲型肝炎细胞受体hHAVcr-1。

