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(54) **ANTIBODIES DIRECTED TO MONOCYTE
CHEMO-ATTRACTANT PROTEIN-1 (MCP-1)
AND USES THEREOF**

Related U.S. Application Data

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(76) Inventors: **Jean M. Gudas**, Pacific Palisades, CA (US); **Mary Haak-Frendscho**, Newark, CA (US); **Orit Foord**, Foster City, CA (US); **Meina L. Liang**, Danville, CA (US); **Kiran Ahluwalia**, Fremont, CA (US); **Sunil Bhakta**, Hayward, CA (US)

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Correspondence Address:
KNOBBE MARTENS OLSON & BEAR LLP
2040 MAIN STREET
FOURTEENTH FLOOR
IRVINE, CA 92614 (US)

ABSTRACT

(57) Embodiments of the invention described herein relate to antibodies directed to the antigen monocyte chemo-attractant protein-1 (MCP-1) and uses of such antibodies. In particular, in accordance with some embodiments, there are provided fully human monoclonal antibodies directed to the antigen MCP-1. Nucleotide sequences encoding, and amino acid sequences comprising, heavy and light chain immunoglobulin molecules, particularly sequences corresponding to contiguous heavy and light chain sequences spanning the framework regions and/or complementarity determining regions (CDRs), specifically from FR1 through FR4 or CDR1 through CDR3, are provided. Hybridomas or other cell lines expressing such immunoglobulin molecules and monoclonal antibodies are also provided.

(21) Appl. No.: **10/644,277**

(22) Filed: **Aug. 19, 2003**

Figure 1

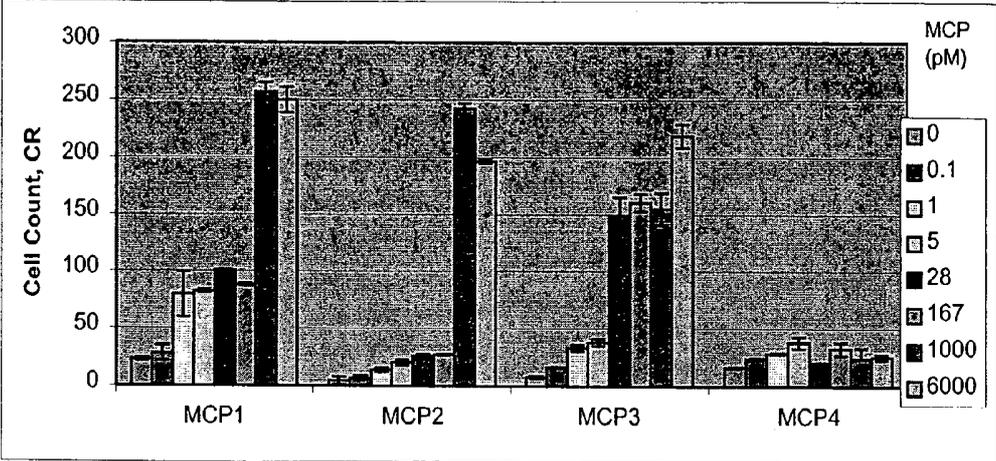


Figure 2

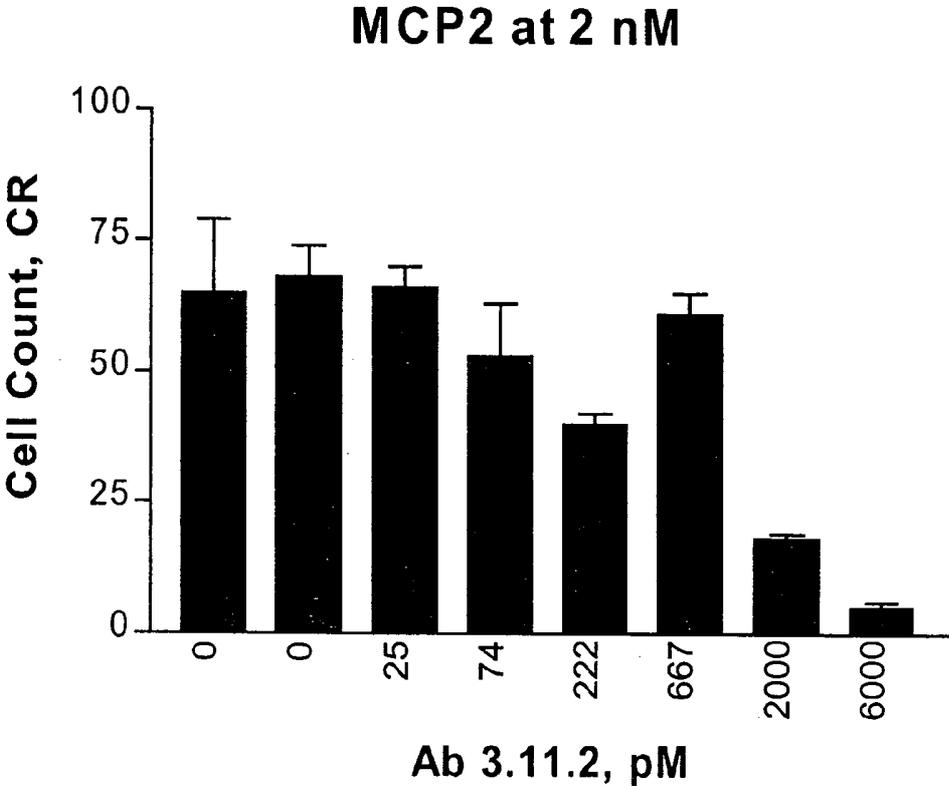


Figure 3

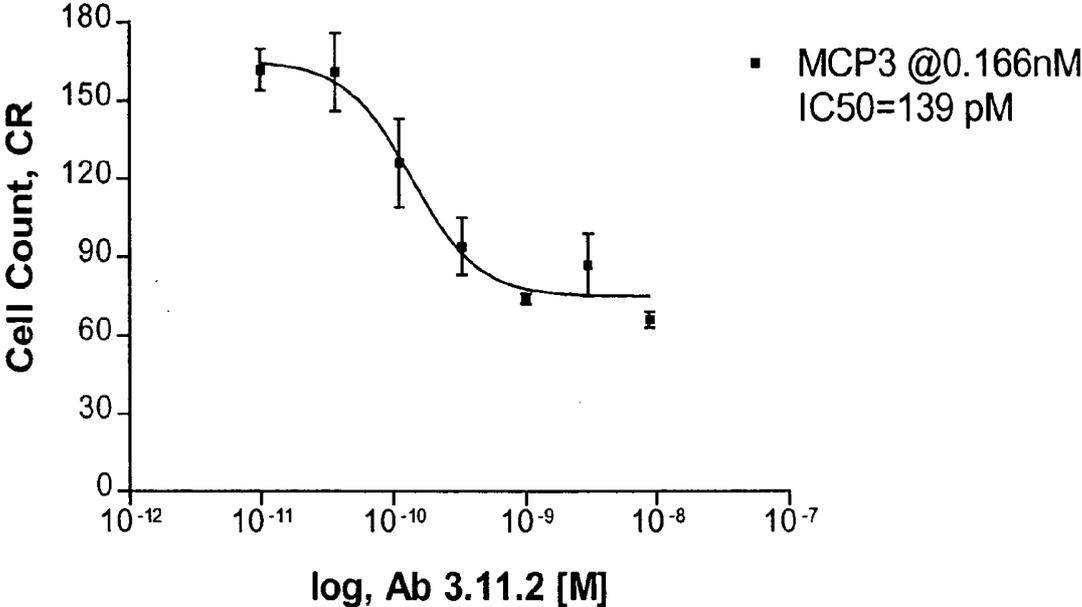


Figure 4

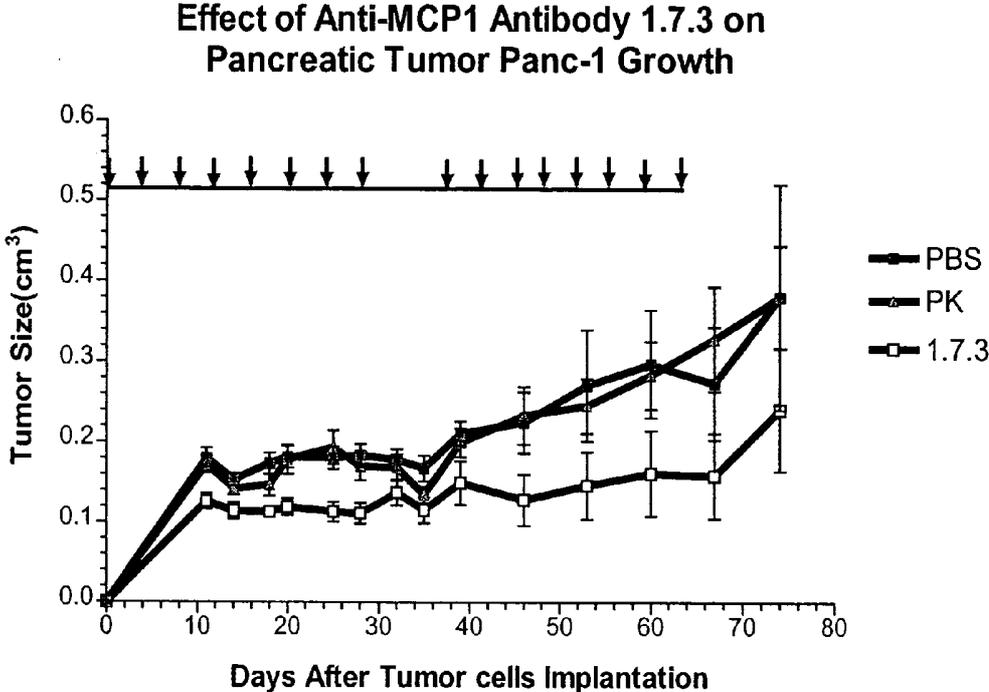


Figure 5

MCP1 mAbs

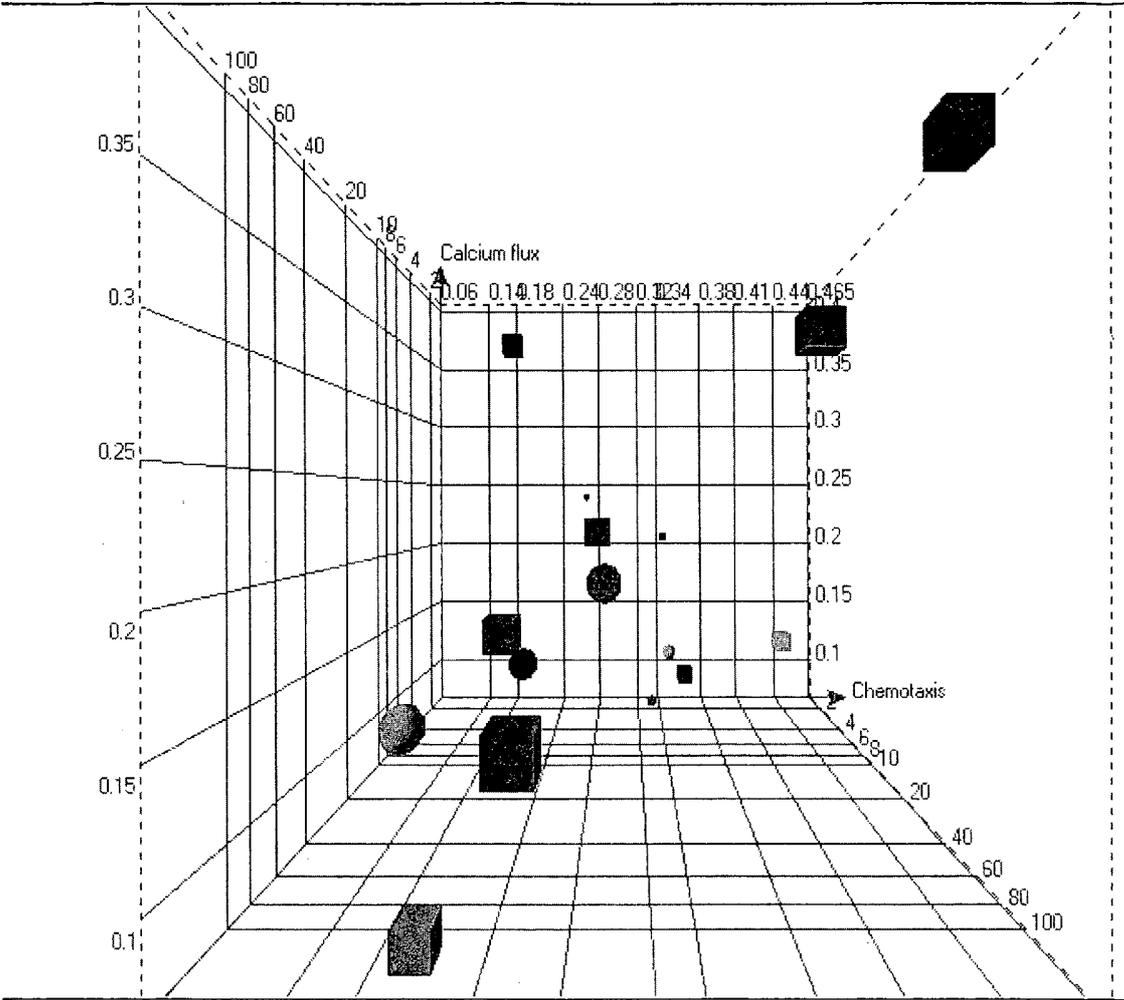


Figure 6

Scatter Plot

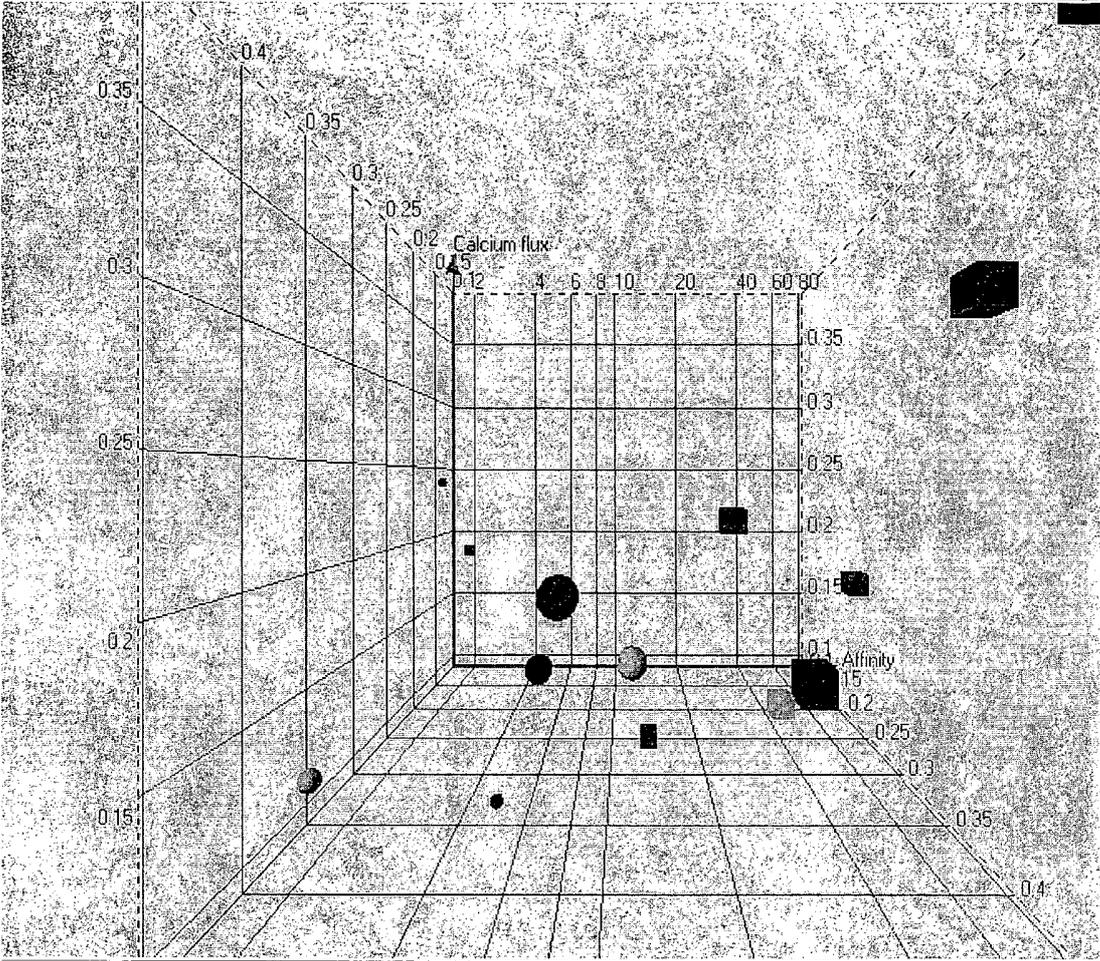


Figure 7A

Alignment of sequences using VH1-24

	<u>CDR1</u>	<u>CDR2</u>
VH1-24	QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIY	
MCP1-1.1.1 _{HC}	QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGNGLWMMGGFDPEDGETIY	
MCP1-1.10 _{HC}	QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIY	
MCP1-1.11 _{HC}	QVQVVQSGAEVKNPGASVKVSCKVSGSTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIY	
MCP1-1.12 _{HC}	QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIY	
MCP1-1.13 _{HC}	QVQLVQSGAEVKKPGASVKVSCKVSGHTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIY	
MCP1-1.18 _{HC}	QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIY	
MCP1-1.2 _{HC}	QVQLVQSGAEVKKPGASVKVSCKVSGYTFTELSMHWVRQAPGKGLEWMGGFDPEDGETSY	
MCP1-1.3 _{HC}	QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRRIPGKGLEWMGGFDPEDGETIY	
MCP1-1.5.1 _{HC}	QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIY	
MCP1-1.6 _{HC}	QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIY	
MCP1-1.7 _{HC}	QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIY	
MCP1-1.8 _{HC}	QVQLVQSGAEVKKPGASVKVSCKVSGHIFTELSIHWVRQAPGKGLEWMGGFDPEDGETIY	
MCP1-1.9 _{HC}	QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIN	
MCP1-2.3 _{HC}	QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIY	
MCP1-3.10 _{HC}	QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIY	
MCP1-3.15 _{HC}	QVQLVQSGAEVKKPGASVQVSKVSGDTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIY	
MCP1-3.16 _{HC}	QVQLVQSGAEVKKPGASVKVSCKVSGYTLTDLTSMHWVRQAPGKGLEWMGGFDPEDGETIY	
MCP1-3.2 _{HC}	QVQLVQSGAEVKKPGASVKVSCKVSGYTLSELSMHWVRQAPGKGLEWMGGFDPEDGETIY	
MCP1-3.4 _{HC}	QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETMY	
MCP1-3.5 _{HC}	QVQLVQSGAEVKKPGASVKVSCKVSGYTLSELSMHWVRQAPGKGLEWMGGFDPEDGETIY	
MCP1-3.6 _{HC}	QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIY	
MCP1-3.7 _{HC}	QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQTPGKGLEWMGGFDPEDGETIY	
MCP1-3.8 _{HC}	QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPENGETIY	
MCP1-4.5 _{HC}	QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIY	
MCP1-4.6.3 _{HC}	QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIY	
MCP1-4.7 _{HC}	QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIY	
MCP1-5.3 _{HC}	QVQLVQSGAEVKKPGASVKVSCKVSGYTLSELSMHWVRQAPGKGLEWMGGFDPEDGETIY	
MCP1-4.8.1 _{HC}	QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIY	

Figure 7A (cont.)

	<u>CDR2</u>	<u>CDR3</u>
VH1-24	AQKFQGRVTMTEDTSTDTAYMELSSLRSED	TAVYYCAT-----
MCP1-1.1.1_HC	AQRFQGRVVMTEDEPSTDTAYMELSSLRSED	TAVYYCATNEFWSGYF-----DYWGQGLV
MCP1-1.10_HC	AQKFQGRVTMTEDTSTDTAYMELSSLRSED	TAVYYCATNEFWSGYF-----DYWGQGLV
MCP1-1.11_HC	AQKFQGRVTMTEDTSTDTVYMESSLRSED	TAVYYCATNDFWSGYF-----DYWGQGLV
MCP1-1.12_HC	AQKFQGRVTMTEDTSTDTAYMELSSLRSED	TAVYYCATNDFWSGYF-----NYWGQGLV
MCP1-1.13_HC	AQKFQDRVTMTEDTSTDTAYMELSSLRSED	TAVYYCATNDFWSGYF-----DCWGQGLV
MCP1-1.18_HC	AQKFQGRVTMTEDTSTDTVYMESSLRSED	TAMYYCATREFWTGYF-----DHWGQGLV
MCP1-1.2_HC	AQKFRGRVTMTEDTSTDTAHMELSSLRSED	TAVYYCATNDFWSGYF-----DYWGQGLV
MCP1-1.3_HC	AQKFQGRVTMTEDTSTDTAYMELSSLRSED	TAVYYCATNDFWSGYW-----GHWGQGLV
MCP1-1.5.1_HC	AQKFQGRVTMTEDTSTDTAYMELSSLRSED	TAVYFCATNDFWSGYF-----DCWDQGLV
MCP1-1.6_HC	AQKFQGRVTMTEDTSTDTAYMELSSLRSED	TAVYYCATWYSGIYLA-----DIWGQGLV
MCP1-1.7_HC	AQKFQGRVSMTEDETSSTAYMELSSLRSED	TAVYFCATNEFWSGYF-----DYWGQGLV
MCP1-1.8_HC	AQKFQGRVTMTEDTSTDTVYMESSLRSED	TAVYYCATNDFWSGYF-----DYWGQGLV
MCP1-1.9_HC	AQKFQGRVTMTEDTSTDTGYMELSSLRSED	TAVYYCATDPGGYSGYF-----DHWGQGLV
MCP1-2.3_HC	AQKFQGRVTMTEDTSTHTAYMELSSLRSED	TAVYYCATHDFWSAYF-----YYWGQGLV
MCP1-3.10_HC	AQKFQGRVMMTEDETSSTAFMDLSSLRSED	TAVYYCATDDMLTPHYLYFGMDVWGQGLTV
MCP1-3.15_HC	ARKFQGRVTMTEDTSTDTVYMESSLRSED	TAVYFCATDSRGYSGYF-----DNWGQGLV
MCP1-3.16_HC	AQKFQGRVTMTEDTSSDTAYMELSSLRSED	TAVYYCATHEFWSGYF-----DYWGQGLV
MCP1-3.2_HC	AQKFQGRVTMTEDTSTDTAYMELSSLRSED	TAVYYCATGDFWSGYL-----DWWGQGLV
MCP1-3.4_HC	AQKFQGRVTMTEDTSTDTAYMELSSLRSED	TAVYYCATDDEFWSGYF-----DYWGQGLV
MCP1-3.5_HC	AQKFQGRVTMTEDTSTDTAFMELSSLRSED	TAVYYCATHDFWSGYF-----HYWGQGLV
MCP1-3.6_HC	AQKFQGRVTMTEDTSTDTAYMELSSLRSED	TAVYYCAIHEFWSGYF-----DYWGQGLV
MCP1-3.7_HC	AQKFQDRVTMTEDTSTDTAYMELSSLRSED	TAVYYCATNDFWTGYF-----DYWGQGLV
MCP1-3.8_HC	AQKFQGRVIMTEDETSSTAYMELSSLRSED	TAVYYCATDQGGYSGYF-----DCWGQGLV
MCP1-4.5_HC	AQKFQGRVTMTEDTSTDTAYMELSSLRSED	TAVYYCATDDEFWSGYF-----DYWGQGLV
MCP1-4.6.3_HC	AQKFQGRVTMTEDTSTDTAYMELSSLRSED	TAVYYCATDDEFWSGYF-----DYWGQGLV
MCP1-4.7_HC	AQKFQGRVTMTEDTSTDTAYMELSSLRSED	TAVYYCATDDEFWSGYF-----DYWGQGLV
MCP1-5.3_HC	AQKFQGRVTMTEDTSTDTAYMELSSLRSED	TAVYFCATKREYSGYF-----DYWGQGLV
MCP1-4.8.1_HC	AQKFQGRVTMTEDTSTDTAYMELSSLRTED	TAVYYCTDDEFWSGYF-----DYWGQGLV

Figure 7A (cont.)

VH1-24	---
MCP1-1.1.1 _{HC}	VSS
MCP1-1.10 _{HC}	VSS
MCP1-1.11 _{HC}	VSS
MCP1-1.12 _{HC}	VSS
MCP1-1.13 _{HC}	VSS
MCP1-1.18 _{HC}	VSS
MCP1-1.2 _{HC}	VSS
MCP1-1.3 _{HC}	VSS
MCP1-1.5.1 _{HC}	VSS
MCP1-1.6 _{HC}	VSS
MCP1-1.7 _{HC}	VSS
MCP1-1.8 _{HC}	VSS
MCP1-1.9 _{HC}	VSS
MCP1-2.3 _{HC}	VSS
MCP1-3.10 _{HC}	VSS
MCP1-3.15 _{HC}	VSS
MCP1-3.16 _{HC}	VSS
MCP1-3.2 _{HC}	VSS
MCP1-3.4 _{HC}	VSS
MCP1-3.5 _{HC}	VSS
MCP1-3.6 _{HC}	VSS
MCP1-3.7 _{HC}	VSS
MCP1-3.8 _{HC}	VSS
MCP1-4.5 _{HC}	VSS
MCP1-4.6.3 _{HC}	VSS
MCP1-4.7 _{HC}	VSS
MCP1-5.3 _{HC}	VSS
MCP1-4.8.1 _{HC}	VSS

Figure 7B

Dendrogram:

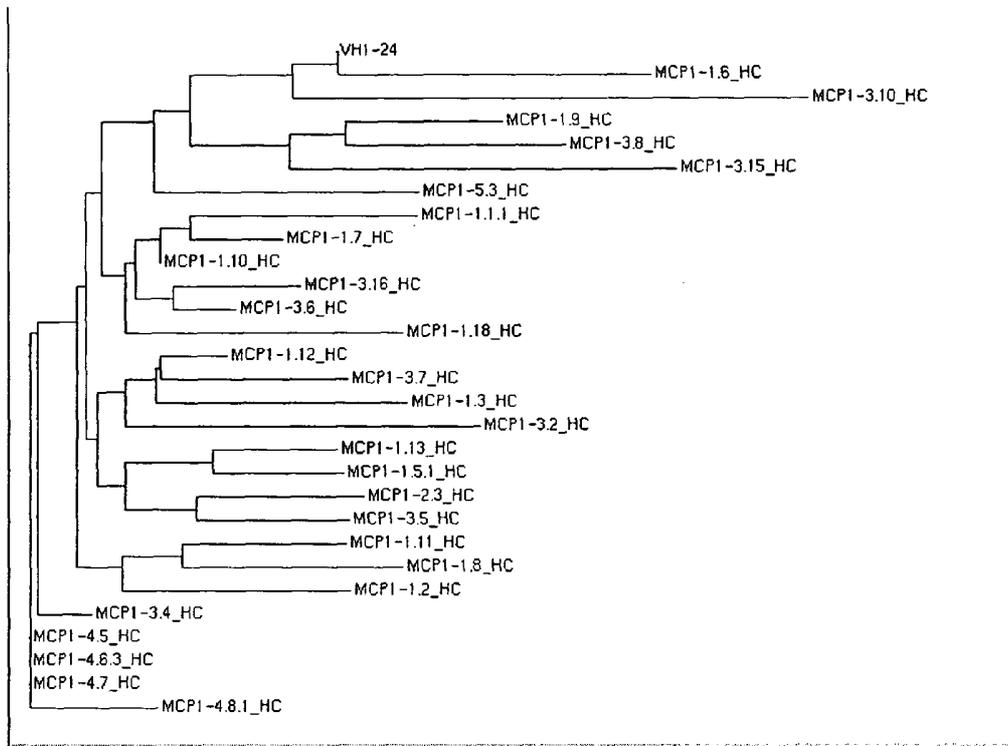


Figure 8A

Alignment of sequences using VK-B3

	CDR1	CDR2
VK-B3	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYWASTR	
MCP1-1.1.1 _{LC}	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLVWYQQKPGQPPKLLIYWASIR	
MCP1-1.10 _{LC}	DIVMTQSPASLAESLGERATINCKSSQSVLYSSNNKNYLVWYQQKLGQPPKLLIYWASTR	
MCP1-1.11 _{LC}	DIVMTQSPDSLAVSLGERATITCKSSQTVLYSSNNKNYLVWYQQKSGQPPKLLIHWASIR	
MCP1-1.12 _{LC}	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLVWYQQKPGQPPKLLIYWASIR	
MCP1-1.13 _{LC}	DIVMTQSPDSLAVCLGERATINCKSSQSVLYSPNNKNFLVWYQQRPGQPPKLLIYWASTR	
MCP1-1.14.1.1 _{LC}	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLVWYQQKPGQPPKLLIYWASTR	
MCP1-1.18 _{LC}	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLVWYQQKPGQPPKLLIYWASIR	
MCP1-1.3 _{LC}	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYXQKPGQPPKLLIYWYIR	
MCP1-1.5.1 _{LC}	DIVMTQSPDSLAASLGERATINCKSSQSVLYRSNNKNYLVWYQQKPGQPPKLLIYWASIR	
MCP1-1.7 _{LC}	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLVWYQQRPGQPPKLLIYWASTR	
MCP1-1.8 _{LC}	DIVMTQSPGSLAVSLGERATINCKSSQSIIFRSNNKNYLTWYQQKPGQPPKLLIYWASIR	
MCP1-1.9 _{LC}	DIVMTQSPDFLAVSLGERPTINCKSSQSVFYSSNNKNYLVWYQQKPGQPPKLLIYWASTR	
MCP1-2.3 _{LC}	DIVMTQSPDSLAVSLGERATINCKSSQSVLYGSSNNKSYLAWYQQKPGQPPKLLIYWASTR	
MCP1-3.14.1.1 _{LC}	DIVMTQSPDSLAVSLGERAAINCKSSQTVLYSSNNKNYLVWYQQKPGQPPKLLIYWASTR	
MCP1-3.15 _{LC}	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNNNYLVWYQQKPGQPPKLLIYWASTR	
MCP1-3.16 _{LC}	DIVMTQSPDSLAVSLGERATINCKSSQSVLFSSNNKSYLTWYQQKPGQPPKLLIFWASIR	
MCP1-3.4 _{LC}	DIVMTQSPDSLAVSLDERATINCKSSQSVLYSPNQKNYLVWYQQKPGQPPKLLIYWASIR	
MCP1-3.5 _{LC}	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSDNKSYLVWYQQKPGQPPKLLIYWASIR	
MCP1-3.6 _{LC}	DIVMTQSPDSLAVSLGERATINCKSSLSVLYSSNNKNYLVWYQLKPGQPPKLLIYWASTR	
MCP1-3.7 _{LC}	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLVWYQQKPGQPPKLLIYWASTR	
MCP1-3.8 _{LC}	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLVWYQQKPGQPPKLLIYWASTR	
MCP1-4.5 _{LC}	DIVMTQSPDSLAVSLGERATINCKSSQSVLYRSNNKSYLVWYQQKLGQSPKLLIYWASTR	
MCP1-4.6.3 _{LC}	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLVWYQQKPGQPPKLLIYWASTR	
MCP1-4.7 _{LC}	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLAWYQQKPGQPPKLLIYWASTR	
MCP1-4.8.1 _{LC}	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNNKNYLVWYQQKPGQPPKLLIYWASTR	
MCP1-5.3 _{LC}	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSNSKNYLAWFQQKPGQPPKLLIYWASTR	

Figure 8A (cont.)

CDR2	CDR3
VK-B3	ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSTP-----
MCP1-1.1.1_LC	ESGVPDRFSSSGSETDFTLTISSLQAEDVAVYYCQQYFSSPWTFGQGTKVEIK
MCP1-1.10_LC	ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYRSPWTFGQGTKVEIK
MCP1-1.11_LC	ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSSPWTFGQGTKVEIK
MCP1-1.12_LC	ESGVPDRFSGSGSGTDFTLTINSLQAEDVAVYYCQQYFSPWTFGQGTKVEIK
MCP1-1.13_LC	ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSSPWTFGQGTKVEIK
MCP1-1.14.1.1_LC	ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYFSSPWTFGQTKVDIK
MCP1-1.18_LC	ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSTPLTFGGGTKVEIK
MCP1-1.3_LC	ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQEHYISIPWTFGQGTKVEIK
MCP1-1.5.1_LC	ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYFCQQYYSSPWTFGQGTKVEIK
MCP1-1.7_LC	ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYFSPWTFGQGTKVEIK
MCP1-1.8_LC	ESGVPDRFSGSGSGSNFTLTITSLQAEDVAIYYCQQYYSSPWTFGQGTKVEIK
MCP1-1.9_LC	ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSSPWTFGQGTKVEIK
MCP1-2.3_LC	ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQHYSTPCSFQGTKLEIK
MCP1-3.14.1.1_LC	ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYKSPWTFGQGTKVEIK
MCP1-3.15_LC	EFGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYFSPWTFGQGTKVEIK
MCP1-3.16_LC	ESGVPDRISGSGSGTDLTLTISSLQAEDAAYYYCQQYYSSPWTFGQGTKVEIK
MCP1-3.4_LC	ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQSYFTPWTFGQGTKVEIK
MCP1-3.5_LC	ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYTSPWTFGQGTKVEIK
MCP1-3.6_LC	ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSSPWTFGQGTKVEIK
MCP1-3.7_LC	ESGVPDRFSGSGSGTDFTLTISSLQAEDVGVYYCQQYYTSPWTFGQGTKVEIK
MCP1-3.8_LC	ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSSPPTFGQGTKVEIK
MCP1-4.5_LC	ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSTPWTFGQGTKVEIK
MCP1-4.6.3_LC	ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSPWTFGQGTKVEIK
MCP1-4.7_LC	ESGVPDRFSGSGSVTDFTLTISSLQAEDVAVYYCQQYYSSPWTFGQGTKVEIK
MCP1-4.8.1_LC	ESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQQYYSSPWTFGQGTKVEIK
MCP1-5.3_LC	ESGVPDRFSGSGSGTDFTLTISRLQAEDVAVYSCQQYFITPWTFGQGTKVELK

Figure 8B

Dendrogram:

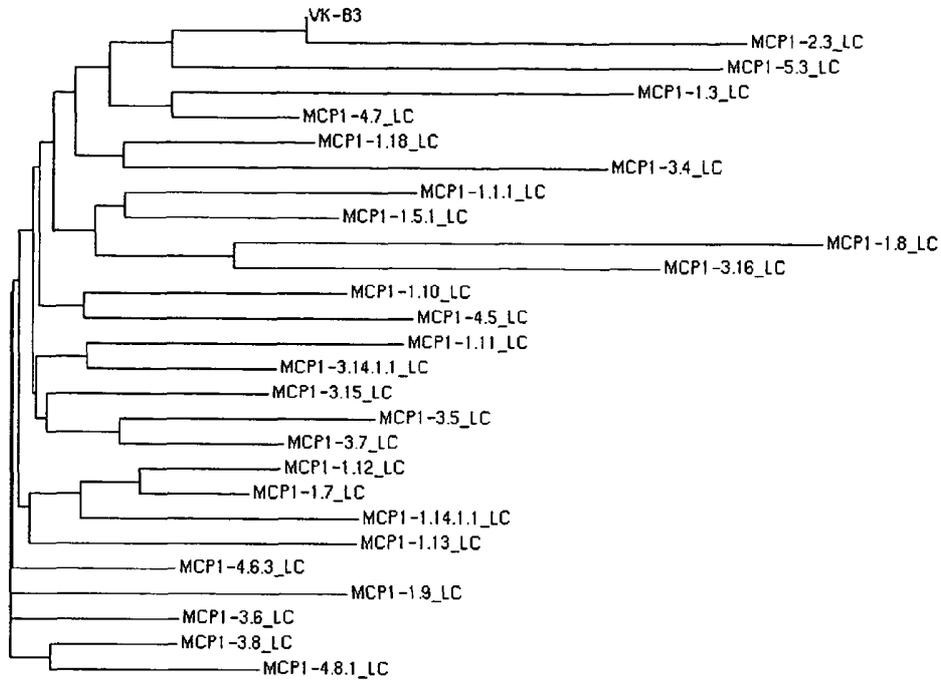


Figure 9A

Alignment of sequences using VK-O8

	<u>CDR1</u>	<u>CDR2</u>
VK-O8	DIQMTQSPSSLSASVGDRTITCQASQDISNYLNWYQQKPGKAPKLLIYDASNLETGVPS	
MCP1-2.4 _{LC}	DIQMTQSPSSLSASVGDRTITCQASQDITTYLNWYQQKPGKAPKLLIYDASNLETGVPS	
MCP1-3.11 _{LC}	DIQMTQSPSSLSASVGDRTITCQASQDISNYLNWYQQKPGKAPKLLIYDASNLETGVPS	
	*****:*****	
	<u>CDR3</u>	
VK-O8	RFSGSGSGTDFTFTISSLQPEDATYYCQQYDNLPI-----	
MCP1-2.4 _{LC}	RFSGSGSGTDFTFTISSLQPEDATYYCQQYDNLPIITFGQTRLEIK	
MCP1-3.11 _{LC}	RFSGSGSGTDFTFTINSLQPEDATYYCQYNNLPYSFGQTKLEIK	
	*****:*****:***	

Figure 9B

Dendrogram:

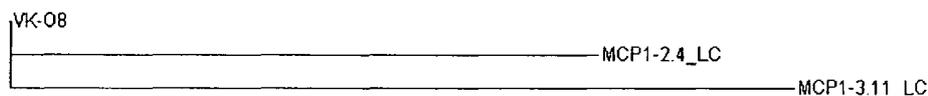


Figure 10A

Alignment of sequences using VH6-1

	<u>CDR1</u>	<u>CDR2</u>
VH6-1	QVQLQQSGPGLVKPSQTLTCAISGDSVSSNSAAWNWI	RQSPSRGLEWLGRTYYRSKWY
MCP1-1.4.1.1_HC	QVQAEQSGPGLVKPSQTLTCAISGDSVSSNSAAWNWI	RQSPSRGLEWLGRTYYRSKWY
MCP1-1.14.1.1_HC	QVQAEQSGPGLVKPSQTLTCAISGDSVSSNSAAWNWI	RQSPSRGLEWLGRTYYRSKWY
MCP1-3.14.1.1_HC	QVQAEQSGPGLVKPSQTLTCAISGDSVSSNSAAWNWI	RQSPSRGLEWLGRTYYRSKWY
	<u>CDR2</u>	<u>CDR3</u>
VH6-1	NDYAVSVKSRITINPDTSKNQFSLQLNSVTPEDTAVYYCAR	-----
MCP1-1.4.1.1_HC	SDHAVSVRSRITIYPDTSKNQFSLQLNSVTPEDTAVYYCARDRISGTYVGMVWGQTTV	
MCP1-1.14.1.1_HC	SDHAVSVRSRITIYPDTSKNQFSLQLNSVTPEDTAVYYCARDRISGTYVGMVWGQTTV	
MCP1-3.14.1.1_HC	SDHAVSVRSRITIYPDTSKNQFSLQLNSVTPEDTAVYYCARDRISGTYVGMVWGQTTV	
VH6-1	---	
MCP1-1.4.1.1_HC	VSS	
MCP1-1.14.1.1_HC	VSS	
MCP1-3.14.1.1_HC	VSS	

Figure 10B

Dendrogram:



**ANTIBODIES DIRECTED TO MONOCYTE
CHEMO-ATTRACTANT PROTEIN-1 (MCP-1) AND
USES THEREOF**

PRIORITY CLAIM

[0001] This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 60/404,802, filed Aug. 19, 2002, which is hereby expressly incorporated by reference.

BACKGROUND OF THE INVENTION

[0002] 1. Field of the Invention

[0003] Embodiments of the invention described herein relate to antibodies directed to the antigen monocyte chemo-attractant protein-1 (MCP-1) and uses of such antibodies. In particular, in accordance with embodiments of the invention, there are provided fully human monoclonal antibodies directed to the antigen MCP-1. Nucleotide sequences encoding, and amino acid sequences comprising, heavy and light chain immunoglobulin molecules, particularly sequences corresponding to contiguous heavy and light chain sequences spanning the framework regions and/or complementarity determining regions (CDRs), specifically from FR1 through FR4 or CDR1 through CDR3, are provided. The antibodies of the invention find use as diagnostics and as treatments for diseases associated with the overproduction of MCP-1. Hybridomas or other cell lines expressing such immunoglobulin molecules and monoclonal antibodies are also provided.

[0004] 2. Description of the Related Art

[0005] An increased production of angiogenic factors and decreased production of angiogenesis inhibitors by cancer cells, vascular endothelial cells and other stromal cell types are believed to induce tumor angiogenesis. Stroma, comprised of interstitial connective tissues, basal lamina, blood cells, blood vessels and fibroblastic cells, surround almost all solid tumor cells. Interactions between the stroma and cancer cells play a critical role in the neovascularization of tumors. Further, macrophage, which are also stromal components, are important in tumor angiogenesis. (M. Ono et al., *Cancer Chemother. Pharmacol.* (1999) 43(Suppl.): S69-S71.)

[0006] Macrophages are the major terminally differentiated cell type of the mononuclear phagocyte system, and are also one of the key angiogenic effector cells, producing a number of growth stimulators and inhibitors. A number of angiogenic cytokines are known to be produced by macrophages, including monocyte chemo-attractant protein 1 (MCP-1).

[0007] MCP-1 is known to be chemotactic for T lymphocytes, basophils and NK cells. MCP-1 is one of the most potent macrophage recruiting molecules. Once recruited to sites of inflammation or tumors, macrophages can generate a number of angiogenic cytokines, thereby stimulating pathologic angiogenesis. A number of studies have shown a relationship between angiogenesis, macrophage recruitment, and prognosis in patients with various kinds of tumors (G. Fantanini et al., *Int. J. Cancer* (1996) 67:615; N. Weidner et al., *J. Natl. Cancer Inst.* (1992) 84:1875). Leek et al. have further demonstrated that focally increased macrophage numbers are closely related to vascularization and

prognosis in breast cancer patients (*Cancer Res.* (1996) 56:4625). R. Huang et al. (*Cancer Res.* (2002) 62:2806-2812) have shown that Connexin 43 suppresses human glioblastoma cell growth by down regulation of MCP-1, as discovered by using protein array technology.

[0008] Goede et al. (*Int. J. Cancer* (1999) 82: 765-770) first demonstrated that MCP-1 had an angiogenic potency which was equivalent to that of VEGF when tested in a rabbit corneal model. In their model, the angiogenic activity induced by MCP-1 was associated with an intense recruitment of macrophages into the rabbit cornea. Salcedo et al. have reported that MCP-1 induced chemotaxis of human endothelial cells at nanomolar concentrations. This chemotactic response was inhibited by a polyclonal antibody to human MCP-1 (R. Salcedo et al., *Blood* (2000) 96(1):34-40).

[0009] MCP-1 is the predominant chemokine expressed in ovarian cancer (Negus, R. P. M. et al., *J. Clin. Investig.* (1995) 95: 2391-96; Sica, A. et al., *J. Immunology* (2000) 164(2):733-8). MCP-1 is also elevated in a number of other human cancers including bladder, breast, lung, and glioblastomas.

[0010] In addition, the importance of MCP-1 in inflammation has been shown in a number of studies. For example, H. J. Anders et al., have demonstrated chemokine and chemokine receptor expression during initiation and resolution of immune complex glomerulonephritis (*J. Am. Soc. Nephrol.* (2001) 12: 919-2001). Segerer et al. (*J. Am. Soc. Nephrol.* (2000) 11:2231-2242) also have studied the expression of MCP-1 and its receptor chemokine receptor 2 in human crescentic glomerulonephritis. J. A. Belperio et al. have shown a critical role for the chemokine MCP-1/CCR2 in the pathogenesis of bronchiolitis obliterans syndrome (*J. Clin. Investig.* (2001) 108: 547-556). N. G. Frangogiannis et al. have delineated the role of MCP-1 in the inflammatory response in myocardial infarction (*Cardiovascular Res.* (2002) 53: 31-47). Gerard and Rollins (*Nature Immunol.* (2001) 2:108-115) and Reape and Groot (*Atherosclerosis* (1999) 147: 213-225) have discussed the role of MCP-1 in atherosclerosis and other diseases. Also, Schmidt and Stem (*Arterioscler. Thromb. Vasc. Biol.* (2001) 21:297-299) describe MCP-1 interactions in restenosis.

[0011] Human MCP-1, a 76-amino-acid CC chemokine with an N-terminal pyroglutamic acid, was originally purified from several sources including phytohemagglutinin-stimulated human lymphocytes (Yoshimura, T. et al., *J. Immunol.* (1989) 142:1956-62), a human glioma cell line (Yoshimura, T., et al., *J. Exp. Med.* (1989) 169:1449-59), and the human myelomonocytic cell line THP-1 (Matsushima, K., et al., (1989) *J. Exp. Med.* (1989) 169: 1485-90). MCP-1 was first described as lymphocyte-derived chemotactic factor (LDCF). Other names for the protein are tumor-cell-derived chemotactic factor (TDCF), glioma-derived monocyte chemotactic factor (TDCF), glioma-derived monocyte chemotactic factor (GDGF), smooth muscle cell-derived chemotactic factor (SMC-CF), monocyte chemotactic activating factor (MCAF) and CCL2. Molecular cloning of the cDNA encoding MCP-1 (Furutani, Y., et al., (1989) *Biochem. Biophys. Res. Comm.* (1989) 169:249-55; B. J. Rollins, et al., *Mol. Cell. Biol.* (1989) 9:4687-95; Chang, H. C., et al., *Int. Immunol.* (1989) 1:388-97) revealed an open reading frame of 99 amino acids, including a signal

peptide of 23 amino acids. The mouse homologue gene of MCP-1 was named JE (B. J. Rollins et al., 1989).

[0012] WO 200189565, published Nov. 29, 2001, discloses polyclonal antibodies to human MCP-1 and describes the inhibition of tumor growth in a nude mouse model by the use of such polyclonal antibodies.

[0013] Embodiments of the invention described herein relate to fully human monoclonal antibodies to human MCP-1 that block MCP-1-induced chemotaxis of THP-1 cells, a cell line derived from a patient with acute monocytic leukemia. These cells are used as a surrogate for assessing the migration of normal human mononuclear cells in circulation. Mononuclear cell infiltration stimulated by MCP-1 plays a pathologic role in a number of inflammatory conditions including rheumatoid arthritis, glomerulonephritis, atherosclerosis, transplant rejection, psoriasis, restenosis, and autoimmune diseases such as multiple sclerosis. An antibody that blocks MCP-1 activity and prevents monocyte infiltration will find use as a treatment for these and other inflammatory diseases.

SUMMARY OF THE INVENTION

[0014] Embodiments of the invention described herein related to monoclonal antibodies that were found to bind MCP-1 and affect MCP-1 function. Other embodiments relate to human anti-MCP-1 antibodies and anti-MCP-1 antibody preparations with desirable properties from a therapeutic perspective, including strong binding affinity for MCP-1, the ability to neutralize MCP-1 in vitro, and the ability to inhibit neovascularization of solid tumors.

[0015] One embodiment of the invention is a fully human monoclonal antibody that binds to MCP-1 and has a heavy chain amino acid sequence selected from the group consisting of SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, 46, 50, 54, 58, 62, 66, 70, 74, 78, 82, 86, 90, 94, 98, 102, 106, 110, 114, 118, 122, 126, 130, 134, 138, 142 and 146. In one embodiment, the antibody further comprises a light chain amino acid sequence selected from the group consisting of SEQ ID NOS: 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 52, 56, 60, 64, 68, 72, 76, 80, 84, 88, 92, 96, 100, 104, 108, 112, 116, 120, 124, 128, 132, 136, 140, 144 and 148.

[0016] Accordingly, one embodiment of the invention described herein provides isolated antibodies, or fragments of those antibodies, that bind to MCP-1. As known in the art, the antibodies can advantageously be, for example, monoclonal, chimeric and/or human antibodies. Embodiments of the invention described herein also provide cells for producing these antibodies.

[0017] Another embodiment of the invention is a fully human antibody that binds to MCP-1 that comprises a heavy chain amino acid sequence having the CDRs comprising the sequences shown in FIGS. 7 and 10. It is noted that CDR determinations can be readily accomplished by those of ordinary skill in the art. In general, CDRs are presented in the invention described herein as defined by Kabat et al., in *Sequences of Proteins of Immunological Interest* vols. 1-3 (Fifth Edition, NIH Publication 91-3242, Bethesda Md. 1991).

[0018] Yet another embodiment of the invention is a fully human antibody that binds to MCP-1 and comprises a light

chain amino acid sequence having the CDRs comprising the sequences shown in FIGS. 8 and 9.

[0019] A further embodiment of the invention is a fully human antibody that binds to MCP-1 and comprises a heavy chain amino acid sequence having the CDRs comprising the sequences shown in FIGS. 7 and 10 and a light chain amino acid sequence having the CDRs comprising the sequences shown in FIGS. 8 and 9.

[0020] Another embodiment of the invention is a fully human antibody that binds to other MCP-1 family members including, but not limited to, MCP-2, MCP-3 and MCP-4. A further embodiment of the invention is an antibody that cross-competes for binding to MCP-1 with the fully human antibodies of the invention.

[0021] It will be appreciated that embodiments of the invention are not limited to any particular form of an antibody or method of generation or production. For example, the anti-MCP-1 antibody may be a full-length antibody (e.g., having an intact human Fc region) or an antibody fragment (e.g., a Fab, Fab' or F(ab')₂). In addition, the antibody may be manufactured from a hybridoma that secretes the antibody, or from a recombinantly produced cell that has been transformed or transfected with a gene or genes encoding the antibody.

[0022] Other embodiments of the invention include isolated nucleic acid molecules encoding any of the antibodies described herein, vectors having an isolated nucleic acid molecule encoding any of such the anti-MCP-1 antibodies, a host cell transformed with any of such nucleic acid molecules. In addition, one embodiment of the invention is a method of producing an anti-MCP-1 antibody by culturing host cells under conditions wherein a nucleic acid molecule is expressed to produce the antibody followed by recovering the antibody.

[0023] A further embodiment of the invention includes a method of producing high affinity antibodies to MCP-1 by immunizing a mammal with human MCP-1 or a fragment thereof and one or more orthologous sequences or fragments thereof.

[0024] Embodiments of the invention described herein are based upon the generation and identification of isolated antibodies that bind specifically to MCP-1. MCP-1 is expressed at elevated levels in neoplastic diseases, such as tumors, and other inflammatory diseases. Inhibition of the biological activity of MCP-1 can prevent further infiltration of mononuclear cells into tissues.

[0025] Another embodiment of the invention includes a method of diagnosing diseases or conditions in which an antibody prepared according to the invention described herein is utilized to detect the level of MCP-1 in a patient sample. In one embodiment, the patient sample is blood or blood serum. In further embodiments, methods for the identification of risk factors, diagnosis of disease, and staging of disease is presented which involves the identification of the overexpression of MCP-1 using anti-MCP-1 antibodies.

[0026] In another embodiment, the invention includes a method for diagnosing a condition associated with the expression of MCP-1 in a cell, comprising contacting the cell with an anti-MCP-1 antibody, and detecting the pres-

ence of MCP-1. Preferred conditions include, but are not limited to, neoplastic diseases including, without limitation, tumors, cancers, such as breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, colorectal, thyroid, pancreatic, prostate and bladder cancer, as well as other inflammatory conditions, including, but not limited to, rheumatoid arthritis, glomerulonephritis, atherosclerosis, psoriasis, organ transplants, restenosis and autoimmune diseases.

[0027] In another embodiment, the invention includes an assay kit for the detection of MCP-1 and MCP-1 family members in mammalian tissues or cells to screen for neoplastic diseases or inflammatory conditions, comprising an antibody that binds to MCP-1 and a means for indicating the reaction of the antibody with the antigen, if present. Preferably the antibody is a monoclonal antibody. In one embodiment, the antibody that binds MCP-1 is labeled. In another embodiment the antibody is an unlabeled first antibody and the means for indicating the reaction comprises a labeled second antibody that is an anti-immunoglobulin. Preferably the antibody is labeled with a marker selected from the group consisting of a fluorochrome, an enzyme, a Radionuclide and a radiopaque material.

[0028] Other embodiments of the invention include pharmaceutical compositions comprising an effective amount of the antibody of the invention in admixture with a pharmaceutically acceptable carrier or diluent. In yet other embodiments, the anti-MCP-1 antibody or fragment thereof is conjugated to a therapeutic agent. The therapeutic agent can be a toxin or a radioisotope. Preferably, such antibodies can be used for the treatment of diseases, such as, for example, tumors, including, without limitation, cancers, such as breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, colorectal, thyroid, pancreatic, prostate and bladder cancer, as well as other inflammatory conditions, including, but not limited to, rheumatoid arthritis, glomerulonephritis, atherosclerosis, psoriasis, organ transplants, restenosis and autoimmune diseases.

[0029] Yet another embodiment of the invention provides a method for treating diseases or conditions associated with the expression of MCP-1 in a patient, comprising administering to the patient an effective amount of an anti-MCP-1 antibody. The method can be performed *in vivo*. The patient is a mammalian patient, preferably a human patient. In a preferred embodiment, the method concerns the treatment of tumors, including, without limitation, cancers, such as breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, colorectal, thyroid, pancreatic, prostate and bladder cancer. In another embodiment, the method concerns the treatment of inflammatory conditions, including, but not limited to, rheumatoid arthritis, glomerulonephritis, atherosclerosis, psoriasis, organ transplants, restenosis and autoimmune diseases. Additional embodiments include methods for the treatment of diseases and conditions associated with the expression of MCP-1, which can include identifying a mammal in need of treatment for overexpression of MCP-1 and administering to the mammal, a therapeutically effective dose of anti-MCP-1 antibodies.

[0030] In another embodiment, the invention provides an article of manufacture comprising a container, comprising a composition containing an anti-MCP-1 antibody, and a package insert or label indicating that the composition can be used to treat neoplastic and inflammatory diseases char-

acterized by the overexpression of MCP-1. Preferably a mammal, and more preferably, a human receives the anti-MCP-1 antibody. In a preferred embodiment, tumors, including, without limitation, cancers, such as breast, ovarian, stomach, endometrial, salivary gland, lung, glioblastomas, kidney, colon, colorectal, thyroid, pancreatic, prostate and bladder cancer, as well as other inflammatory conditions, including, but not limited to, rheumatoid arthritis, glomerulonephritis, atherosclerosis, psoriasis, organ transplants, restenosis and autoimmune diseases such as multiple sclerosis are treated.

[0031] In some embodiments, the anti-MCP-1 antibody is administered, followed by a clearing agent to remove circulating antibody from the blood.

[0032] In some embodiments, anti-MCP-1 antibodies can be modified to enhance their capability of fixing complement and participating in complement-dependent cytotoxicity (CDC). In one embodiment, the anti-MCP-1 antibody can be modified, such as by an amino acid substitution, to alter antibody clearance. For example, certain amino acid substitutions may accelerate clearance of the antibody from the body. Alternatively, the amino acid substitutions may slow the clearance of antibody from the body. In other embodiments, the anti-MCP-1 antibody can be altered such that it is eliminated less rapidly from the body.

[0033] Yet another embodiment is the use of an anti-MCP-1 antibody in the preparation of a medicament for the treatment of diseases such as neoplastic diseases and inflammatory conditions. In one embodiment, the neoplastic diseases include tumors and cancers, such as breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, colorectal, thyroid, pancreatic, prostate and bladder cancer. In an alternative embodiment, the inflammatory condition includes, but is not limited to, rheumatoid arthritis, glomerulonephritis, atherosclerosis, psoriasis, organ transplants, restenosis and autoimmune diseases.

BRIEF DESCRIPTION OF THE DRAWINGS

[0034] FIG. 1 shows results of THP-1 monocyte migration studies in response to MCP-1, MCP-2, MCP-3 and MCP-4.

[0035] FIG. 2 shows inhibition by antibody 3.11.2 in a dose-dependent manner of the migration ability of THP-1 cells in response to MCP-2.

[0036] FIG. 3 shows inhibition by antibody 3.11.2 in a dose-dependent manner of the migration ability of THP-1 cells in response to MCP-3.

[0037] FIG. 4 shows the effect of anti-MCP-1 antibody 1.7.3 on pancreatic tumor Panc-1 growth.

[0038] FIG. 5 shows a 3-dimensional scatter plot of calcium flux, chemotaxis and affinity data for the MCP-1 antibodies.

[0039] FIG. 6 shows another orientation of a 3-dimensional scatter plot of calcium flux, chemotaxis and affinity data for the MCP-1 antibodies.

[0040] FIG. 7A shows a Clustal W comparison of anti-MCP-1 sequences using VH1-24, indicating the CDR1, CDR2, and CDR3 regions, and the associated dendrogram (FIG. 7B).

[0041] FIG. 8A shows a Clustal W comparison of anti-MCP-1 sequences using VK-B3, indicating the CDR1, CDR2, and CDR3 regions, and the associated dendrogram (FIG. 8B).

[0042] FIG. 9A shows a Clustal W comparison of anti-MCP-1 sequences using VK-08, indicating the CDR1, CDR2, and CDR3 regions, and the associated dendrogram (FIG. 9B).

[0043] FIG. 10A shows a Clustal W comparison of anti-MCP-1 sequences using VH6-1, indicating the CDR1, CDR2, and CDR3 regions, and the associated dendrogram (FIG. 10B).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0044] Embodiments of the invention described herein relate to monoclonal antibodies that bind to MCP-1. In some embodiments, the antibodies bind to MCP-1 and affect MCP-1 function. Other embodiments provide fully human anti-MCP-1 antibodies and anti-MCP-1 antibody preparations with desirable properties from a therapeutic perspective, including strong binding affinity for MCP-1, the ability to neutralize MCP-1 in vitro, and the ability to inhibit the growth and neovascularization of solid tumors in vivo.

[0045] Accordingly, embodiments of the invention provide isolated antibodies, or fragments of those antibodies, that bind to MCP-1. As known in the art, the antibodies can advantageously be, e.g., monoclonal, chimeric and/or human antibodies. Embodiments of the invention also provide cells for producing these antibodies.

[0046] In some embodiments, the antibodies described herein possess therapeutic utilities. An anti-MCP-1 antibody can potentially block or limit the extent of tumor neovascularization and tumor growth. Many cancer cells including those from glioblastomas and renal cancers express the receptor for MCP-1, CCR2. The co-expression of ligand and receptor in the same tumor cell suggests that MCP-1 may regulate an autocrine growth loop in cancer cells that express both components. Huang et al. (*Cancer Res.* (2002) 62:2806-2812) have recently reported that MCP-1 can directly influence the growth and survival of tumor cells that express the CCR2 receptor for MCP-1. Thus, in addition to its effects on angiogenesis, MCP-1 may also directly regulate tumor cell growth, migration and invasion.

[0047] In addition, embodiments of the invention provide for using these antibodies as a diagnostic or treatment for disease. For example, embodiments of the invention provide

methods and antibodies for inhibition expression of MCP-1 associated with tumors and inflammatory conditions. Preferably, the antibodies are used to treat cancers, such as breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, colorectal, thyroid, pancreatic, prostate and bladder cancer, as well as other inflammatory conditions, including, but not limited to, rheumatoid arthritis, glomerulonephritis, atherosclerosis, psoriasis, organ transplants, restenosis and autoimmune diseases. In association with such treatment, articles of manufacture comprising antibodies of the invention described herein are provided. Additionally, an assay kit comprising antibodies in accordance with the invention described herein is provided to screen for tumors and inflammatory conditions.

[0048] Additionally, the nucleic acids described herein, and fragments and variants thereof, may be used, by way of nonlimiting example, (a) to direct the biosynthesis of the corresponding encoded proteins, polypeptides, fragments and variants as recombinant or heterologous gene products, (b) as probes for detection and quantification of the nucleic acids disclosed herein, (c) as sequence templates for preparing antisense molecules, and the like. Such uses are described more fully in the following disclosure.

[0049] Furthermore, the proteins and polypeptides described herein, and fragments and variants thereof, may be used, in ways that include (a) serving as an immunogen to stimulate the production of an anti-MCP-1 antibody, (b) a capture antigen in an immunogenic assay for such an antibody, (c) as a target for screening for substances that bind to a MCP-1 polypeptide described herein, and (d) a target for a MCP-1 specific antibody such that treatment with the antibody affects the molecular and/or cellular function mediated by the target.

[0050] In view of its strong effects in modulating cell growth, an increase of MCP-1 polypeptide expression or activity can be used to promote cell survival. Conversely, a decrease in MCP-1 polypeptide expression can be used to induce cell death.

[0051] Further embodiments, features, and the like regarding the antibodies of the invention are provided in additional detail below.

[0052] Sequence Listing

[0053] The heavy chain and light chain variable region nucleotide and amino acid sequences of representative human anti-MCP-1 antibodies are provided in the sequence listing, the contents of which are summarized in Table 1 below.

TABLE 1

mAb ID No.:	Sequence	SEQ ID NO:
1.1.1	Nucleotide sequence encoding the variable region of the heavy chain	1
	Amino acid sequence encoding the variable region of the heavy chain	2
	Nucleotide sequence encoding the variable region of the light chain	3
	Amino acid sequence encoding the variable region of the light chain	4
1.10.1	Nucleotide sequence encoding the variable region of the heavy chain	5
	Amino acid sequence encoding the variable region of the heavy chain	6
	Nucleotide sequence encoding the variable region of the light chain	7
	Amino acid sequence encoding the variable region of the light chain	8

[0054] Definitions

[0055] Unless otherwise defined, scientific and technical terms used in connection with the invention described herein shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures utilized in connection with, and techniques of, cell and tissue culture, molecular biology, and protein and oligo- or polynucleotide chemistry and hybridization described herein are those well known and commonly used in the art. Standard techniques are used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the instant application. See, e.g., Sambrook et al., *Molecular Cloning. A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. 1989), which is incorporated herein by reference. The nomenclatures utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

[0056] As utilized in accordance with the embodiments provided herein, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

[0057] The term "isolated polynucleotide" as used herein shall mean a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, which by virtue of its origin the "isolated polynucleotide" (1) is not associated with all or a portion of a polynucleotide in which the "isolated polynucleotide" is found in nature, (2) is operably linked to a polynucleotide which it is not linked to in nature, or (3) does not occur in nature as part of a larger sequence.

[0058] The term "isolated protein" referred to herein means a protein of cDNA, recombinant RNA, or synthetic origin or some combination thereof, which by virtue of its origin, or source of derivation, the "isolated protein" (1) is not associated with proteins found in nature, (2) is free of other proteins from the same source, e.g. free of murine proteins, (3) is expressed by a cell from a different species, or (4) does not occur in nature.

[0059] The term "polypeptide" is used herein as a generic term to refer to native protein, fragments, or analogs of a polypeptide sequence. Hence, native protein, fragments, and analogs are species of the polypeptide genus. Preferred polypeptides in accordance with the invention comprise the human heavy chain immunoglobulin molecules and the human kappa light chain immunoglobulin molecules, as well as antibody molecules formed by combinations comprising the heavy chain immunoglobulin molecules with light chain immunoglobulin molecules, such as the kappa light chain immunoglobulin molecules, and vice versa, as well as fragments and analogs thereof.

[0060] The term "naturally occurring" as used herein as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory or otherwise is naturally occurring.

[0061] The term "operably linked" as used herein refers to positions of components so described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

[0062] The term "control sequence" as used herein refers to polynucleotide sequences which are necessary to effect the expression and processing of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence; in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term "control sequences" is intended to include, at a minimum, all components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

[0063] The term "polynucleotide" as referred to herein means a polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

[0064] The term "oligonucleotide" referred to herein includes naturally occurring, and modified nucleotides linked together by naturally occurring, and non-naturally occurring oligonucleotide linkages. Oligonucleotides are a polynucleotide subset generally comprising a length of 200 bases or fewer. Preferably oligonucleotides are 10 to 60 bases in length and most preferably 12, 13, 14, 15, 16, 17, 18, 19, or 20 to 40 bases in length. Oligonucleotides are usually single stranded, e.g. for probes; although oligonucleotides may be double stranded, e.g. for use in the construction of a gene mutant. Oligonucleotides of the invention can be either sense or antisense oligonucleotides.

[0065] The term "naturally occurring nucleotides" referred to herein includes deoxyribonucleotides and ribonucleotides. The term "modified nucleotides" referred to herein includes nucleotides with modified or substituted sugar groups and the like. The term "oligonucleotide linkages" referred to herein includes oligonucleotides linkages such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoramidate, phosphoramidate, and the like. See e.g., LaPlanche et al. *Nucl. Acids Res.* 14:9081 (1986); Stec et al. *J. Am. Chem. Soc.* 106:6077 (1984); Stein et al. *Nucl. Acids Res.* 16:3209 (1988); Zon et al. *Anti-Cancer Drug Design* 6:539(1991); Zon et al. *Oligonucleotides and Analogues: A Practical Approach*, pp. 87-108 (F. Eckstein, Ed., Oxford University Press, Oxford England (1991)); Stec et al. U.S. Pat. No. 5,151,510; Uhlmann and Peyman *Chemical Reviews* 90:543 (1990), the disclosures of which are hereby

incorporated by reference. An oligonucleotide can include a label for detection, if desired.

[0066] The term “selectively hybridize” referred to herein means to detectably and specifically bind. Polynucleotides, oligonucleotides and fragments thereof in accordance with the invention selectively hybridize to nucleic acid strands under hybridization and wash conditions that minimize appreciable amounts of detectable binding to nonspecific nucleic acids. High stringency conditions can be used to achieve selective hybridization conditions as known in the art and discussed herein. Generally, the nucleic acid sequence homology between the polynucleotides, oligonucleotides, and fragments of the invention and a nucleic acid sequence of interest will be at least 80%, and more typically with preferably increasing homologies of at least 85%, 90%, 95%, 99%, and 100%. Two amino acid sequences are homologous if there is a partial or complete identity between their sequences. For example, 85% homology means that 85% of the amino acids are identical when the two sequences are aligned for maximum matching. Gaps (in either of the two sequences being matched) are allowed in maximizing matching; gap lengths of 5 or less are preferred with 2 or less being more preferred. Alternatively and preferably, two protein sequences (or polypeptide sequences derived from them of at least 30 amino acids in length) are homologous, as this term is used herein, if they have an alignment score of at more than 5 (in standard deviation units) using the program ALIGN with the mutation data matrix and a gap penalty of 6 or greater. See M. O. Dayhoff, in *Atlas of Protein Sequence and Structure*, Vol. 5, 101-110 and Supplement 2 to Vol. 5, 1-10 (National Biomedical Research Foundation 1972). The two sequences or parts thereof are more preferably homologous if their amino acids are greater than or equal to 50% identical when optimally aligned using the ALIGN program. The term “corresponds to” is used herein to mean that a polynucleotide sequence is homologous (i.e., is identical, not strictly evolutionarily related) to all or a portion of a reference polynucleotide sequence, or that a polypeptide sequence is identical to a reference polypeptide sequence. In contradistinction, the term “complementary to” is used herein to mean that the complementary sequence is homologous to all or a portion of a reference polynucleotide sequence. For illustration, the nucleotide sequence “TATAC” corresponds to a reference sequence “TATAC” and is complementary to a “GTATA”.

[0067] The following terms are used to describe the sequence relationships between two or more polynucleotide or amino acid sequences: “reference sequence,” “comparison window,” “sequence identity,” “percentage of sequence identity,” and “substantial identity”. A “reference sequence” is a defined sequence used as a basis for a sequence comparison; a reference sequence may be a subset of a larger sequence, for example, as a segment of a full-length cDNA or gene sequence given in a sequence listing or may comprise a complete cDNA or gene sequence. Generally, a reference sequence is at least 18 nucleotides or 6 amino acids in length, frequently at least 24 nucleotides or 8 amino acids in length, and often at least 48 nucleotides or 16 amino acids in length. Since two polynucleotides or amino acid sequences may each (1) comprise a sequence (i.e., a portion of the complete polynucleotide or amino acid sequence) that is similar between the two molecules, and (2) may further comprise a sequence that is divergent between the two

polynucleotides or amino acid sequences, sequence comparisons between two (or more) molecules are typically performed by comparing sequences of the two molecules over a “comparison window” to identify and compare local regions of sequence similarity. A “comparison window,” as used herein, refers to a conceptual segment of at least 18 contiguous nucleotide positions or 6 amino acids wherein a polynucleotide sequence or amino acid sequence may be compared to a reference sequence of at least 18 contiguous nucleotides or 6 amino acid sequences and wherein the portion of the polynucleotide sequence in the comparison window may comprise additions, deletions, substitutions, and the like (i.e., gaps) of 20 percent or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by the local homology algorithm of Smith and Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman and Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson and Lipman, *Proc. Natl. Acad. Sci. (U.S.A.)* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, (Genetics Computer Group, 575 Science Dr., Madison, Wis.), Geneworks, or MacVector software packages), or by inspection, and the best alignment (i.e., resulting in the highest percentage of homology over the comparison window) generated by the various methods is selected.

[0068] The term “sequence identity” means that two polynucleotide or amino acid sequences are identical (i.e., on a nucleotide-by-nucleotide or residue-by-residue basis) over the comparison window. The term “percentage of sequence identity” is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, U, or I) or residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the comparison window (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The terms “substantial identity” as used herein denotes a characteristic of a polynucleotide or amino acid sequence, wherein the polynucleotide or amino acid comprises a sequence that has at least 85 percent sequence identity, preferably at least 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison window of at least 18 nucleotide (6 amino acid) positions, frequently over a window of at least 24-48 nucleotide (8-16 amino acid) positions, wherein the percentage of sequence identity is calculated by comparing the reference sequence to the sequence which may include deletions or additions which total 20 percent or less of the reference sequence over the comparison window. The reference sequence may be a subset of a larger sequence.

[0069] As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. See *Immunology—A Synthesis* (2d ed., Golub, E. S. and Gren, D. R. eds., Sinauer Associates, Sunderland, Mass. 1991), which is incorporated herein by reference. Stereoisomers (e.g., D-amino acids) of the twenty conventional amino acids, unnatural amino acids such as α -, α -disubstituted amino acids, N-alkyl amino acids, lactic acid, and other unconven-

tional amino acids may also be suitable components for polypeptides of the invention described herein. Examples of unconventional amino acids include: 4-hydroxyproline, γ -carboxyglutamate, ϵ -N,N,N-trimethyllysine, ϵ -N-acetyllsine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, σ -N-methylarginine, and other similar amino acids and imino acids (e.g., 4-hydroxyproline). In the polypeptide notation used herein, the left-hand direction is the amino terminal direction and the right-hand direction is the carboxy-terminal direction, in accordance with standard usage and convention.

[0070] Similarly, unless specified otherwise, the left-hand end of single-stranded polynucleotide sequences is the 5' end; the left-hand direction of double-stranded polynucleotide sequences is referred to as the 5' direction. The direction of 5' to 3' addition of nascent RNA transcripts is referred to as the transcription direction; sequence regions on the DNA strand having the same sequence as the RNA and which are 5' to the 5' end of the RNA transcript are referred to as "upstream sequences"; sequence regions on the DNA strand having the same sequence as the RNA and which are 3' to the 3' end of the RNA transcript are referred to as "downstream sequences".

[0071] As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80 percent sequence identity, preferably at least 90 percent sequence identity, more preferably at least 95 percent sequence identity, and most preferably at least 99 percent sequence identity. Preferably, residue positions that are not identical differ by conservative amino acid substitutions. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. Preferred conservative amino acid substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamic-aspartic, and asparagine-glutamine.

[0072] As discussed herein, minor variations in the amino acid sequences of antibodies or immunoglobulin molecules are contemplated as being encompassed by the invention described herein, providing that the variations in the amino acid sequence maintain at least 75%, more preferably at least 80%, 90%, 95%, and most preferably 99%. In particular, conservative amino acid replacements are contemplated. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Genetically encoded amino acids are generally divided into families: (1) acidic=aspartate, glutamate; (2) basic=lysine, arginine, histidine; (3) non-polar=alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar=glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. More preferred families are: serine and threonine are aliphatic-hydroxy family;

asparagine and glutamine are an amide-containing family; alanine, valine, leucine and isoleucine are an aliphatic family; and phenylalanine, tryptophan, and tyrosine are an aromatic family. For example, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the binding or properties of the resulting molecule, especially if the replacement does not involve an amino acid within a framework site. Whether an amino acid change results in a functional peptide can readily be determined by assaying the specific activity of the polypeptide derivative. Assays are described in detail herein. Fragments or analogs of antibodies or immunoglobulin molecules can be readily prepared by those of ordinary skill in the art. Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. Preferably, computerized comparison methods are used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known. Bowie et al., *Science* 253:164 (1991). Thus, the foregoing examples demonstrate that those of skill in the art can recognize sequence motifs and structural conformations that may be used to define structural and functional domains in accordance with the invention.

[0073] Preferred amino acid substitutions are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and (4) confer or modify other physicochemical or functional properties of such analogs. Analogs can include various muteins of a sequence other than the naturally occurring peptide sequence. For example, single or multiple amino acid substitutions (preferably conservative amino acid substitutions) may be made in the naturally occurring sequence (preferably in the portion of the polypeptide outside the domain(s) forming intermolecular contacts. A conservative amino acid substitution should not substantially change the structural characteristics of the parent sequence (e.g., a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in *Proteins, Structures and Molecular Principles* (Creighton, ed., W. H. Freeman and Company, New York 1984); *Introduction to Protein Structure* (Branden, C. and Tooze, J. eds., Garland Publishing, New York, N.Y. 1991); and Thornton et al., *Nature* 354:105 (1991), which are each incorporated herein by reference.

[0074] The term "polypeptide fragment" as used herein refers to a polypeptide that has an amino-terminal and/or carboxy-terminal deletion, but where the remaining amino acid sequence is identical to the corresponding positions in the naturally occurring sequence deduced, for example, from a full-length cDNA sequence. Fragments typically are at least 5, 6, 8 or 10 amino acids long, preferably at least 14 amino acids long, more preferably at least 20 amino acids long, usually at least 50 amino acids long, and even more

preferably at least 70 amino acids long. The term “analog” as used herein refers to polypeptides which are comprised of a segment of at least 25 amino acids that has substantial identity to a portion of a deduced amino acid sequence and which has at least one of the following properties: (1) specific binding to a MCP-1, under suitable binding conditions, (2) ability to block appropriate MCP-1 binding, or (3) ability to inhibit MCP-1 expressing cell growth in vitro or in vivo. Typically, polypeptide analogs comprise a conservative amino acid substitution (or addition or deletion) with respect to the naturally occurring sequence. Analogs typically are at least 20 amino acids long, preferably at least 50 amino acids long or longer, and can often be as long as a full-length naturally occurring polypeptide.

[0075] Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. These types of non-peptide compound are termed “peptide mimetics” or “peptidomimetics.” Fauchere, *J. Adv. Drug Res.* 15:29 (1986); Veber and Freidinger, *TINS* p.392 (1985); and Evans et al., *J. Med. Chem.* 30:1229 (1987), which are incorporated herein by reference. Such compounds are often developed with the aid of computerized molecular modeling. Peptide mimetics that are structurally similar to therapeutically useful peptides may be used to produce an equivalent therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to a paradigm polypeptide (i.e., a polypeptide that has a biochemical property or pharmacological activity), such as human antibody, but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of: $-\text{CH}_2\text{NH}-$, $-\text{CH}_2\text{S}-$, $-\text{CH}_2-\text{CH}_2-$, $-\text{CH}=\text{CH}-$ (cis and trans), $-\text{COCH}_2-$, $-\text{CH}(\text{OH})\text{CH}_2-$, and $-\text{CH}_2\text{SO}-$, by methods well known in the art. Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (e.g., D-lysine in place of L-lysine) may be used to generate more stable peptides. In addition, constrained peptides comprising a consensus sequence or a substantially identical consensus sequence variation may be generated by methods known in the art (Rizo and Gierasch *Ann. Rev. Biochem.* 61:387 (1992), incorporated herein by reference); for example, by adding internal cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide.

[0076] “Antibody” or “antibody peptide(s)” refer to an intact antibody, or a binding fragment thereof that competes with the intact antibody for specific binding. Binding fragments are produced by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact antibodies. Binding fragments include Fab, Fab', F(ab')₂, Fv, and single-chain antibodies. An antibody other than a “bispecific” or “bifunctional” antibody is understood to have each of its binding sites identical. An antibody substantially inhibits adhesion of a receptor to a counterreceptor when an excess of antibody reduces the quantity of receptor bound to counterreceptor by at least about 20%, 40%, 60% or 80%, and more usually greater than about 85% (as measured in an in vitro competitive binding assay).

[0077] The term “epitope” includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific

three-dimensional structural characteristics, as well as specific charge characteristics. An antibody is said to specifically bind an antigen when the dissociation constant is $\leq 1 \mu\text{M}$, preferably $\leq 100 \text{ nM}$ and most preferably $\leq 10 \text{ nM}$.

[0078] The term “agent” is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials.

[0079] “Active” or “activity” for the purposes herein refers to form(s) of MCP-1 polypeptide which retain a biological and/or an immunological activity of native or naturally occurring MCP-1 polypeptides, wherein “biological” activity refers to a biological function (either inhibitory or stimulatory) caused by a native or naturally occurring MCP-1 polypeptide other than the ability to induce the production of an antibody against an antigenic epitope possessed by a native or naturally occurring MCP-1 polypeptide and an “immunological” activity refers to the ability to induce the production of an antibody against an antigenic epitope possessed by a native or naturally occurring MCP-1 polypeptide.

[0080] “Treatment” refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the targeted pathologic condition or disorder. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented.

[0081] “Mammal” refers to any animal classified as a mammal, including humans, other primates, such as monkeys, chimpanzees and gorillas, domestic and farm animals, and zoo, sports, laboratory, or pet animals, such as dogs, cats, cattle, horses, sheep, pigs, goats, rabbits, rodents, etc. For purposes of treatment, the mammal is preferably human.

[0082] “Carriers” as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are non-toxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

[0083] Papain digestion of antibodies produces two identical antigen-binding fragments, called “Fab” fragments, each with a single antigen-binding site, and a residual “Fc” fragment, a designation reflecting the ability to crystallize readily. Pepsin treatment yields an “F(ab')₂” fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

[0084] “Fv” is the minimum antibody fragment that contains a complete antigen-recognition and binding site of the

antibody. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, for example, even a single variable domain (e.g., the VH or VL portion of the Fv dimer or half of an Fv comprising only three CDRs specific for an antigen) may have the ability to recognize and bind antigen, although, possibly, at a lower affinity than the entire binding site.

[0085] A Fab fragment also contains the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab fragments differ from Fab' fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region. F(ab')₂ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0086] "Solid phase" means a non-aqueous matrix to which the antibodies described herein can adhere. Examples of solid phases encompassed herein include those formed partially or entirely of glass (e.g., controlled pore glass), polysaccharides (e.g., agarose), polyacrylamides, polystyrene, polyvinyl alcohol and silicones. In certain embodiments, depending on the context, the solid phases can comprise the well of an assay plate; in others it is a purification column (e.g., an affinity chromatography column). This term also includes a discontinuous solid phase of discrete particles, such as those described in U.S. Pat. No. 4,275,149.

[0087] The term "liposome" is used herein to denote a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug (such as a MCP-1 polypeptide or antibody thereto) to a mammal. The components of the liposomes are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

[0088] The term "small molecule" is used herein to describe a molecule with a molecular weight below about 500 Daltons.

[0089] As used herein, the terms "label" or "labeled" refers to incorporation of a detectable marker, e.g., by incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). In certain situations, the label or marker can also be therapeutic. Various methods of labeling polypeptides and glycoproteins are known in the art and may be used. Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (e.g., ³H, ¹⁴C, ¹⁵N, ³⁵S, ⁹⁰Y, ⁹⁹Tc, ¹¹¹In, ¹²¹I, ¹³¹I), fluorescent labels (e.g., FITC, rhodamine, lanthanide phosphors), enzymatic labels (e.g., horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase), chemiluminescent, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

[0090] The term "pharmaceutical agent or drug" as used herein refers to a chemical compound or composition capable of inducing a desired therapeutic effect when properly administered to a patient. Other chemistry terms herein are used according to conventional usage in the art, as exemplified by *The McGraw-Hill Dictionary of Chemical Terms* (Parker, S., Ed., McGraw-Hill, San Francisco (1985)), incorporated herein by reference.

[0091] As used herein, "substantially pure" means an object species is the predominant species present (i.e., on a molar basis it is more abundant than any other individual species in the composition), and preferably a substantially purified fraction is a composition wherein the object species comprises at least about 50 percent (on a molar basis) of all macromolecular species present. Generally, a substantially pure composition will comprise more than about 80 percent of all macromolecular species present in the composition, more preferably more than about 85%, 90%, 95%, and 99%. Most preferably, the object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) wherein the composition consists essentially of a single macromolecular species.

[0092] The term "patient" includes human and veterinary subjects.

[0093] Antibody Structure

[0094] The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50 to 70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See generally, *Fundamental Immunology* Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). The variable regions of each light/heavy chain pair form the antibody-binding site. Thus, an intact antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are the same.

[0095] The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hyper variable regions, also called complementarity determining regions or CDRs. The CDRs from the two chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. 1991) (1987), or Chothia and Lesk, *J. Mol. Biol.* 196:901-17 (1987); Chothia et al., *Nature* 342:878-83 (1989).

[0096] A bispecific or bifunctional antibody is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. See, e.g., Song-sivilai and Lachmann, *Clin. Exp. Immunol.* 79: 315-21 (1990); Kostelny et al., *J. Immunol.* 148:1547-53 (1992). Production of bispecific antibodies can be a relatively labor intensive process compared with production of conventional antibodies and yields and degree of purity are generally lower for bispecific antibodies. Bispecific antibodies do not exist in the form of fragments having a single binding site (e.g., Fab, Fab', and Fv).

[0097] Human Antibodies and Humanization of Antibodies

[0098] Human antibodies avoid certain of the problems associated with antibodies that possess murine or rat variable and/or constant regions. The presence of such murine or rat derived proteins can lead to the rapid clearance of the antibodies or can lead to the generation of an immune response against the antibody by a patient. In order to avoid the utilization of murine or rat derived antibodies, fully human antibodies can be generated through the introduction of human antibody function into a rodent so that the rodent produces fully human antibodies.

Human Antibodies

[0099] One method for generating fully human antibodies is through the use of XenoMouse® strains of mice that have been engineered to contain human heavy chain and light chain genes within their genome. For example, a XenoMouse® mouse containing 245 kb and 190 kb-sized germline configuration fragments of the human heavy chain locus and kappa light chain locus is described in Green et al., *Nature Genetics* 7:13-21 (1994). The work of Green et al. was extended to the introduction of greater than approximately 80% of the human antibody repertoire through utilization of megabase-sized, germline configuration YAC fragments of the human heavy chain loci and kappa light chain loci, respectively. See Mendez et al., *Nature Genetics* 15:146-56 (1997) and U.S. patent application Ser. No. 08/759,620, filed Dec. 3, 1996, the disclosures of which are hereby incorporated by reference. Further, XenoMouse® mice have been generated that contain the entire lambda light chain locus (U.S. patent application Ser. No. 60/334,508, filed Nov. 30, 2001). And, XenoMouse® mice have been generated that produce multiple isotypes (see, e.g., WO 00/76310). XenoMouse® strains are available from Abgenix, Inc. (Fremont, Calif.).

[0100] The production of XenoMouse® mice is further discussed and delineated in U.S. patent application Ser. Nos. 07/466,008, filed Jan. 12, 1990, Ser. No. 07/610,515, filed Nov. 8, 1990, Ser. No. 07/919,297, filed Jul. 24, 1992, Ser. No. 07/922,649, filed Jul. 30, 1992, filed Ser. No. 08/031,801, filed Mar. 15, 1993, Ser. No. 08/112,848, filed Aug. 27, 1993, Ser. No. 08/234,145, filed Apr. 28, 1994, Ser. No. 08/376,279, filed Jan. 20, 1995, Ser. No. 08/430,938, Apr. 27, 1995, Ser. No. 08/464,584, filed Jun. 5, 1995, Ser. No. 08/464,582, filed Jun. 5, 1995, Ser. No. 08/463,191, filed Jun. 5, 1995, Ser. No. 08/462,837, filed Jun. 5, 1995, Ser. No. 08/486,853, filed Jun. 5, 1995, Ser. No. 08/486,857, filed Jun. 5, 1995, Ser. No. 08/486,859, filed Jun. 5, 1995,

Ser. No. 08/462,513, filed Jun. 5, 1995, Ser. No. 08/724,752, filed Oct. 2, 1996, and Ser. No. 08/759,620, filed Dec. 3, 1996 and U.S. Pat. Nos. 6,162,963, 6,150,584, 6,114,598, 6,075,181, and 5,939,598 and Japanese Patent Nos. 3 068 180 B2, 3 068 506 B2, and 3 068 507 B2. See also Mendez et al. *Nature Genetics* 15:146-156 (1997) and Green and Jakobovits *J. Exp. Med.*, 188:483-495 (1998). See also European Patent No., EP 463,151 B1, grant published Jun. 12, 1996, International Patent Application No., WO 94/02602, published Feb. 3, 1994, International Patent Application No., WO 96/34096, published Oct. 31, 1996, WO 98/24893, published Jun. 11, 1998, WO 00/76310, published Dec. 21, 2000. The disclosures of each of the above-cited patents, applications, and references are hereby incorporated by reference in their entirety.

[0101] In an alternative approach, others, including GenPharm International, Inc., have utilized a "minilocus" approach. In the minilocus approach, an exogenous Ig locus is mimicked through the inclusion of pieces (individual genes) from the Ig locus. Thus, one or more V_H genes, one or more D_H genes, one or more J_H genes, a mu constant region, and a second constant region (preferably a gamma constant region) are formed into a construct for insertion into an animal. This approach is described in U.S. Pat. No. 5,545,807 to Surani et al. and U.S. Pat. Nos. 5,545,806, 5,625,825, 5,625,126, 5,633,425, 5,661,016, 5,770,429, 5,789,650, 5,814,318, 5,877,397, 5,874,299, and 6,255,458 each to Lonberg and Kay, U.S. Pat. Nos. 5,591,669 and 6,023,010 to Krimpenfort and Berns, U.S. Pat. Nos. 5,612,205, 5,721,367, and 5,789,215 to Berns et al., and U.S. Pat. No. 5,643,763 to Choi and Dunn, and GenPharm International U.S. Patent Application Serial Nos. 07/574,748, filed Aug. 29, 1990, 07/575,962, filed Aug. 31, 1990, 07/810,279, filed Dec. 17, 1991, Ser. No. 07/853,408, filed Mar. 18, 1992, Ser. No. 07/904,068, filed Jun. 23, 1992, Ser. No. 07/990,860, filed Dec. 16, 1992, Ser. No. 08/053,131, filed Apr. 26, 1993, Ser. No. 08/096,762, filed Jul. 22, 1993, Ser. No. 08/155,301, filed Nov. 18, 1993, Ser. No. 08/161,739, filed Dec. 3, 1993, Ser. No. 08/165,699, filed Dec. 10, 1993, Ser. No. 08/209,741, filed Mar. 9, 1994, the disclosures of which are hereby incorporated by reference. See also European Patent No. 546,073 B1, International Patent Application Nos. WO 92/03918, WO 92/22645, WO 92/22647, WO 92/22670, WO 93/12227, WO 94/00569, WO 94/25585, WO 96/14436, WO 97/13852, and WO 98/24884 and U.S. Pat. No. 5,981,175, the disclosures of which are hereby incorporated by reference in their entirety. See further Taylor et al., (1992), Chen et al., (1993), Tuailon et al., (1993), Choi et al., (1993), Lonberg et al., (1994), Taylor et al., (1994), and Tuailon et al., (1995), Fishwild et al., (1996), the disclosures of which are hereby incorporated by reference in their entirety.

[0102] Kirin has demonstrated the generation of human antibodies from mice in which, through microcell fusion, large pieces of chromosomes, or entire chromosomes, have been introduced. See European Patent Application Nos. 773,288 and 843,961, the disclosures of which are hereby incorporated by reference.

[0103] Lidak Pharmaceuticals (now Xenorex) has also demonstrated the generation of human antibodies in SCID mice modified by injection of non-malignant mature peripheral leukocytes from a human donor. The modified mice exhibit an immune response characteristic of the human

donor upon stimulation with an immunogen, which consists of the production of human antibodies. See U.S. Pat. Nos. 5,476,996 and 5,698,767, the disclosures of which are herein incorporated by reference.

[0104] Human anti-mouse antibody (HAMA) responses have led the industry to prepare chimeric or otherwise humanized antibodies. While chimeric antibodies have a human constant region and a murine variable region, it is expected that certain human anti-chimeric antibody (HACA) responses will be observed, particularly in chronic or multi-dose utilizations of the antibody. Thus, it would be desirable to provide fully human antibodies against MCP-1 in order to vitiate concerns and/or effects of HAMA or HACA response.

Humanization and Display Technologies

[0105] As discussed above in connection with human antibody generation, there are advantages to producing antibodies with reduced immunogenicity. To a degree, this can be accomplished in connection with techniques of humanization and display techniques using appropriate libraries. It will be appreciated that murine antibodies or antibodies from other species can be humanized or primatized using techniques well known in the art. See e.g., Winter and Harris, *Immunol Today* 14:43-46 (1993) and Wright et al., *Crit. Reviews in Immunol.* 12:125-168 (1992). The antibody of interest may be engineered by recombinant DNA techniques to substitute the CH1, CH2, CH3, hinge domains, and/or the framework domain with the corresponding human sequence (see WO 92/02190 and U.S. Pat. Nos. 5,530,101, 5,585,089, 5,693,761, 5,693,792, 5,714,350, and 5,777,085). Also, the use of Ig cDNA for construction of chimeric immunoglobulin genes is known in the art (Liu et al., *P.N.A.S.* 84:3439 (1987) and *J. Immunol.* 139:3521 (1987)). mRNA is isolated from a hybridoma or other cell producing the antibody and used to produce cDNA. The cDNA of interest may be amplified by the polymerase chain reaction using specific primers (U.S. Pat. Nos. 4,683,195 and 4,683,202). Alternatively, a library is made and screened to isolate the sequence of interest. The DNA sequence encoding the variable region of the antibody is then fused to human constant region sequences. The sequences of human constant regions genes may be found in Kabat et al., "Sequences of Proteins of Immunological Interest," N.I.H. publication no. 91-3242 (1991). Human C region genes are readily available from known clones. The choice of isotype will be guided by the desired effector functions, such as complement fixation, or activity in antibody-dependent cellular cytotoxicity. Preferred isotypes are IgG1, IgG3 and IgG4. Either of the human light chain constant regions, kappa or lambda, may be used. The chimeric, humanized antibody is then expressed by conventional methods.

[0106] Antibody fragments, such as Fv, F(ab').sub.2 and Fab may be prepared by cleavage of the intact protein, e.g., by protease or chemical cleavage. Alternatively, a truncated gene is designed. For example, a chimeric gene encoding a portion of the F(ab')₂ fragment would include DNA sequences encoding the CH1 domain and hinge region of the H chain, followed by a translational stop codon to yield the truncated molecule.

[0107] Consensus sequences of H and L J regions may be used to design oligonucleotides for use as primers to intro-

duce useful restriction sites into the J region for subsequent linkage of V region segments to human C region segments. C region cDNA can be modified by site directed mutagenesis to place a restriction site at the analogous position in the human sequence.

[0108] Expression vectors include plasmids, retroviruses, YACs, EBV derived episomes, and the like. A convenient vector is one that encodes a functionally complete human CH or CL immunoglobulin sequence, with appropriate restriction sites engineered so that any VH or VL sequence can be easily inserted and expressed. In such vectors, splicing usually occurs between the splice donor site in the inserted J region and the splice acceptor site preceding the human C region, and also at the splice regions that occur within the human CH exons. Polyadenylation and transcription termination occur at native chromosomal sites downstream of the coding regions. The resulting chimeric antibody may be joined to any strong promoter, including retroviral LTRs, e.g., SV-40 early promoter, (Okayama et al., *Mol. Cell. Bio.* 3:280 (1983)), Rous sarcoma virus LTR (Gorman et al., *P.N.A.S.* 79:6777 (1982)), and moloney murine leukemia virus LTR (Grosschedl et al., *Cell* 41:885 (1985)). Also, as will be appreciated, native Ig promoters and the like may be used.

[0109] Further, human antibodies or antibodies from other species can be generated through display-type technologies, including, without limitation, phage display, retroviral display, ribosomal display, and other techniques, using techniques well known in the art and the resulting molecules can be subjected to additional maturation, such as affinity maturation, as such techniques are well known in the art. Wright and Harris, supra., Hanes and Plutchau, *PNAS USA* 94:4937-4942 (1997) (ribosomal display), Parmley and Smith, *Gene* 73:305-318 (1988) (phage display), Scott, *TIBS* 17:241-245 (1992), Cwirla et al., *PNAS USA* 87:6378-6382 (1990), Russel et al., *Nucl. Acids Res.* 21:1081-1085 (1993), Hog- anboom et al., *Immunol. Reviews* 130:43-68 (1992), Chiswell and McCafferty, *TIBTECH* 10:80-84 (1992), and U.S. Pat. No. 5,733,743. If display technologies are utilized to produce antibodies that are not human, such antibodies can be humanized as described above.

[0110] Using these techniques, antibodies can be generated against MCP-1 expressing cells, MCP-1 itself, forms of MCP-1, epitopes or peptides thereof, and expression libraries thereto (see, e.g., U.S. Pat. No. 5,703,057) which can thereafter be screened as described above for the activities described above.

[0111] Preparation of Antibodies

[0112] Antibodies in accordance with the invention were prepared through the utilization of the XenoMouse® technology, as described below. Such mice, then, are capable of producing human immunoglobulin molecules and antibodies and are deficient in the production of murine immunoglobulin molecules and antibodies. Technologies utilized for achieving the same are disclosed in the patents, applications, and references disclosed in the Background, herein. In particular, however, a preferred embodiment of transgenic production of mice and antibodies therefrom is disclosed in U.S. patent application Ser. No. 08/759,620, filed Dec. 3, 1996 and International Patent Application Nos. WO 98/24893, published Jun. 11, 1998 and WO 00/76310, published Dec. 21, 2000, the disclosures of which are hereby

incorporated by reference. See also Mendez et al., *Nature Genetics* 15:146-156 (1997), the disclosure of which is hereby incorporated by reference.

[0113] Antibodies, as described herein, are neutralizing high affinity antibodies to human MCP-1. Further, in some embodiments, the antibodies cross react with rat MCP-1. Several different methods have been used historically to generate monoclonal antibodies or polyclonal antibodies against the N-terminus of human MCP-1. These approaches have included immunizing with full length human MCP-1 (hMCP-1) or bovine MCP-1 (bMCP-1) (Vieira et al., *Braz. J. Med. Biol. Res.* 21:1005-1011 (1988)), synthetic peptides of human MCP-1 (1-34 or 1-37) (Visser et al., *Acta Endocrinol.* 90:90-102 (1979)); Logue et al., *J. Immunol. Methods* 137:159-66 (1991)), and multiple antigenic peptides (MAP) of hMCP-1 (1-10), hMCP-1 (9-18) and hMCP-1 (24-37) (Magerlein et al., *Drug Res.* 48:783-87 (1998)). These approaches did not produce antibodies suitable for human therapeutics. (See section entitled "Therapeutic Administration and Formulation" herein for therapeutic criteria.) High affinity antibodies to hMCP-1 are difficult to make because of B cell tolerance to the peptide. However, Bradwell et al., (1999) have demonstrated that immunization with a mixture of human MCP-1 (1-34) and bovine MCP-1 (1-34) MAPs followed by a mixture of human and bovine MAPs targeting the hMCP-1(51-84) and bMCP-1(51-86) was effective in breaking B-cell tolerance to MCP-1 in a human patient with an inoperable parathyroid tumor.

[0114] The approach described herein was designed to overcome B-cell tolerance to hMCP-1 as well as to produce a fully human monoclonal antibody suitable for therapeutic and diagnostic use. XenoMouse® animals were immunized with synthetic peptides of MCP-1 (hMCP-1(1-34) and rMCP-1(1-34)), because synthetic peptides have been successfully used to generate antibodies specific to endogenous human MCP-1 (Visser et al., (1979)). Furthermore, because the N-terminus of murine MCP-1 is highly conserved with human MCP-1 (85% identity) and rat MCP-1 (91%), the combination of peptides was used as an immunogen to break B-cell tolerance to murine MCP-1 through molecular mimicry, thereby allowing the generation of high affinity human anti-human MCP-1 antibodies. These peptides were both coupled to keyhole limpet hemocyanin and emulsified in complete Freund's adjuvant or incomplete Freund's adjuvant to enhance the immunogenicity of these proteins.

[0115] After immunization, lymphatic cells (such as B cells) were recovered from the mice that expressed antibodies, and such recovered cell lines fused with a myeloid-type cell line to prepare immortal hybridoma cell lines. Such hybridoma cell lines were screened and selected to identify hybridoma cell lines that produced antibodies specific to the antigen of interest. Herein, the production of multiple hybridoma cell lines that produce antibodies specific to MCP-1 is described. Further, a characterization of the antibodies produced by such cell lines is provided, including nucleotide and amino acid sequence analyses of the heavy and light chains of such antibodies.

[0116] Embodiments of the invention provide for the production of multiple hybridoma cell lines that produce antibodies specific to MCP-1. Further embodiments relate to antibodies that bind to and neutralize the activity of other MCP-1 family members including MCP-2, MCP-3, and

MCP-4. The supernatants are also screened for immunoreactivity against fragments of MCP-1 to further epitope map the different antibodies against related human chemokines and against rat MCP-1 and the mouse ortholog of MCP-1, JE, to determine species cross-reactivity. Further embodiments provide a characterization of the antibodies produced by such cell lines, including nucleotide and amino acid sequence analyses of the heavy and light chains of such antibodies.

[0117] Alternatively, instead of being fused to myeloma cells to generate hybridomas, B cells may be directly assayed. For example, CD19+B cells may be isolated from hyperimmune XenoMouse® mice and allowed to proliferate and differentiate into antibody-secreting plasma cells. Antibodies from the cell supernatants are then screened by ELISA for reactivity against the MCP-1 immunogen. The supernatants are also screened for immunoreactivity against fragments of MCP-1 to further epitope map the different antibodies against related human chemokines and against rat MCP-1 and the mouse ortholog of MCP-1, JE, to determine species cross-reactivity. Single plasma cells secreting antibodies with the desired specificities are then isolated using a MCP-1-specific hemolytic plaque assay (Babcock et al., *Proc. Natl. Acad. Sci. USA*, 93:7843-7848 (1996)). Cells targeted for lysis are preferably sheep red blood cells (SRBCs) coated with the MCP-1 antigen. In the presence of a B cell culture containing plasma cells secreting the immunoglobulin of interest and complement, the formation of a plaque indicates specific MCP-1-mediated lysis of the sheep red blood cells surrounding the plasma cell of interest. The single antigen-specific plasma cell in the center of the plaque can be isolated and the genetic information that encodes the specificity of the antibody is isolated from the single plasma cell. Using reverse-transcriptase PCR, the DNA encoding the heavy and light chain variable regions of the antibody can be cloned. Such cloned DNA can then be further inserted into a suitable expression vector, preferably a vector cassette such as a pcDNA, more preferably such a pcDNA vector containing the constant domains of immunoglobulin heavy and light chain. The generated vector can then be transfected into host cells, preferably CHO cells, and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. The isolation of multiple single plasma cells that produce antibodies specific to MCP-1 is described below. Further, the genetic material that encodes the specificity of the anti-MCP-1 antibody can be isolated, introduced into a suitable expression vector that can then be transfected into host cells.

[0118] In general, antibodies produced by the fused hybridomas were human IgG2 heavy chains with fully human kappa or lambda light chains. In some embodiments, antibodies possess human IgG4 heavy chains as well as IgG2 heavy chains. Antibodies may also be of other human isotypes, including IgG1. The antibodies possessed high affinities, typically possessing a K_D of from about 10^{-6} through about 10^{-12} M or below, when measured by either solid phase and solution phase. Antibodies possessing a K_D of at least 10^{11} M are preferred to inhibit the activity of MCP-1.

[0119] Regarding the importance of affinity to therapeutic utility of anti-MCP-1 antibodies, it will be understood that one can generate anti-MCP-1 antibodies, for example, com-

binatorially, and assess such antibodies for binding affinity. One approach that can be utilized is to take the heavy chain cDNA from an antibody, prepared as described above and found to have good affinity to MCP-1, and combine it with the light chain cDNA from a second antibody, prepared as described above and also found to have good affinity to MCP-1, to produce a third antibody. The affinities of the resulting third antibodies can be measured as described herein and those with desirable dissociation constants isolated and characterized. Alternatively, the light chain of any of the antibodies described above can be used as a tool to aid in the generation of a heavy chain that when paired with the light chain will exhibit a high affinity for MCP-1, or vice versa. These heavy chain variable regions in this library could be isolated from naive animals, isolated from hyper-immune animals, generated artificially from libraries containing variable heavy chain sequences that differ in the CDR regions, or generated by any other methods that produce diversity within the CDR regions of any heavy chain variable region gene (such as random or directed mutagenesis). These CDR regions, and in particular CDR3, may be a significantly different length or sequence identity from the heavy chain initially paired with the original antibody. The resulting library could then be screened for high affinity binding to MCP-1 to generate a therapeutically relevant antibody molecule with similar properties as the original antibody (high affinity and neutralization). A similar process using the heavy chain or the heavy chain variable region can be used to generate a therapeutically relevant antibody molecule with a unique light chain variable region. Furthermore, the novel heavy chain variable region, or light chain variable region, can then be used in a similar fashion as described above to identify a novel light chain variable region, or heavy chain variable region, that allows the generation of a novel antibody molecule.

[0120] Another combinatorial approach that can be utilized is to perform mutagenesis on germ line heavy and/or light chains that are demonstrated to be utilized in the antibodies in accordance with the invention described herein, particularly in the complementarity determining regions (CDRs). The affinities of the resulting antibodies can be measured as described herein and those with desirable dissociation constants isolated and characterized. Upon selection of a preferred binder, the sequence or sequences encoding the same may be used to generate recombinant antibodies as described above. Appropriate methods of performing mutagenesis on an oligonucleotide are known to those skilled in the art and include chemical mutagenesis, for example, with sodium bisulfite, enzymatic misincorporation, and exposure to radiation. It is understood that the invention described herein encompasses antibodies with substantial identity, as defined herein, to the antibodies explicitly set forth herein, whether produced by mutagenesis or by any other means. Further, antibodies with conservative or non-conservative amino acid substitutions, as defined herein, made in the antibodies explicitly set forth herein, are included in embodiments of the invention described herein.

[0121] Another combinatorial approach that can be used is to express the CDR regions, and in particular CDR3, of the antibodies described above in the context of framework regions derived from other variable region genes. For example, CDR1, CDR2, and CDR3 of the heavy chain of one anti-MCP-1 antibody could be expressed in the context of the framework regions of other heavy chain variable

genes. Similarly, CDR1, CDR2, and CDR3 of the light chain of an anti-MCP-1 antibody could be expressed in the context of the framework regions of other light chain variable genes. In addition, the germline sequences of these CDR regions could be expressed in the context of other heavy or light chain variable region genes. The resulting antibodies can be assayed for specificity and affinity and may allow the generation of a novel antibody molecule.

[0122] As will be appreciated, antibodies prepared in accordance with the invention described herein can be expressed in various cell lines. Sequences encoding particular antibodies can be used for transformation of a suitable mammalian host cell. Transformation can be by any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus (or into a viral vector) and transducing a host cell with the virus (or vector) or by transfection procedures known in the art, as exemplified by U.S. Pat. Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455 (which patents are hereby incorporated herein by reference). The transformation procedure used depends upon the host to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are well known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

[0123] Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), and a number of other cell lines. Cell lines of particular preference are selected through determining which cell lines have high expression levels and produce antibodies with constitutive MCP-1 binding properties.

[0124] Additional Criteria for Antibody Therapeutics

[0125] As discussed herein, the function of the MCP-1 antibody appears important to at least a portion of its mode of operation. The anti-MCP-1 antibodies of the instant invention may be made capable of effector function, including complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC). There are a number of isotypes of antibodies that are capable of the same, including, without limitation, the following: murine IgM, murine IgG2a, murine IgG2b, murine IgG3, human IgM, human IgG1, and human IgG3. It will be appreciated that antibodies that are generated need not initially possess such an isotype but, rather, the antibody as generated can possess any isotype and the antibody can be isotype switched thereafter using conventional techniques that are well known in the art. Such techniques include the use of direct recombinant techniques (see, e.g., U.S. Pat. Nos. 4,816,397 and 6,331,415), cell-cell fusion techniques (see, e.g., U.S. Pat. Nos. 5,916,771 and 6,207,418), among others.

[0126] In the cell-cell fusion technique, a myeloma or other cell line is prepared that possesses a heavy chain with any desired isotype and another myeloma or other cell line is prepared that possesses the light chain. Such cells can, thereafter, be fused and a cell line expressing an intact antibody can be isolated.

[0127] By way of example, the MCP-1 antibodies discussed herein are human anti-MCP-1 IgG2 and IgG4 antibodies. If such antibody possessed desired binding to the MCP-1 molecule, it could be readily isotype switched to generate a human IgM, human IgG1, or human IgG3, IgA1 or IgGA2 isotypes, while still possessing the same variable region (which defines the antibody's specificity and some of its affinity). Such molecule would then be capable of fixing complement and participating in CDC.

[0128] Accordingly, as antibody candidates are generated that meet desired "structural" attributes as discussed above, they can generally be provided with at least certain of the desired "functional" attributes through isotype switching.

[0129] Epitope Mapping

Immunoblot Analysis

[0130] The binding of the antibodies described herein to MCP-1 can be examined by a number of methods. For example, MCP-1 may be subjected to SDS-PAGE and analyzed by immunoblotting. The SDS-PAGE may be performed either in the absence or presence of a reduction agent. Such chemical modifications may result in the methylation of cysteine residues. Accordingly, it is possible to determine whether the anti-MCP-1 antibodies described herein bind to a linear epitope on MCP-1.

Surface-enhanced Laser Desorption/ionization (SELDI)

[0131] Epitope mapping of the epitope for the MCP-1 antibodies described herein can also be performed using SELDI. SELDI ProteinChip® arrays are used to define sites of protein-protein interaction. Antigens are specifically captured on antibodies covalently immobilized onto the Protein Chip array surface by an initial incubation and wash. The bound antigens can be detected by a laser-induced desorption process and analyzed directly to determine their mass. Such fragments of the antigen that bind are designated as the "epitope" of a protein.

[0132] The SELDI process enables individual components within complex molecular compositions to be detected directly and mapped quantitatively relative to other components in a rapid, highly-sensitive and scalable manner. SELDI utilizes a diverse array of surface chemistries to capture and present large numbers of individual protein molecules for detection by a laser-induced desorption process. The success of the SELDI process is defined in part by the miniaturization and integration of multiple functions, each dependent on different technologies, on a surface ("chip"). SELDI BioChips and other types of SELDI probes are surfaces "enhanced" such that they become active participants in the capture, purification (separation), presentation, detection, and characterization of individual target molecules (e.g., proteins) or population of molecules to be evaluated.

[0133] A single SELDI protein BioChip, loaded with only the original sample, can be read thousands of times. The SELDI protein BioChips from LumiCyte hold as many as 10,000 addressable protein docking locations per 1 square centimeter. Each location may reveal the presence of dozens of individual proteins. When the protein composition information from each location is compared and unique infor-

mation sets combined, the resulting composition map reveals an image with sets of features that are used collectively to define specific patterns or molecular "fingerprints." Different fingerprints may be associated with various stages of health, the onset of disease, or the regression of disease associated with the administration of appropriate therapeutics.

[0134] The SELDI process may be described in further detail in four parts. Initially, one or more proteins of interest are captured or "docked" on the ProteinChip Array, directly from the original source material, without sample preparation and without sample labeling. In a second step, the "signal-to-noise" ratio is enhanced by reducing the chemical and biomolecular "noise." Such "noise" is reduced through selective retention of target on the chip by washing away undesired materials. Further, one or more of the target protein(s) that are captured are read by a rapid, sensitive, laser-induced process (SELDI) that provides direct information about the target (molecular weight). Lastly, the target protein at any one or more locations within the array may be characterized in situ by performing one or more on-the-chip binding or modification reactions to characterize protein structure and function.

Phage Display

[0135] The epitope for the anti-MCP-1 antibodies described herein can be determined by exposing the ProteinChip Array to a combinatorial library of random peptide 12-mer displayed on Filamentous phage (New England Biolabs).

[0136] Phage display describes a selection technique in which a peptide is expressed as a fusion with a coat protein of a bacteriophage, resulting in display of the fused protein on the surface of the virion. Panning is carried out by incubation of a library of phage displayed peptide with a plate or tube coated with the target, washing away the unbound phage, and eluting the specifically bound phage. The eluted phage is then amplified and taken through additional binding and amplification cycles to enrich the pool in favor of binding sequences. After three or four rounds, individual clones binding are further tested for binding by phage ELISA assays performed on antibody-coated wells and characterized by specific DNA sequencing of positive clones.

[0137] After multiple rounds of such panning against the anti-MCP-1 antibodies described herein, the bound phage may be eluted and subjected to further studies for the identification and characterization of the bound peptide.

[0138] Monoclonal antibodies of the invention were shown to bind important residues in the core domain of MCP-1. The neutralizing monoclonal antibodies studied discriminate two functionally important sites in human MCP-1, involved with two residues that were previously shown to be required for binding to the receptor. One site was recognized by all tested antibodies, which competed with the receptor protein for MCP-1 binding and involved Arg 24. The second site was detected by the group of six antibodies that bound the conformational epitope, and their binding site appeared to involve Arg24 and Lys35, which are held in close proximity to the N-terminus by virtue of a disulfide bond between C11 and C36.

[0139] The MCP-1 variants described herein have been analyzed before with respect to biological activity, physical receptor binding and structural integrity (Jarnagin et al., (1999) *Biochemistry* 38: 16167-16177; Hemmerich et al., (1999) *Biochemistry* 38: 13013-13025) and provided valuable tools in determining the binding epitopes of the antibodies as described below.

[0140] Anti MCP-1 antibody 3.11.1 recognizes a conformational epitope and differs from other antibodies by its unique sequence of heavy and light chain, and its ability to cross-react with, and to cross-neutralize, other members of the MCP family, such as MCP-2, MCP-3 and MCP-4. As shown by the mutagenesis experiments, the binding site of mAb 3.11.1 was affected by the change R24A but not by K35A. These data are confirmed by the Lyc-C on chip digest result with SELDI, which delimits the binding epitope to be between residues 20-35 of MCP-1.

[0141] Determination that the epitope for 3.11.1 is between residues 20-35 was also supported by sequence alignment showing that R24, but not K35, was conserved across other members of the MCP family, specifically MCP-2, MCP-3 and MCP-4. Binding analyses by means of SPOTs peptide synthesized on membrane (Sigma-Genosys, The Woodlands, Tex.) revealed that binding site for at least eight mAbs with linear epitopes involved residues 20-25, and included R24. Given the similarities in the results in these binding studies and the significant homology between the variable gene structures for all the mAbs binding to linear epitopes on MCP-1, it appears that the antibodies all bind to this neutralizing epitope.

[0142] The cluster of the epitope around R24 and K35 explains the neutralizing activity of all 36 antibodies. The recognized epitope on MCP-1 does not appear to extend to the N-terminal residues up to Pro9. This residue appears to affect receptor signaling, but not binding affinity.

[0143] Diagnostic Use

[0144] Antibodies prepared in accordance with embodiments of the invention described herein are useful for assays, particularly in vitro diagnostic assays, for example, for use in determining the level of MCP-1 and all MCP-1 family members in patient samples. The patient samples can be, for example, bodily fluids, preferably blood, more preferably blood serum, synovial fluid, tissue lysates, and extracts prepared from diseased tissues. Examples of diagnostic assays include measuring the level of MCP family chemokines in, for example, human serum, synovial fluid and tissue lysates. Monitoring the level of specific MCP family members may be used as a surrogate measure of patient response to treatment and as a method of monitoring the severity of the disease in a patient. Elevated levels of MCP-1 compared to levels of other soluble markers would indicate the presence of inflammation. The concentration of the MCP-1 antigen present in patient samples is determined using a method that specifically determines the amount of the antigen that is present. Such a method includes an ELISA method in which, for example, antibodies of the invention may be conveniently immobilized on an insoluble matrix, such as a polymer matrix. Using a population of samples that provides statistically significant results for each stage of progression or therapy, a range of concentrations of the antigen that may be considered characteristic of each stage of disease can be designated.

[0145] In order to determine the degree of inflammation in a subject under study, or to characterize the response of the subject to a course of therapy, a sample of blood is taken from the subject and the concentration of the MCP-1 antigen present in the sample is determined. The concentration so obtained is used to identify in which range of concentrations the value falls. The range so identified correlates with a stage of disease progression or a stage of therapy identified in the various populations of diagnosed subjects, thereby providing a stage in the subject under study.

[0146] Gene amplification and/or expression may be measured in a sample directly, for example, by conventional Southern blotting, Northern blotting to quantitate the transcription of mRNA (Thomas, *Proc. Natl. Acad. Sci. USA*, 77:5201-5205 (1980)), dot blotting (DNA analysis), or in situ hybridization, using an appropriately labeled probe, based on the sequences provided herein. Alternatively, antibodies may be employed that can recognize specific duplexes, including DNA duplexes, RNA duplexes, and DNA-RNA hybrid duplexes or DNA-protein duplexes. The antibodies in turn may be labeled and the assay can be carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected.

[0147] For example, antibodies, including antibody fragments, can be used to qualitatively or quantitatively detect the expression of MCP-1 proteins. As noted above, the antibody preferably is equipped with a detectable, e.g., fluorescent label, and binding can be monitored by light microscopy, flow cytometry, fluorimetry, or other techniques known in the art. These techniques are particularly suitable if the amplified gene encodes a cell surface protein, e.g., a growth factor. Such binding assays are performed as known in the art.

[0148] In situ detection of antibody binding to the MCP-1 protein can be performed, for example, by immunofluorescence or immunoelectron microscopy. For this purpose, a tissue specimen is removed from the patient, and a labeled antibody is applied to it, preferably by overlaying the antibody on a biological sample. This procedure also allows for determining the distribution of the marker gene product in the tissue examined. It will be apparent for those skilled in the art that a wide variety of histological methods are readily available for in situ detection.

[0149] One of the most sensitive and most flexible quantitative methods for quantitating differential gene expression is RT-PCR, which can be used to compare mRNA levels in different sample populations, in normal and tumor tissues, with or without drug treatment, to characterize patterns of gene expression, to discriminate between closely related mRNAs, and to analyze RNA structure.

[0150] The first step in this process is the isolation of mRNA from a target sample. The starting material is typically total RNA isolated from a disease tissue and corresponding normal tissues, respectively. Thus, mRNA can be extracted, for example, from frozen or archived paraffin-embedded and fixed (e.g. formalin-fixed) samples of diseased tissue for comparison with normal tissue of the same type. Methods for mRNA extraction are well known in the art and are disclosed in standard textbooks of molecular biology, including Ausubel et al., *Current Protocols of Molecular Biology*, John Wiley and Sons (1997). Methods

for RNA extraction from paraffin embedded tissues are disclosed, for example, in Rupp and Locker, *Lab Invest*, 56:A67 (1987), and De Andrés et al., *BioTechniques*, 18:42044 (1995). In particular, RNA isolation can be performed using purification kit, buffer set and protease from commercial manufacturers, such as Qiagen, according to the manufacturer's instructions. For example, total RNA from cells in culture can be isolated using Qiagen RNeasy minicolumns. Total RNA from tissue samples can be isolated using RNA Stat-60 (Tel-Test).

[0151] As RNA cannot serve as a template for PCR, the first step in differential gene expression analysis by RT-PCR is the reverse transcription of the RNA template into cDNA, followed by its exponential amplification in a PCR reaction. The two most commonly used reverse transcriptases are avilo myeloblastosis virus reverse transcriptase (AMV-RT) and Moloney murine leukemia virus reverse transcriptase (MMLV-RT). The reverse transcription step is typically primed using specific primers, random hexamers, or oligo-dT primers, depending on the circumstances and the goal of expression profiling. For example, extracted RNA can be reverse-transcribed using a GeneAmp RNA PCR kit (Perkin Elmer, Calif., USA), following the manufacturer's instructions. The derived cDNA can then be used as a template in the subsequent PCR reaction.

[0152] Although the PCR step can use a variety of thermostable DNA-dependent DNA polymerases, it typically employs the Taq DNA polymerase, which has a 5'-3' nuclease activity but lacks a 3'-5' endonuclease activity. Thus, TaqMan PCR typically utilizes the 5'-nuclease activity of Taq or Tth polymerase to hydrolyze a hybridization probe bound to its target amplicon, but any enzyme with equivalent 5' nuclease activity can be used. Two oligonucleotide primers are used to generate an amplicontypical of a PCR reaction. A third oligonucleotide, or probe, is designed to detect nucleotide sequence located between the two PCR primers. The probe is non-extendible by Taq DNA polymerase enzyme, and is labeled with a reporter fluorescent dye and a quencher fluorescent dye. Any laser-induced emission from the reporter dye is quenched by the quenching dye when the two dyes are located close together as they are on the probe. During the amplification reaction, the Taq DNA polymerase enzyme cleaves the probe in a template-dependent manner. The resultant probe fragments disassociate in solution, and signal from the released reporter dye is free from the quenching effect of the second fluorophore. One molecule of reporter dye is liberated for each new molecule synthesized, and detection of the unquenched reporter dye provides the basis for quantitative interpretation of the data.

[0153] TaqMan RT-PCR can be performed using commercially available equipments, such as, for example, ABI PRIZM 7700™ Sequence Detection System™ (Perkin-Elmer-Applied Biosystems, Foster City, Calif., USA), or Lightcycler (Roche Molecular Biochemicals, Mannheim, Germany). In a preferred embodiment, the 5' nuclease procedure is run on a real-time quantitative PCR device such as the ABI PRIZM 7700™ Sequence Detection System™. The system consists of a thermocycler, laser, charge-coupled device (CCD), camera and computer. The system amplifies samples in a 96-well format on a thermocycler. During amplification, laser-induced fluorescent signal is collected in real-time through fiber optics cables for

all 96 wells, and detected at the CCD. The system includes software for running the instrument and for analyzing the data.

[0154] 5'-Nuclease assay data are initially expressed as Ct, or the threshold cycle. As discussed above, fluorescence values are recorded during every cycle and represent the amount of product amplified to that point in the amplification reaction. The point when the fluorescent signal is first recorded as statistically significant is the threshold cycle (Ct). The ΔC_t values are used as quantitative measurement of the relative number of starting copies of a particular target sequence in a nucleic acid sample when comparing the expression of RNA in a cell from a diseased tissue with that from a normal cell.

[0155] To minimize errors and the effect of sample-to-sample variation, RT-PCR is usually performed using an internal standard. The ideal internal standard is expressed at a constant level among different tissues, and is unaffected by the experimental treatment. RNAs most frequently used to normalize patterns of gene expression are mRNAs for the housekeeping genes glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) and β -actin.

[0156] Differential gene expression can also be identified, or confirmed using the microarray technique. In this method, nucleotide sequences of interest are plated, or arrayed, on a microchip substrate. The arrayed sequences are then hybridized with specific DNA probes from cells or tissues of interest.

[0157] In a specific embodiment of the microarray technique, PCR amplified inserts of cDNA clones are applied to a substrate in a dense array. Preferably at least 10,000 nucleotide sequences are applied to the substrate. The microarrayed genes, immobilized on the microchip at 10,000 elements each, are suitable for hybridization under stringent conditions. Fluorescently labeled cDNA probes may be generated through incorporation of fluorescent nucleotides by reverse transcription of RNA extracted from tissues of interest. Labeled cDNA probes applied to the chip selectively hybridize to each spot of DNA on the array. After stringent washing to remove non-specifically bound probes, the chip is scanned by confocal laser microscopy. Quantitation of hybridization of each arrayed element allows for assessment of corresponding mRNA abundance. With dual color fluorescence, separately labeled cDNA probes generated from two sources of RNA are hybridized pairwise to the array. The relative abundance of the transcripts from the two sources corresponding to each specified gene is thus determined simultaneously. The miniaturized scale of the hybridization affords a convenient and rapid evaluation of the expression pattern for large numbers of genes. Such methods have been shown to have the sensitivity required to detect rare transcripts, which are expressed at a few copies per cell, and to reproducibly detect at least approximately two-fold differences in the expression levels (Schena et al., *Proc. Natl. Acad. Sci. USA*, 93(20)L106-49). The methodology of hybridization of nucleic acids and microarray technology is well known in the art.

[0158] MCP-1 Agonists and Antagonists

[0159] Embodiments of the invention described herein also pertain to variants of a MCP-1 protein that function as either MCP-1 agonists (mimetics) or as MCP-1 antagonists.

Variants of a MCP-1 protein can be generated by mutagenesis, e.g., discrete point mutation or truncation of the MCP-1 protein. An agonist of the MCP-1 protein can retain substantially the same, or a subset of, the biological activities of the naturally occurring form of the MCP-1 protein. An antagonist of the MCP-1 protein can inhibit one or more of the activities of the naturally occurring form of the MCP-1 protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade which includes the MCP-1 protein. Thus, specific biological effects can be elicited by treatment with a variant of limited function. In one embodiment, treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein has fewer side effects in a subject relative to treatment with the naturally occurring form of the MCP-1 protein.

[0160] Variants of the MCP-1 protein that function as either MCP-1 agonists (mimetics) or as MCP-1 antagonists can be identified by screening combinatorial libraries of mutants, e.g., truncation mutants, of the MCP-1 protein for protein agonist or antagonist activity. In one embodiment, a variegated library of MCP-1 variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of MCP-1 variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential MCP-1 sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g., for phage display) containing the set of MCP-1 sequences therein. There are a variety of methods which can be used to produce libraries of potential MCP-1 variants from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential MCP-1 variant sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (see, e.g., Narang, *Tetrahedron* 39:3 (1983); Itakura et al., *Annu. Rev. Biochem.* 53:323 (1984); Itakura et al, *Science* 198:1056 (1984); Ike et al., *Nucl. Acid Res.* 11:477 (1983).

[0161] Design and Generation of Other Therapeutics

[0162] In accordance with embodiments of the invention described herein and based on the activity of the antibodies that are produced and characterized herein with respect to MCP-1, the design of other therapeutic modalities beyond antibody moieties is facilitated. Such modalities include, without limitation, advanced antibody therapeutics, such as bispecific antibodies, immunotoxins, and radiolabeled therapeutics, generation of peptide therapeutics, gene therapies, particularly intrabodies, antisense therapeutics, and small molecules.

[0163] In connection with the generation of advanced antibody therapeutics, where complement fixation is a desirable attribute, it may be possible to sidestep the dependence on complement for cell killing through the use of bispecifics, immunotoxins, or radiolabels, for example.

[0164] For example, in connection with bispecific antibodies, bispecific antibodies can be generated that comprise (i) two antibodies one with a specificity to MCP-1 and another to a second molecule that are conjugated together, (ii) a single antibody that has one chain specific to MCP-1 and a second chain specific to a second molecule, or (iii) a single chain antibody that has specificity to MCP-1 and the other molecule. Such bispecific antibodies can be generated using techniques that are well known for example, in connection with (i) and (ii) see e.g., Fanger et al. *Immunol Methods* 4:72-81 (1994) and Wright and Harris, supra. and in connection with (iii) see e.g., Traunecker et al. *Int. J. Cancer* (Suppl.) 7:51-52 (1992). In each case, the second specificity can be made to the heavy chain activation receptors, including, without limitation, CD16 or CD64 (see e.g., Deo et al. 18:127 (1997)) or CD89 (see e.g., Valerius et al. *Blood* 90:4485-4492 (1997)).

[0165] In connection with immunotoxins, antibodies can be modified to act as immunotoxins utilizing techniques that are well known in the art. See e.g., Vitetta *Immunol Today* 14:252 (1993). See also U.S. Pat. No. 5,194,594. In connection with the preparation of radiolabeled antibodies, such modified antibodies can also be readily prepared utilizing techniques that are well known in the art. See e.g., Junghans et al. in *Cancer Chemotherapy and Biotherapy* 655-686 (2d edition, Chafner and Longo, eds., Lippincott Raven (1996)). See also U.S. Pat. Nos. 4,681,581, 4,735,210, 5,101,827, 5,102,990 (RE 35,500), 5,648,471, and 5,697,902.

[0166] Therapeutic Administration and Formulations

[0167] Biologically active anti-MCP-1 antibodies prepared in accordance with the invention described herein may be used in a sterile pharmaceutical preparation or formulation to neutralize the activity of MCP-1 produced in diseased and inflamed tissues, thereby preventing the further infiltration of mononuclear cells into tissues. Such diseased and inflamed tissues occur in many types of human cancer, including breast, ovarian and lung cancer, and in conditions such as glomerulonephritis, arteriosclerosis, and multiple sclerosis. The biologically active anti-MCP-1 antibody of the instant invention may be employed alone or in combination with other therapeutic agents. For cancer, the anti-MCP-1 antibodies may be combined with traditional modes of chemotherapy such as taxol, doxorubicin, cis-platinum, 5-fluorouracil and other novel inhibitors of the angiogenic process. For treating inflammatory disease, the MCP-1 antibodies may be combined with steroids or antibodies to other cytokines and chemokines that contribute to the disease state.

[0168] When used for in vivo administration, the antibody formulation may be sterile. This can be readily accomplished by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution. The antibody ordinarily will be stored in lyophilized form or in solution. Therapeutic antibody compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[0169] The route of antibody administration can be in accord with known methods, e.g., injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, intrathecal, inhalation or intraleisional routes, or by sustained release systems as noted below. The antibody is preferably administered continuously by infusion or by bolus injection.

[0170] An effective amount of antibody to be employed therapeutically will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the patient. Accordingly, it will be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. Typically, the clinician will administer antibody until a dosage is reached that achieves the desired effect. The progress of this therapy is easily monitored by conventional assays or by the assays described herein.

[0171] The antibodies of the invention may be prepared in a mixture with a pharmaceutically acceptable carrier. This therapeutic composition can be administered intravenously or through the nose or lung, preferably as a liquid or powder aerosol (lyophilized). The composition may also be administered parenterally or subcutaneously as desired. When administered systematically, the therapeutic composition should be sterile, pyrogen-free and in a parenterally acceptable solution having due regard for pH, isotonicity, and stability. These conditions are known to those skilled in the art. Briefly, dosage formulations of the compounds of embodiments of the invention described herein are prepared for storage or administration by mixing the compound having the desired degree of purity with physiologically acceptable carriers, excipients, or stabilizers. Such materials are non-toxic to the recipients at the dosages and concentrations employed, and include buffers such as TRIS HCl, phosphate, citrate, acetate and other organic acid salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) peptides such as polyarginine, proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium and/or nonionic surfactants such as TWEEN, PLURONICS or polyethyleneglycol.

[0172] Sterile compositions for injection can be formulated according to conventional pharmaceutical practice as described in *Remington's Pharmaceutical Sciences* (18th ed, Mack Publishing Company, Easton, Pa. (1990)). For example, dissolution or suspension of the active compound in a vehicle such as water or naturally occurring vegetable oil like sesame, peanut, or cottonseed oil or a synthetic fatty vehicle like ethyl oleate or the like may be desired. Buffers, preservatives, antioxidants and the like can be incorporated according to accepted pharmaceutical practice.

[0173] Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the polypeptide, which matrices are in the form of shaped articles, films or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (e.g., poly(2-hydroxyethyl-methacrylate) as described by Langer et al., *J. Biomed Mater. Res.*, 15:167-277 (1981) and Langer, *Chem. Tech.*, 12:98-105 (1982) or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman et al., *Biopolymers*, 22:547-556 (1983)), non-degradable ethylene-vinyl acetate (Langer et al., supra), degradable lactic acid-glycolic acid copolymers such as the LUPRON DepotTM (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D(-)-3-hydroxybutyric acid (EP 133,988).

[0174] While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods. When encapsulated proteins remain in the body for a long time, they may denature or aggregate as a result of exposure to moisture at 37° C., resulting in a loss of biological activity and possible changes in immunogenicity. Rational strategies can be devised for protein stabilization depending on the mechanism involved. For example, if the aggregation mechanism is discovered to be intermolecular S—S bond formation through disulfide interchange, stabilization may be achieved by modifying sulfhydryl residues, lyophilizing from acidic solutions, controlling moisture content, using appropriate additives, and developing specific polymer matrix compositions.

[0175] Sustained-release compositions also include liposomally entrapped antibodies of the invention. Liposomes containing such antibodies are prepared by methods known per se: U.S. Pat. No. DE 3,218,121; Epstein et al., *Proc. Natl. Acad. Sci. USA*, 82:3688-3692 (1985); Hwang et al., *Proc. Natl. Acad. Sci. USA*, 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; 142,641; Japanese patent application 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. The dosage of the antibody will be determined by the attending physician taking into consideration various factors known to modify the action of drugs including severity and type of disease, body weight, sex, diet, time and route of administration, other medications and other relevant clinical factors. Therapeutically effective dosages may be determined by either in vitro or in vivo methods.

[0176] The dosage of the antibody formulation for a given patient will be determined by the attending physician taking into consideration various factors known to modify the action of drugs including severity and type of disease, body weight, sex, diet, time and route of administration, other medications and other relevant clinical factors. Therapeutically effective dosages may be determined by either in vitro or in vivo methods.

[0177] An effective amount of the antibody of the invention to be employed therapeutically will depend, for example, upon the therapeutic objectives, the route of administration, and the condition of the patient. Accordingly, it will be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. A typical daily dosage might range from about 0.001 mg/kg to up to 100 mg/kg or more, depending on the factors mentioned above. Desirable dosage concentrations include 0.001 mg/kg, 0.005 mg/kg, 0.01 mg/kg, 0.05 mg/kg, 0.1 mg/kg, 0.5 mg/kg, 1 mg/kg, 5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 35 mg/kg, 40 mg/kg, 45 mg/kg, 50 mg/kg, 55 mg/kg, 60 mg/kg, 65 mg/kg, 70 mg/kg, 75 mg/kg, 80 mg/kg, 85 mg/kg, 90 mg/kg, 95 mg/kg, and 100 mg/kg or more. Typically, the clinician will administer the therapeutic antibody until a dosage is reached that achieves the desired effect. The progress of this therapy is easily monitored by conventional assays or as described herein.

EXAMPLES

[0178] The following examples, including the experiments conducted and results achieved are provided for illustrative purposes only and are not to be construed as limiting upon the embodiments of the invention described herein.

Example 1

MCP-1 Antigen Preparation

[0179] The human MCP-1 peptide used as the antigen in these studies had the following amino acid sequence:

QPDAINAPVTCCYNFTNRKISVQRLASYYRRITSSKCPKEAVIFKTIIVAKEICADP (SEQ ID NO: 149)
KQKRWVQDSMDHLDKQTQTPKT

[0180] This peptide was expressed recombinantly in *E. coli* and purchased from Prepro Tech (Rocky Hill, N.J.).

Example 2

Anti-MCP-1 Antibodies

[0181] Antibody Generation

[0182] Immunization and selection of animals for harvesting by ELISA. Monoclonal antibodies against MCP-1 were developed by sequentially immunizing Xenomouse® mice (Xenomouse® strains XMG2, XMG4 (3C-1 strain), and a hybrid strain produced through the crossing of XMG2 with an XMG4 (3C-1 strain) mouse, Abgenix, Inc. Fremont, Calif.) according to the schedule shown in Table 2. For instance, the initial immunization was with 10 µg antigen admixed 1:1 v/v with TiterMax Gold. Subsequent boosts were made with 5 or 10 µg antigen admixed 1:1 v/v with 100 µg alum gel in pyrogen-free D-PBS. Some boosts were done with 50% TiterMax Gold, followed by three injections with 10 µg antigen admixed 1:1 v/v with 10 µg MCP-1 antigen in alum gel, and then a final boost of 10 µg antigen in PBS. In particular, each mouse was immunized in the footpad by subcutaneous injection. The animals were immunized on days 0, 4, 7, 10, 14, 18, 27, 31, 35 and 42. The animals were bled on days 13 and 26 to obtain sera for harvest selection as described below.

TABLE 2

Group	Strain	# of mice	1 st injection	2 nd boost	3 rd boost	4 th boost	5 th Boost	6 th boost	7 th Boost	8 th boost	9 th boost	10 th boost	Fu-sion		
1	xmg2	7	10 µg/ mouse	5 µg/ mouse	5 µg/ mouse	5 µg/ mouse	5 µg/ mouse	5 µg/ mouse	10 µg/ mouse	10 µg/ mouse	10 µg/ mouse	10 µg/ mouse			
2	3C-1	7	10 µg/ mouse	5 µg/ mouse	5 µg/ mouse	5 µg/ mouse	5 µg/ mouse	5 µg/ mouse	10 µg/ mouse	10 µg/ mouse	10 µg/ mouse	10 µg/ mouse			
3	(3C-1) × xmg2	7	10 µg/ mouse	5 µg/ mouse	5 µg/ mouse	5 µg/ mouse	5 µg/ mouse	5 µg/ mouse	10 µg/ mouse	10 µg/ mouse	10 µg/ mouse	10 µg/ mouse			
			TiterMax	Alum Gel	Alum Gel	Alum Gel	Alum Gel	TiterMax	Alum Gel	Alum Gel	Alum Gel	D-PBS			
Day			0	4	7	10	13	14	18	26	27	31	35	42	46

[0183] Similarly, other XenoMouse® mice (XenoMouse® strains XMG2 and XMG2L3) were sequentially immunized according to the schedule shown in Table 3.

TABLE 3

Group	Strain	# of mice	1 st injection	2 nd boost	3 rd boost	4 th boost	Bleed	5 th boost	6 th boost	Fusion
4	xmg2	4	10 µg/ mouse	10 µg/ mouse	10 µg/ mouse	10 µg/ mouse	10 µg/ mouse	10 µg/ mouse	10 µg/ mouse	
5	xmg2L3	4	10 µg/ mouse TiterMax	10 µg/ mouse Alum	10 µg/ mouse Alum Gel					
Day		0		3	6	10	13	14	17	21

[0184] Anti-MCP-1 antibody titers were determined by indirect ELISA. The titer value is the reciprocal of the greatest dilution of sera with an OD reading two-fold that of background. Briefly, MCP-1 (84 mer; 1 µg/mL) was coated onto Costar Labcoat Universal Binding Polystyrene 96 well plates overnight at four degrees. The solution containing unbound MCP-1 was removed and the plates were treated with UV light (365 nm) for 4 minutes (4000 microjoules). The plates were washed five times with dH₂O. XenoMouse® sera from the MCP-1 immunized animals, or naive XenoMouse® animals, were titrated in 2% milk/PBS at 1:2 dilutions in duplicate from a 1:100 initial dilution. The last well was left. The plates were washed five times with dH₂O.

[0185] A goat anti-human IgG Fc-specific HRP-conjugated antibody was added at concentration of 1 µg/mL for 1 hour at room temperature. The plates were washed five times with dH₂O. The plates were developed with the addition of TMB for 30 minutes and the ELISA was stopped by the addition of 1 M phosphoric acid. The specific titer of individual XenoMouse® animals was determined from the optical density at 450 nm and is shown in Tables 4, 5, 6, 7, and 8. The titer represents the reciprocal dilution of the serum and therefore the higher the number the greater the humoral immune response to MCP-1. Lymph nodes from all immunized XenoMouse® animals were harvested for fusion.

TABLE 4

Group 1, footpad, xmg2, 7 mice			
Mouse ID	bleed of Day 13	bleed of Day 26	fusion of Day 46
	After 4 injections	After 6 injections	After 10 injections
	Reactivity to MCP-1		
	Titers via hIgG		
N160-1	1,000	73,000	300,000
N160-2	6,500	600,000	600,000
N160-3	2,300	250,000	125,000
N160-4	1,400	125,000	75,000
N160-5	4,000	200,000	225,000
N160-6	250	2,400	18,000
N160-7	60	1,600	35,000
NC	175	<100	200

[0186]

TABLE 5

Group 2, footpad, 3c-1, 7 mice		
Mouse ID	bleed of Day 13	fusion of Day 46
	After 6 injections	After 10 injections
	Reactivity to MCP-1	
	Titers via hIgG	
M724-1	35,000	24,000
M724-3	8,000	7,500
M724-5	8,000	20,000
N600-4	9,000	7,500
N600-5	1,800	75,000
N600-6	2,200	20,000
N600-7	800	25,000
NC	<100	<100

[0187]

TABLE 6

Group 3, footpad, 3c-1/xmg2 (F1), 7 mice			
Mouse ID	bleed of Day 13	bleed of Day 26	fusion of Day 46
	After 4 injections	After 6 injections	After 10 injections
	Reactivity to MCP-1		
	Titers via hIgG		
M219-1	50	2,200	8,000
M219-2	<100	9,000	18,000
M246-3	800	7,000	18,000
M246-5	850	18,000	65,000
M246-9	<100	18,000	55,000
M344-6	<100	800	12,000
M344-10	<100	6,000	25,000
NC	200	225	175

[0188]

TABLE 7

Group 4, XMG2, footpad, 4 mice			
Capture:			
Mouse ID	bleed of Day 13	bleed of Day 21	
	after 4 injections	after 6 injections	
	Human MCP-1	Human MCP-1	Human MCP-1
	Reactivity to MCP-1	Reactivity to MCP-1	Reactivity to MCP-1
	Titers via hIgG	Titers via hL	Titers via hIgG
N493-1	<100	<100	2,500
N493-2	<100	<100	1,000

TABLE 7-continued

Group 4, XMG2, footpad, 4 mice				
Capture:				
Mouse ID	bleed of Day 13 after 4 injections		bleed of Day 21 after 6 injections	
	Human MCP-1 Reactivity to MCP-1 Titers via hIgG	Human MCP-1 Reactivity to MCP-1 Titers via hL	Human MCP-1 Reactivity to MCP-1 Titers via hIgG	Human MCP-1 Reactivity to MCP-1 Titers via hL
N493-3	300	<100	4,500	<100
N493-4	800	<100	10,000	<100
NC	900	100	600	<100
*PC	8,000		3,000	

[0189]

TABLE 8

Group 5, XMG2L3, footpad, 4 mice				
Capture:				
Mouse ID	bleed after 4 injections		bleed of after 6 injections	
	Human MCP-1 Reactivity to MCP-1 Titers via hIgG	Human MCP-1 Reactivity to MCP-1 Titers via hL	Human MCP-1 Reactivity to MCP-1 Titers via hIgG	Human MCP-1 Reactivity to MCP-1 Titers via hL
N259-12	300	300	2,000	700
N259-14	100	400	2,500	650
N269-2	700	200	2,800	500
N263-3	900	900	24,000	8,000
NC	900	100	600	<100
*PC	8,000		3,000	

*For Tables 4-8, NC (negative control) = XMG2 KLH group 1, footpad L627-6 PC (positive control) = XMG2 MCP-1 group 1, footpad N160-1

[0190] Recovery of lymphocytes, B-cell isolations, fusions and generation of hybridomas. Immunized mice were sacrificed by cervical dislocation, and the lymph nodes harvested and pooled from each cohort. The lymphoid cells were dissociated by grinding in DMEM to release the cells from the tissues and the cells were suspended in DMEM. The cells were counted, and 0.9 mL DMEM per 100 million lymphocytes added to the cell pellet to resuspend the cells gently but completely. Using 100 μ L of CD90⁺ magnetic beads per 100 million cells, the cells were labeled by incubating the cells with the magnetic beads at 4° C. for 15 minutes. The magnetically labeled cell suspension containing up to 10⁸ positive cells (or up to 2 \times 10⁹ total cells) was loaded onto a LS⁺ column and the column washed with DMEM. The total effluent was collected as the CD90-negative fraction (most of these cells are B cells).

[0191] P3 myeloma cells and B cell-enriched lymph node cells were combined in a ratio of 1:1 (myeloma: lymph nodes) into a 50 mL conical tube in DMEM. The combined cells were centrifuged at 800 \times g (2000 rpm) for 5-7 minutes and the supernatant immediately removed from the resulting pellet. Two to four mL of Pronase solution (CalBiochem, Cat. #53702; 0.5 mg/mL in PBS) was added to the cells to resuspend the cell pellet gently. The enzyme treatment was

allowed to proceed for no more than two minutes and the reaction stopped by the addition of 3-5 mL of FBS. Enough ECF solution was added to bring the total volume to 40 mL and the mixture was centrifuged at 800 \times g (2000 rpm) for 5-7 minutes. The supernatant was removed and the cell pellet gently resuspended with a small volume of ECF solution, followed by enough ECF solution to make a total volume of 40 mL. The cells were mixed well and counted, then centrifuged at 800 \times g (2000 rpm) for 5-7 minutes. The supernatant was removed and the cells resuspended in a small volume of ECF solution. Enough additional ECF solution was added to adjust the concentration to 2 \times 10⁶ cells/mL.

[0192] The cells were then placed in an Electro-Cell-Fusion (ECF) generator (Model ECM2001, Genetric, Inc., San Diego, Calif.) and fused according to the manufacturer's instructions. After ECF, the cell suspensions were carefully removed from the fusion chamber under sterile conditions and transferred into a sterile tube containing the same volume of Hybridoma Medium in DMEM. The cells were incubated for 15-30 minutes at 37° C., then centrifuged at 400 \times g (1000 rpm) for five minutes. The cells were gently resuspended in a small volume of 1/2 HA medium (1 bottle of 50 \times HA from Sigma, Cat. #A9666 and 1 liter of Hybridoma Medium) and the volume adjusted appropriately with more 1/2 HA medium (based on 5 \times 10⁶ B cells per 96-well plate and 200 μ L per well). The cells were mixed well and pipetted into 96-well plates and allowed to grow. On day 7 or 10, one-half the medium was removed, and the cells re-fed with 1/2 HA medium.

[0193] Selection of candidate antibodies for ELISA. After 14 days of culture, hybridoma supernatants were screened for MCP-1-specific monoclonal antibodies. The ELISA plates (Fisher, Cat. No. 12-565-136) were coated with 50 μ L/well of MCP-1 (2 μ g/mL) in Coating Buffer (0.1 M Carbonate Buffer, pH 9.6, NaHCO₃ 8.4 g/L), then incubated at 4° C. overnight. After incubation, the plates were washed with Washing Buffer (0.05% Tween 20 in PBS) three times. 200 μ L/well Blocking Buffer (0.5% BSA, 0.1% Tween 20, 0.01% Thimerosal in 1 \times PBS) were added and the plates incubated at room temperature for 1 hour. After incubation, the plates were washed with Washing Buffer three times. 50 μ L/well of hybridoma supernatants, and positive and negative controls were added and the plates incubated at room temperature for 2 hours.

[0194] The positive control used throughout was XMG2 MCP-1 Group 1, footpad N160-7 and the negative control was XMG2 KLH Group 1, footpad L627-6. After incubation, the plates were washed three times with Washing Buffer. 100 μ L/well of detection antibody goat anti-huIgGfc-HRP (Caltag, Cat. #H10507), (and goat anti-hlgkappa-HRP (Southern Biotechnology, Cat. # 2060-05) and goat anti-hlglambda (Southern Biotechnology, Cat. # 2070-05) in secondary screening) were added and the plates incubated at room temperature for 1 hour. In the secondary screen, three sets of samples (positives in first screening) were screened, one set for hIgG detection, one set for hKappa detection, and one set for hlambda detection. After incubation, the plates were washed three times with Washing Buffer. 100 μ L/well of TMB (BioFX Lab. Cat. #TMSK-0100-01) were added and the plates allowed to develop for about 10 minutes (until negative control wells barely started to show color), then 50 μ L/well stop solution (TMB Stop Solution (BioFX Lab. Cat.

#STPR-0100-01) were added and the plates read on an ELISA plate reader at wavelength 450 nm. The OD readings from the positive wells are presented in Table 9.

TABLE 9

mAb Clone	ELISA OD-MCP-1	IC50 Ca++ Flux ($\mu\text{g}/\text{mL}$)	IC50 Chemotaxis ($\mu\text{g}/\text{mL}$)	Affinity (pMol)	Cross-Reactivity
1.1.1	3.638	0.24 + 0.034	0.27 + 0.034	2.7	
1.2.1	3.466	0.18 + 0.008	0.24 + 0.034	77	
1.3.1	4	0.12 + 0.012	0.24 + 0.059	55	
1.4.1	4	0.11 + 0.005	0.51 + 0.035	96	
1.5.1	0.51	0.21 + 0.027	0.34 + 0.054	4.2	
1.6.1	3.918	1 + 0.24	12 + 5.8	228	
1.7.1	3.521	0.11 + 0.013	0.35 + 0.064	4.9	
1.8.1	3.472	0.26 + 0.076	0.88 + 0.21	4	
1.9.1	3.6561	1.2 + 0.38	35 + 54	96	
1.10.1	3.845	0.18 + 0.11	1.2 + 0.55	9.6	
1.11.1	3.905	0.098 + 0.008	0.81 + 0.24	4.2	
1.12.1	4	0.13 + 0.02	0.35 + 0.039	13	
1.13.1	4	0.11 + 0.015	0.5 + 0.091	71	
1.14.1	2.064	0.41 + 0.1	0.58 + 0.18	6	
1.18.1	0.9984	0.18 + 0.055	0.29 + 0.07	3.8	
2.3.1	3.876	0.14 + 0.021	0.58 + 0.085	96	
2.4.1	3.892	0.26 + 0.18	>5	14	mouse JE
3.2	3.96			ND	MCP-2, MCP-3, eotaxin
3.4.1	3.86	0.24 + 0.019	0.51 + 0.1	45	
3.5.1	3.765	0.58 + 0.29	3.1 + 1.1	100	
3.6.1	3.593	0.17 + 0.04	0.52 + 0.18	15	
3.7.1	4	0.094 + 0.023	0.98 + 0.019	4.8	
3.8.1	3.603	0.27 + 0.028	0.7 + 0.19	3.4	
3.10.1	3.634	0.3 + 0.1	0.25 + 0.1	90	MCP-2, MCP-3, eotaxin
3.11.1	4	0.092 + 0.023	0.33 + 0.47	3.3	MCP-2, MCP-3, MCP-4 eotaxin
3.14.1	4	1.3 + 0.3	1.4 + 0.47	ND	
3.15.1	4	0.12 + 0.034	0.89 + 0.1	3.4	
3.16.1	3.921	0.16 + 0.08	0.4 + 0.081	25	
4.5.1	3.38	0.27 + 0.074	0.75 + 0.18	61	
4.6.1	3.51	0.31 + 0.06	0.4 + 0.056	330	
4.7.1	3.843	0.39 + 0.063	0.45 + 0.11	280	
4.8.1	4	0.22 + 0.77	0.29 + 0.032	102	
4.9.1	3.415	0.083 + .0094	0.21 + 0.035	ND	
5.1	4	3.5 + 2.1	1.3 + 1.2	1610	
5.2.1	3.714	2.5 + 0.66	2.1 + 1.7	319	Rantes
5.3.1	4	1.8 + 0.56	2.6 + 0.31	450	

ND = not done

[0195] Characterization of Anti-MCP-1 Antibodies for Biologic Activity.

[0196] Neutralization of MCP-1 bioactivity with anti-MCP-1 antibodies—FLIPR assay. DMSO and Pluronic Acid (20% DMSO solution) were added to a vial of Fluo-4 (Molecular Probes) to yield a final concentration of 5 mM Fluo4. THP-1 cells were resuspended in prewarmed (37° C.) loading buffer at $3 \times 10^6/\text{mL}$ and 1 μL of Fluo-4 dye per ml of cells was added to give a final concentration of dye at 5 μM . The cells were incubated in the dark at 37° C. for 45-50 minutes. After incubation, the cells were centrifuged at 1000 RPM for 5-10 min. The cells were resuspended in loading buffer and the centrifugation was repeated. The cells were resuspended at $1.667 \times 10^6/\text{mL}$. At a concentration of 200,000 cells/well, the cells were added to a 96-well plate and centrifuged gently. After taking a baseline reading, a second reading was taken upon subsequent addition of 3.5 nM MCP-1 in the presence or absence of varying concentrations

of anti-MCP-1 antibodies. Addition of MCP-1 to the THP-1 cells resulted in a rise of intracellular calcium leading to enhancement of fluorescence intensity of Fluo-4 dye. Upon addition of increasing concentrations of neutralizing antibody, the fluorescent dye intensity within the cells was decreased, thus indicating that the antibody tested was neutralizing. The concentration of antibody that yielded a 50% decrease in MCP-1 induced fluorescence intensity is presented in Table 9.

[0197] Neutralization of MCP-1-induced cell migration. An automated 96-well chemotaxis assay was developed using THP-1 cells and a Beckman Biomek F/X robotic system. Using a specially designed 96-well plate, a framed filter with the filter membrane bonded to a rigid frame, the chemotaxis assay was run in a NeuroProbe 96-well disposable microplate with a well volume of either 30 μL or 300 μL and pore diameter ranging from 2-14 μm . The Neuroprobe 96-well plate provides bottom wells for placing the MCP-1 chemoattractant and other reagents such as anti-MCP-1 antibodies in cell-migration assays. No top wells were required because the framed filter was coated with a hydrophobic mask that confines each cell-suspension sample to its site on top of the filter.

[0198] The optimum conditions for this assay were: 100,000 cells/well with 90 min incubation at 37° C. Suspensions of THP-1 cells that had been pre-loaded with dye from Molecular Probes were pipetted directly onto the sites on the upper side of the filter and incubated at 37° C. for 1-2 hours. After incubation, the cells that had migrated to the bottom of the filter and into the microplate were counted by placing the microplate into an FMAT purchased from Applied Biosystems.

[0199] MCP-1 induced cell migration for THP-1 cells and the maximal cell migration was reached at 1 nM with a signal to noise ratio of 10-15 fold. Using either hybridoma supernatants or fresh hybridoma media, MCP-1-dependent migration was detected. The variability of the assay was minimal (C.V.~15). The number of cells migrating to the bottom of the filters was decreased in a dose dependent manner when antibodies to MCP-1 were included with the chemoattractant.

[0200] Determination of anti-MCP-1 antibody affinity using Biacore analysis. The antibody/MCP-1 interaction analysis was performed at 25° C. using two CM5 chips docked in Biacore 3000 optical biosensors. Individual flow cells on each chip were activated with a 7-minute injection of NHS/EDC, carbonyldiimidazole was coupled through the NHS ester using a 7-minute injection, and the residual activated groups were blocked with a 7-minute injection of ethanolamine. The monosaccharide residues of each antibody were oxidized using 1 mM sodium metaperiodate in 100 mM sodium acetate, pH 5.5 at 4° C. for 30 minutes. The oxidized antibody was desalted into 100 mM sodium acetate, pH 5.0, to couple the antibody to the carbonyldiimidazole-modified surface. The mAb surfaces were stabilized by reducing the hydrazone bond with 0.1 M sodium cyanoborohydride. The antigen/antibody interaction was tested by injecting 0, 0.049, 0.15, 0.4, 1.3, 4 and 12 nM of MCP-1 (Peprotech, N.J.) in running buffer (10 mM HEPES, 150 mM NaCl, 0.005% surfactant, 200 $\mu\text{g}/\text{mL}$ BSA, pH 7.4). The surfaces were regenerated with a 12-second pulse of 15 mM H_3PO_4 . The antigen/antibody interaction was tested by injecting

duplicate antigen samples diluted in running buffer (10 mM HEPES, 50 mM NaCl, 0.005% surfactant, 200 µg/mL BSA, pH 7.4), in a 300-fold concentration range. The surfaces were regenerated with a 12-second pulse of 15 mM H₃PO₄. To determine the kinetics of each interaction, the data sets were fit globally to a 1:1 interaction model that included a parameter for mass transport. The calculated affinities of interaction are reported in Table 9.

[0201] Determining cross-reactivity of anti-MCP-1 antibodies with other chemokines. ELISA plates (Fisher Cat. No. 12-565-136) were coated with 50 µl/well of MCP-1, MCP-2, MCP-3, MCP-4, RANTES, GRO-alpha, MIP-1 alpha, eotaxin, rat MCP-1 and mouse JE (2 µg/ml) in coating buffer (0.1 M carbonate buffer, pH 9.6, NaHCO₃ 8.4 g/L, then incubated at 4° C. overnight. After incubation, the plates were washed with washing buffer (0.05% Tween 20 in PBS) three times. 200 µL/well blocking buffer (0.5% BSA, 0.1% Tween 20, 0.01% Thimerosal in 1xPBS) were added and the plates incubated at room temperature for 1 hour. After incubation, the plates were washed with washing buffer three times. 50 µL/well of hybridoma supernatants, and positive and negative controls (positive control was anti-MCP-1 antibody purchased from R&D Sciences, and negative control was an antibody to Keyhole Limpet Hemocyanin produced at Abgenix) were added and the plates incubated at room temperature for 2 hours. After incubation, the plates were washed three times with washing buffer. 100 µL/well of detection antibody goat anti-huIgGf-HRP (Caltag, Cat. #H10507), (goat anti-hlgkappa-HRP (Southern Biotechnology, Cat. #2060-05) and goat anti-hlglambda (Southern Biotechnology, Cat. #2070-05) in secondary screening) were added and the plates incubated at room temperature for 1 hour. After incubation, the plates were washed three times with washing buffer and 100 µL/well of TMB (BioFX Lab. Cat. #TMSK-0 100-01) was added and the plates allowed to develop for about 10 minutes. At this time, 50 µL/well stop solution (TMB Stop Solution (BioFX Lab. Cat. #STPR-0100-01) were added and the plates read on an ELISA plate reader at wavelength 450 nm. The results presented in Table 10 demonstrate that several of the anti-MCP-1 antibodies cross-reacted with related chemokines.

TABLE 10

mAb	rmJE/MCP-1 2 µg/mL	rat MCP-1 1 µg/mL	rhMCP-2 2 µg/mL	rhMCP-3 2 µg/mL	rhMCP-4 2 µg/mL
1.1.1	0.045	0.051	0.051	0.064	0.052
1.2.1	0.041	0.044	0.056	0.048	0.055
1.3.1	0.046	0.048	0.065	0.052	0.048
1.4.1	0.042	0.05	0.046	0.049	0.045
1.5.1	0.043	0.045	0.047	0.069	0.05
1.6.1	0.042	0.062	0.042	0.046	0.044
1.7.1	0.041	0.042	0.044	0.053	0.041
1.8.1	0.045	0.049	0.048	0.054	0.046
1.9.1	0.053	0.065	0.04	0.044	0.042
1.10.1	0.041	0.059	0.04	0.047	0.052
1.11.1	0.041	0.052	0.041	0.043	0.043
1.12.1	0.042	0.062	0.042	0.046	0.044
1.13.1	0.043	0.06	0.046	0.047	0.045
1.14.1	0.042	0.062	0.042	0.046	0.044
1.18.1	0.044	0.058	0.04	0.045	0.045
2.3.1	0.054	0.058	0.052	0.059	0.064
2.4.1	0.129	0.077	0.045	0.066	0.06
3.4.1	0.044	0.053	0.042	0.05	0.047
3.5.1	0.042	0.053	0.042	0.045	0.044

TABLE 10-continued

3.6.1	0.047	0.046	0.052	0.045	0.048
3.7.1	0.046	0.048	0.043	0.048	0.048
3.8	0.042	0.062	0.042	0.046	0.044
3.10.1	0.054	0.045	0.845	0.167	0.042
3.11.1	0.063	0.057	0.336	1.317	0.981
3.14.1	0.044	0.046	0.045	0.05	0.045
3.15.1	0.041	0.05	0.043	0.046	0.051
3.16.1	0.042	0.046	0.049	0.043	0.043
4.5.1	0.049	0.055	0.042	0.046	0.046
4.6.1	0.049	0.05	0.047	0.05	0.047
4.7.1	0.042	0.062	0.042	0.046	0.044
4.8.1	0.042	0.091	0.041	0.043	0.039
4.9.1	0.05	0.05	0.046	0.049	0.05
5.1	0.044	0.054	0.051	0.05	0.043
5.2.1	0.04	0.054	0.041	0.048	0.041
5.3.1	0.05	0.047	0.043	0.045	0.043
3.2	0.059	0.07	0.535	0.449	0.041
(neat)					
nc	0.042	0.134	0.045	0.084	0.074
pc	0.263	ND	ND	1.084	0.215

mAb	hGRO/ MGSA 1 µg/mL	hMIP-1-alpha 1 µg/mL	hRANTES 1 µg/mL	hEotaxin 1 µg/mL	Positive control hMCP- 1(MCAF) 2 µg/mL
1.1.1	0.047	0.044	0.044	0.042	0.944
1.2.1	0.044	0.04	0.04	0.044	1.159
1.3.1	0.051	0.049	0.049	0.046	1.158
1.4.1	0.044	0.041	0.046	0.043	0.738
1.5.1	0.048	0.041	0.049	0.043	1.178
1.6.1	0.046	0.046	0.046	0.042	0.375
1.7.1	0.041	0.04	0.039	0.04	1.17
1.8.1	0.06	0.045	0.045	0.047	1.159
1.9.1	0.043	0.044	0.042	0.042	0.446
1.10.1	0.043	0.043	0.042	0.05	1.259
1.11.1	0.042	0.042	0.042	0.049	1.336
1.12.1	0.046	0.046	0.046	0.044	0.933
1.13.1	0.046	0.042	0.046	0.044	1.16
1.14.1	0.046	0.046	0.046	0.042	1.129
1.18.1	0.049	0.043	0.04	0.043	1.228
2.3.1	0.062	0.067	0.055	0.045	0.087
2.4.1	0.048	0.061	0.046	0.084	0.462
3.4.1	0.065	0.055	0.046	0.048	1.153
3.5.1	0.048	0.047	0.044	0.043	0.194
3.6.1	0.047	0.047	0.043	0.043	0.342
3.7.1	0.045	0.049	0.067	0.043	1.276
3.8	0.046	0.046	0.046	0.042	0.275
3.10.1	0.042	0.043	0.04	0.306	0.71
3.11.1	0.054	0.053	0.064	0.339	0.803
3.14.1	0.046	0.046	0.045	0.043	0.549
3.15.1	0.044	0.045	0.049	0.045	0.948
3.16.1	0.043	0.043	0.042	0.043	0.633
4.5.1	0.045	0.046	0.049	0.041	0.957
4.6.1	0.046	0.055	0.053	0.049	0.686
4.7.1	0.046	0.046	0.046	0.042	0.744
4.8.1	0.042	0.041	0.044	0.043	1.136
4.9.1	0.043	0.049	0.057	0.045	0.822
5.1	0.044	0.043	0.043	0.042	0.521
5.2.1	0.045	0.043	0.262	0.043	0.663
5.3.1	0.045	0.042	0.045	0.042	0.272
3.2	0.042	0.041	0.043	0.194	0.235
(neat)					
nc	0.357	0.065	0.072	0.063	0.042
pc	1.075	0.794	1.219	0.221	0.281

Coat: Ag @ 2 µg/mL or 1 µg/mL; O/N

Ab: MCP-1 purified clones 1:50

pc: 1 µg/mL;

nc: D39.2 IL8 @1 µg/mL

Detect samples with gxhG-Fc HRP 1:2K; controls with mix xmIgG1, 2a, 2b, 3 1:1K

[0202] To determine whether anti-MCP-1 antibody 3.11.2 could block the function of other MCP family members,

migration assays as described above were performed. First, the ability of THP-1 monocytes to migrate in response to MCP-1, MCP-2, MCP-3, and MCP-4 was determined. MCP-1, -2 and -3 effectively induced migration of THP-1 cells, but MCP-4 was not active in this assay (see FIG. 1). When antibody 3.11.2 was added to the bottom side of the well at varying concentrations, the ability of the THP-1 cells to migrate in response to MCP-2 and MCP-3 was inhibited in a dose dependent manner (FIGS. 2 and 3).

Example 3

Epitope Mapping of MCP-1

[0203] Monocyte chemo-attractant protein-1 (MCP-1) is a member of the beta chemokine family that acts through a specific seven-transmembrane receptor to recruit monocytes, basophils, and T lymphocytes to the site of inflammation. The antigen, a 76-amino-acid residue is nonglycosylated and has a predicted molecular mass of 8.7 kD. Human MCP-1, expressed in *E. coli*, was purchased from R&D #279MC/CF. Monkey MCP was expressed in 293F cells, and three monkey MCP-1 variants were used to analyze how defined amino-acid replacements affect binding affinity for each individual mAb.

[0204] Sequence analysis showed that the antibodies fell into five classes. The largest class included 28 antibodies highly related by their use of VH1-24, of which, 24 also use Vk gene B3. A class comprised of three antibodies use the VH6-1 gene, two of which use Vk B3. Three other classes are represented by one antibody each, using VH1-2, VH3-33 and VH4-3 1, of which two of these mAbs use the Vk08 gene. It should be noted that antibody names beginning with 1, 2, 3, or 4 represent different hybridoma fusions from independent cohorts of XenoMouse® mice. Therefore, these monoclonal antibodies arose from independent lineages of B cells maturing during independent primary and secondary immune responses in XenoMouse® mice. Because of their independence, the similarity in nucleotide and amino acid sequence of the antibody VH and Vk genes likely represents a convergent evolution and selection for a similar variable region structure that can bind to and potentially neutralize MCP-1 (see Table 11).

TABLE 11

Samples	Iso-type	VH	DH	JH	VK	JK	Epitope
1.1.1	γ2/k	VH1-24	D3-3(17)	JH4b	VK-B3	JK1	Conf.
1.2.1	γ2/k	VH1-24	D3-3(17)	JH4b	VK-L5	JK1	Linear
1.3.1	γ2/k	VH1-24	D3-3(15)	JH4b	VK-B3	JK1	Conf.
1.4.1	γ2/k	VH6-1	D1-26	JH4b	VK-A2	JH4	linear
1.5.1	γ2/k	VH1-24	D3-3(17)	JH4b	VK-B3	JK1	Linear
1.6.1	γ2/k	VH1-24	D1-26(18)	JH3b	VK-A10	JK4	Conf.
1.7.1	γ2/k	VH1-24	D3-3(17)	JH4b	VK-B3	JK1	Conf.
1.8.1	γ2/k	VH1-24	D3-3(17)	JH4b	VK-B3	JK1	Linear
1.9.1	γ2/k	VH1-24	D5-12(13)	JH4b	VK-B3	JK1	no binding
1.10.1	γ2/k	VH1-24	D3-3(17)	JH4b	VK-B3	JK1	Linear
1.11.1	γ2/k	VH1-24	D3-3	JH4B	VK-B3	JK1	Linear
1.12.1	γ2/k	VH1-24	D3-3(16)	JH4b	VK-B3	JK1	Conf.
1.13.1	γ2/k	VH1-24	D3-3(17)	JH4b	VK-B3	JK1	Linear
1.14.1	γ2/k	VH6-1	D1-26	JH6b	VK-B3	JK1	Linear
1.18.1	γ2/k	VH1-24	D3-3(15)	JH4b	VK-B3	JK4	Linear
2.3.1	γ4/k	VH1-24	D3-3(16)	JH4b	VK-B3	JK2	no binding
3.2	γ2/k	VH1-24	D3-3(17)	JH4b	VK-L16	JK4	Conf.
2.4.1	γ4/k	VH1-2	D6-13(15)	JH4b	VK-08	JK5	no binding

TABLE 11-continued

Samples	Iso-type	VH	DH	JH	VK	JK	Epitope
3.4.1	γ2/k	VH1-24	D3-3(16)	JH4b	VK-B3	JK1	Linear
3.5.1	γ4/k	VH1-24	D3-3(17)	JH4b	VK-B3	JK1	no binding
3.6.1	γ4/k	VH1-24	D3-3(17)	JH4b	VK-B3	JK1	no binding
3.7.1	γ2/k	VH1-24	D3-3(16)	JH4b	VK-B3	JK1	Conf.
3.8	γ4/k	VH1-24	D3-3	JH4B	VK-B3	JK1	no binding
3.10.1	γ4/k	VH1-24	D3-9(12)	JH6b	VK-A30	JK3	Conf.
3.11.1	γ4/k	VH4-31	D2-21(10)	JH3b	VK-08	JK2	Conf.
3.14.1	γ4/k	VH6-1	D1-26	JH6B	VK-B3	JK1	Conf.
3.15.1	γ4/k	VH1-24	D5-12(13)	JH4b	VK-B3	JK1	Linear
3.16.1	γ4/k	VH1-24	D3-3(17)	JH4b	VK-B3	JK1	Conf.
4.5.1	γ2/k	VH1-24	D3-3(16)	JH4b	VK-B3	JK1	Conf.
4.6.1	γ2/k	VH1-24	D3-3	JH3B	VK-B3	JK1	ND
4.7.1	γ2/k	VH1-24	D3-3(16)	JH4b	VK-B3	JK1	Conf.
4.8.1	γ2/k	VH1-24	D3-3	JH4b	VK-B3	JK1	Conf.
4.9.1	γ2/k	ND	ND	ND	ND	ND	Conf.
5.1	γ2/λ	VH3-33	D6-6(15)	JH6B	V1-22	JK2	ND
5.3.1	γ2/k	VH1-24	D5-12(13)	JH4b	VK-B3	JK1	no binding

Conf. = conformational

ND = Not Done

No binding = No binding on western blot.

[0205] Whether each antibody bound to a linear or conformational epitope was determined by Western blot analysis. To determine whether disruption of the intramolecular bonds by a reducing agent changed the reactivity of selected anti-MCP-1 antibodies, purified MCP-1 was loaded on SDS/PAGE (4-20% gel) under non-reducing (NR) or reducing (R) conditions. SDS/PAGE was performed by the method of Laemmli, using a mini-gel system. Separated proteins were transferred onto nitrocellulose membrane. Membranes were blocked using PBS containing 5% (w/v) non-fat dried milk for at least 1 hour before developing, and probed for 1 hour with each antibody. Anti-MCP-1 antibodies were detected using HRP-conjugated goat anti-human immunoglobulins (1:8,000 dilution; Sigma Catalog No. A-8667). Membranes were developed by using enhanced Chemiluminescence (ECL®; Amersham Bioscience) according to the manufacturer's instructions.

[0206] Antibody-MCP-1 complexes were analyzed by three methods: (1) Surface Enhanced Laser Desorption Ionization (SELDI) (Protein chip technology) for linear and conformational epitopes; (2) Site Directed Mutagenesis for linear and conformational epitopes; and (3) SPOTs Peptide Array for linear epitopes. SELDI is a recently developed method for accurate, rapid and sensitive determination of the molecular weights of peptides and proteins. Linear and conformational epitopes were mapped based on the mass of the bound fragment to immobilized antibody by SELDI protein chip technology. Mapping of linear epitopes by SELDI was carried out in three steps. In the first step, MCP-1 was digested by highly specific proteolytic enzymes to generate sets of peptide fragments. In the second step, peptide fragments containing the linear epitopes were selected by their specific binding to the immobilized antibody on the protein chip. In this step, peptides that contain the epitope form complexes with the antibody, while other peptides that do not bind the antibody were removed by stringency wash. In the final step, the identity of the antibody-binding peptide was determined by its molecular weight by SELDI and the known digestion sites of the specific protease.

[0207] Antibodies 1.4.1, 1.8.1, 1.14.1, 1.18.1 reacted equally with native and denatured MCP-1 on the Western blot, indicating that these have a linear epitope. Their epitope was mapped by SELDI. The experiments were carried out by carboxymethylation of MCP-1 antigen to prevent the formation of disulfide bonds between cysteine residues in the protein. Methylated MCP-1 was digested with Glu-C, an endoproteinase that specifically cleaves peptide bonds on the carboxy-terminal side of glutamic acid (E) residues. mAbs were covalently coupled to the Protein chip array, PS20. The chip surface was blocked with 1M ethanolamine and washed with PBS, 0.5% Triton. Glu-C fragments of methylated MCP-1 antigen were bound to the immobilized antibody. Unbound fragments were washed off with detergent (PBS, 0.1% Tween). Bound Glu-C fragments (epitope) were analyzed and identified by SELDI based on their mass. Table 12 summarizes the expected mass of each peptide generated from complete digest of methylated MCP-1 with Glu-C. MCP-1 was completely digested into three fragments. The theoretical pI was: 9.39/Mw (average mass): 8685.03/Mw (monoisotopic mass): 8679.44. After the wash, the fragment with the mass 4635, corresponding to the residues 1-39, remained bound to the antibody, indicating that the epitope of all these antibodies lies in the first 39 residues as same pattern was seen with each of these antibodies.

bound to PS2 Protein chip arrays (Ciphergen Biosystems) was used to capture the mAbs, and subsequently incubated with MCP-1. After removal of unbound material, the complexes were digested with high concentration of specific proteases. MCP-1 antibodies (1.7.2, 3.11.2 and 3.7.2) do not bind to the reduced, denatured antigen on Western blots, indicating that the epitope is likely to be conformational. Antibodies 1.7.2 and 3.7.2 were first covalently coupled to the PS20 chip. Native MCP-1 was bound to the antibody and then digested with an endoproteinase (Lys-C in one experiment and Asp-N in the other). Unbound fragments were washed off with PBS+, 0.2% Triton followed with PBS and HPLC water wash. The epitope was determined by SELDI and identified by the mass of the fragment. Both these antibodies 1.7.2 and 3.7.2 had a fragment of mass 5712 corresponding to the residues 3-53 (Table 13; Theoretical pI: 9.39/Mw (average mass): 8685.03/Mw (monoisotopic mass): 8679.44) bound to it after the wash, indicating that

TABLE 12

Mass	Position in	Artif.	Peptide sequence
	SEQ ID		
	NO: 149	#MCmodification(s)	
4458.2591	1-39	0	Cys_CM: 11, 12, 36 QPDAINAPVTCCYNFTNRKI
		4632.2755	SVQRLASYRRITSSKCPKE
3041.4819	51-76	0	Cys_CM: 52 ICADPKQKWVQDSMDHLDKQ
		3099.4873	TQTPKT
1218.7456	40-50	0	AVIFKTIVAKE

[0208] The SELDI approach was also used to map conformational epitopes. In this case, the protein A covalently

the epitope lies in the 3 to 53 amino acid residues of the native MCP-1 antigen.

TABLE 13

Mass	Position in SEQ	#MCPeptide sequence
	ID NO: 149	
5720.0059	3-53	0 DAINAPVTCCYNFTNRKISV
		QRLASYRRITSSKCPKEAVI FKTIVAKEICA
1046.5476	68-76	0 DKQTQTPKT
1028.5523	54-61	0 DPKQKWVQ

[0209] For mapping the epitope of the antibody 3.11.2, the size of the binding domain was minimized by using a different protease. Protein A (Calbiochem, 539202) was immobilized covalently to a PS20 chip. Residual binding sites were blocked with ethanolamine, pH 8.0. Antibody 3.11.2 was bound to protein A. The chip was washed with PBS and then with 50 mM Hepes, pH 7.5. MCP-1 antigen was bound to the antibody. Unbound antigen was removed by washing with 0.1% Tween in PBS, followed by 50 mM Hepes, pH 7.5, and 100 mM ammonium bicarbonate. One chip digestion of MCP-1 was carried out with the endoprotease, Lys-C. The chip was washed with 0.1% Triton in PBS to remove the unbound fragments. The bound fragment was analyzed based on its mass on SELDI. Only one peak of mass 1861.8 was bound to the antibody, representing a 15-amino-acid sequence, located at residues 20 to 35 (Table 14; Theoretical pI: 9.39/Mw (average mass): 8685.03/Mw (monoisotopic mass): 8679.44) of MCP-1, with the mass of 1865 and the sequence ISVQRLASYRRITSSK (Position 20-35 of SEQ ID NO.: 149) was identified as the most tightly bound fragment.

TABLE 14

Mass	Position in SEQ		#MC	Peptide sequence
	ID NO:	149		
2155.0059	1-19	0	QPDAINAPVTCCYNFTNRK	
1865.0715	20-35	0	ISVQRLASYRRITSSK	
1373.6154	59-69	0	WVQDSMDHLDK	
775.3654	50-56	0	EICADPK	
706.4134	39-44	0	EAVIFK	
702.3781	70-75	0	QTQTPK	
531.3500	45-49	0	TIVAK	

[0210] Mutagenesis of MCP-1. It was previously shown that two clusters of primarily basic residues (R24, K35, K38, K49, and Y13) appear to make the largest contributions to the interaction between MCP-1 and its receptor (Hemmerich et al., (1999) *Biochemistry* 38, 13013-13025). Binding data revealed that the N-terminal residues contribute little to binding activity and that two important residues are important for signaling activity of the MCP-1: K35 and R24. K35

is the most functionally important residue, because K35A mutation has a significant effect on binding and activity, as well as alanine mutants of R24 (Hemmerich et al., (1999) *Biochemistry* 38, 13013-13025). Arg24 is conserved across different species of MCP-1 as well as in human MCP-2-4, but varies widely in other CC chemokines and therefore maybe involved in receptor specificity. To identify individual residues within the first 39 residues of MCP-1, representing the Glu-C digest, that were important for antibody binding, three MCP-1 mutants were generated: the three basic residues, R24, K35, and K38, were mutated by site-directed mutagenesis and mutant protein was further analyzed for binding to all 36 neutralizing antibodies by ELISA. Arg24 was mutated to alanine (R24A) and glutamic acid (R24E). Lys35 and K38 were mutated to alanine (K35A, K38A respectively). All mutations were introduced in Monkey MCP-1 background. The monkey MCP-1 construct was generated recovered by performing RT-PCR on RNA isolated from monkey peripheral blood lymphocytes (cynomologus MCP-1 PCR3.1 bidirectional). Protein sequence alignment between human and Monkey MCP-1 revealed 99% homology with two amino-acids changes at the C-terminal (positions 71 and 76). The C-terminal residues 59-76 are not involved in interaction with the receptor and did not affect the binding of all 36 antibodies.

[0211] ELISA assays were performed using supernatant from 293 cells transfected with different MCP-1 mutated constructs. ELISA plates were coated with anti-human MCP-1 goat IgG Polyclonal antibody (R&D catalog No. AF279NA) diluted to 1 μ g/mL in ELISA plate coating buffer. Expression of mutant MCP-1 constructs in 293 cells was confirmed by detection with biotinylated goat anti-human MCP-1 (R&D catalog No. BAF279) followed by streptavidin HRP. Binding of mutant MCP-1 to MCP-1 antibodies was detected with HRP conjugated goat anti-human IgG (Fc specific, Caltag Catalog No. H10507). ELISA results have shown that changing K38 did not have any effect of binding activity of all 36 antibodies. Binding of all antibodies to R24E and R24A MCP-1 mutant antigen was completely abolished (see Table 15). However, the K35A mutation inhibited the binding of only six antibodies (1.6.1, 1.9.1, 3.6.1, 3.10.1). All of these antibodies appear to have a conformational epitope, binding to which is affected by mutation of either Arg24 or Lys35. These data suggest that these four antibodies recognize a conformational epitope different, but overlapping with, the other antibodies.

TABLE 15

mAb	Epitope	Glu-C digest	Lys-C	Asp-N digest	Peptide	Residues	R24A/E	K35A
1.1.1	Conf.	ND	ND	ND	ND	ND	Inhibition	Inhibition
1.2.1	Linear	ND	ND	ND	7_11	21-25	Inhibition	No Inhibition
1.3.1	Conf.	ND	ND	ND	ND	ND	Inhibition	No Inhibition
1.4.1	Linear	1_39	ND	ND	7_11	21-25	Inhibition	No Inhibition
1.5.1	Linear	ND	ND	ND	7_11	21-25	Inhibition	No Inhibition
1.6.1	Conf.	ND	ND	ND	ND	ND	Inhibition	Inhibition
1.7.1	Conf.	ND	ND	3-53/5712	ND	ND	Inhibition	No Inhibition
1.8.1	Linear	1_39	ND	ND	7_11	21-25	Inhibition	No Inhibition
1.9.1	no binding	ND	ND	ND	ND	ND	Inhibition	Inhibition
1.10.1	Linear	ND	ND	ND	7_11	21-25	Inhibition	No Inhibition
1.11.1	Linear	ND	ND	ND	ND	ND	Inhibition	No Inhibition
1.12.1	Conf.	ND	ND	ND	ND	ND	Inhibition	No Inhibition
1.13.1	Linear	ND	ND	ND	7_11	21-25	Inhibition	No Inhibition

TABLE 15-continued

mAb	Epitope	Glu-C digest	Lys-C	Asp-N digest	Peptide	Residues	R24A/E	K35A
1.14.1	Linear	1_39	ND	ND	7_11	21-25	Inhibition	No Inhibition
1.18.1	Linear	1_39	ND	ND	7_11	21-25	Inhibition	No Inhibition
2.3.1	no binding	ND	ND	ND	ND	ND	Inhibition	No Inhibition
3.2	Conf.	ND	ND	ND	ND	ND	Inhibition	No Inhibition
2.4.1	no binding	ND	ND	ND	ND	ND	Inhibition	No Inhibition
3.4.1	Linear	ND	ND	ND	ND	ND	Inhibition	No Inhibition
3.5.1	no binding	ND	ND	ND	ND	ND	Inhibition	No Inhibition
3.6.1	no binding	ND	ND	ND	ND	ND	Inhibition	Inhibition
3.7.1	Conf.	ND	ND	3-53/5712	ND	ND	Inhibition	No Inhibition
3.8	no binding	ND	ND	ND	ND	ND	Inhibition	Inhibition
3.10.1	Conf.	ND	ND	ND	ND	ND	Inhibition	Inhibition
3.11.1	Conf.	ND	20-35(1864)	ND	ND	ND	Inhibition	No Inhibition
3.14.1	Conf.	ND	ND	ND	ND	ND	Inhibition	No Inhibition
3.15.1	Linear	ND	ND	ND	7_11	21-25	Inhibition	No Inhibition
3.16.1	Conf.	ND	ND	ND	ND	ND	Inhibition	No Inhibition
4.5.1	Conf.	ND	ND	ND	ND	ND	Inhibition	No Inhibition
4.6.1	ND	ND	ND	ND	ND	ND	Inhibition	No Inhibition
4.7.1	Conf.	ND	ND	ND	ND	ND	Inhibition	No Inhibition
4.8.1	Conf.	ND	ND	ND	ND	ND	Inhibition	No Inhibition
5.1	ND	ND	ND	ND	ND	ND	Inhibition	No Inhibition
5.3.1	no binding	ND	ND	ND	ND	ND	Inhibition	No Inhibition

ND = Not Done

No binding = No binding on Western blot.

[0212] For those antibodies binding to a linear epitope, their binding to a peptide epitope was studied in detail using the SPOTs technology. SPOTs is a technology that allows the solid-phase synthesis of hundreds of peptides in a format suitable for the systematic analysis of antibody epitopes. The system is simple, extremely rapid and economic in its use of reagents. A custom-made peptide array was obtained from Sigma-Genosys (The Woodlands, Tex.). A series of 32, 13-mer peptides were synthesized spanning residues 1-76 of the MCP-1 sequence. Each consecutive peptide was offset by two amino acids from the previous one, yielding a nested, overlapping library. The membrane carrying the 32 peptides was probed with eight MCP-1 antibodies (1 $\mu\text{g}/\text{mL}$), detected with HRP-conjugated secondary antibody and followed by enhanced chemiluminescence (ECL). Reaction was observed with five consecutive peptide spots (7 to 11) corresponding to amino acids 21 to 25 of MCP-1. From these results, it appears that the core of the epitope for all of the tested MCP-1 antibodies binding to a linear epitope is SVQRL (21-25). The MCP-1 sequence is:

[0213] QPDAINAPVTCCYNFTNRKISVQR-
LASYRRITSSKCPKEAVIFKTIKAIKADPK
QKWVQDSMDHLDKQTQTPKT (SEQ ID NO:
149)

[0214] Eight antibodies, which recognized a linear epitope, reacted with the same SPOTs: 1.2.1, 1.4.1, 1.5.1, 1.8.1, 1.10.1, 1.13.1, 1.14.1, and 1.18.1.

Example 4

Affinity Determination of Cross-Reacting
Antibodies by High-Resolution Biacore Analysis

[0215] The interaction analysis was performed at 25° C. using two CM5 chips docked in Biacore 2000 optical biosensors. Individual flow cells on each chip were activated with a 7-minute injection of NHS/EDC, carbonyldiimidazole was coupled through the NHS ester using a 7-minute injection,

and the residual activated groups were blocked with a 7-minute injection of ethanolamine. The monosaccharide residues of mAb 3.11.2, diluted 1/50, were oxidized using 1 mM sodium metaperiodate in 100 mM sodium acetate, pH 5.5 at 4° C. for 30 minutes. The oxidized antibody was desalted into 10 mM sodium acetate, pH 5.0, to couple the antibody to the carbonyldiimidazole-modified surface. A surface density of 250 RU mAb 3.11.2 was used to measure the reported interactions of MCP-1 and MCP-4, while a surface of 110 RU was used to measure the interactions of antigens MCP-2 and MCP-3 with mAb 3.11.2. The mAb surfaces were stabilized by reducing the hydrazone bond with 0.1 M sodium cyanoborohydride. The antigen/antibody interaction was tested by injecting duplicate antigen samples diluted in running buffer (10 mM HEPES, 150 mM NaCl, 0.005% surfactant, 200 $\mu\text{g}/\text{mL}$ BSA, pH 7.4), in a 300-fold concentration range. The surfaces were regenerated with a 12-second pulse of 15 mM H_3PO_4 .

[0216] To determine the kinetics of each interaction, the data sets were fit globally to a 1:1 interaction model that included a parameter for mass transport. The estimated rate constants and the calculated affinities of interaction for antibody 3.11.2 are reported in Table 16. The data for all the other antibodies are presented in Table 8.

TABLE 16

Ag	k_a ($\text{M}^{-1} \text{s}^{-1}$)	k_d (s^{-1})	K_D (pM)
MCP-1	3.0×10^8	1.0×10^{-3}	3.3
MCP-2	2.6×10^8	1.2×10^{-2}	46
MCP-3	1.5×10^8	7.4×10^{-3}	49
MCP-4	1.5×10^8	5.5×10^{-4}	3.7

Example 5

Prevention of Angiogenesis with Antibodies to MCP-1

[0217] Angiogenesis was induced in a mouse model by admixing Matrigel with human bFGF (10 ng/mL), human VEGF165 (100 ng/mL) and 10 g/mL heparin or MCP-1 (250 ng/mL) and MCP-3 (100 ng/mL). About 0.5 mL of the suspension was subcutaneously injected into the right flank of 6-8 week-old, athymic, female, nude mice. Five mice were used for each dose of MCP-1 and MCP-3. In addition, as a negative control, Matrigel alone (no growth factors) was included. The Matrigel implants solidified in situ and were left undisturbed for 7 days. At the end of 7 days, the mice were anesthetized, and the Matrigel plugs were removed carefully using microsurgical instruments. Gels were photographed under transillumination. One part of the plugs was processed for paraffin embedded sectioning. Sections were cut at two different levels and stained with H/E. Another part of the gel was snap frozen in liquid nitrogen and subjected to immunocytochemical staining with rat monoclonal antibody directed against mouse CD31 antigen conjugated with phycoerythrin. H+E stained slides were elevated for the formation of the distinct, endothelial lined vessels. Anti-CD31-PE stained slides were observed under Fluorescence microscope (red filter) attached to a Spot Camera. Images were captured digitally using Metamorph software program. Microvessel density was determined by the method published by Wild et al. (2000).

[0218] Both MCP-1 and MCP-3 were found to show equivalent angiogenesis as the well-characterized angiogenic factors VEGF and bFGF. In addition, angiogenesis induced by MCP-1 or MCP-3 in animals, and by inference in human tumors or diseased tissue, can be prevented by treating with antibodies to MCP-1 or an antibody such as 3.11.2, which neutralizes the activity of all MCP family members. Accordingly, one would inject the anti-MCP antibodies into animals at different doses ranging from approximately 0.1 to 0.5 mg per animal to obtain a dose-response relationship for treatment.

Example 6

MCP-1 Production by Tumor Cells

[0219] To determine whether tumor cells produced MCP-1 in cell culture, a panel of cell lines was examined for their ability to secrete MCP-1 into the culture medium. Cells were cultured in Dulbecco's Modified Eagles Medium (DMEM) containing 10% fetal bovine serum or an equivalent until confluent. The supernatant was removed and an aliquot tested for reactivity to MCP-1 using a commercially available ELISA kit from R & D Sciences. Table 17 shows a series of cancer cell lines that constitutively secrete MCP-1 and their respective MCP-1 levels as determined by ELISA.

TABLE 17-continued

	Cell Line	MCP-1 (pg/mL)	
6	Colon Carcinoma	SW707	31
7	Colon Carcinoma	SW948	13
8	Colon Carcinoma	KM-12	6
9	Colon Carcinoma	HCC-2998	39
10	Gastric Carcinoma	NCI-N87	37
11	Gastric Carcinoma	NCI-SNU-1 4	0
12	Gastric Carcinoma	NCI-SNU-5	<10
13	CNS Carcinoma	SF-268	94
14	CNS Carcinoma	SF-295	223
15	CNS Carcinoma	SF-593	>2500
16	CNS Carcinoma	SNB-19	>2500
17	CNS Carcinoma	SNB-75	>2500
18	CNS Carcinoma	U251	>2500
63	CNS	XF-498(Curg)	>2500
61	Glioblastoma	SF-295(Curg)	>2500
21	Medulloblastoma	TE 671 (u)	>2500
25	Leukemia	SR	25
26	Leukemia	A 673	>2501
27	Leukemia	K562	287
28	Leukemia	RPMI-8226	528
29	Leukemia	Jurkats	184
30	Leukemia	THP-1	113
31	Leukemia	HUT 78	35
32	Leukemia	JY	0
33	Leukemia	CEM	0
34	Lung Carcinoma	MV 522	74
35	Lung adenocarcinoma	EKVX	>2500
36	Lung adenocarcinoma	HOP-62	>2500
37	Lung Carcinoma NSC	HOP-92	897
38	Lung Carcinoma NSC	NCI-H1299	384
39	Lung Carcinoma NSC	NCI-H2126	107
55	Lung adenocarcinoma	NCI-H522	0
42	Lung adenocarcinoma	NCI-H322M	0
40	IPF Lung fibroblasts	A 549	>2501
57	Lung adenocarcinoma	NCI-H292	245
43	Lung Carcinoma NSC	NCI-H460	118
45	Lung Squamous NSC	Skmes-1	410
44	Lung Carcinoma Small Cell	SHF-77	1663
58	Lung Carcinoma Small Cell	NCI-H510A	>2500
56	Lung Carcinoma Small Cell	NCI-H69	
53	Mammary Gland Carcinoma	HCC-2218	129
54	Mammary Gland Carcinoma	HCC-1954	113
46	Mammary Gland Carcinoma	ZR-75-30	357
47	Mammary Gland Carcinoma	MCF-7	0
48	Mammary Gland Carcinoma	MDA-MB-453	40
49	Mammary Gland Carcinoma	MDA-MB-231	>2501
50	Mammary Gland Carcinoma	MDA-MB-468	9
51	Mammary Gland Carcinoma	NCI/ADR	0
52	Mammary Gland Carcinoma	T47D	61
22	Mammary Gland Carcinoma	SK-BR-3	475
20	Mammary Gland Carcinoma	Hs 605T	>2500
53	Melanoma	A431	56
54	Melanoma	LOX IMVI	105
55	Melanoma	M14	786
56	Melanoma	RPMI 7591	>2501
57	Melanoma	SK-MEL-28	29
58	Melanoma	UACC-62	119
59	Melanoma	UACC-257	265
41	Melanoma	Hs 936.T	15
24	Melanoma	SK-mel-5	38
25	Melanoma	Hs 940.T	>2500
26	Melanoma	A375	136
6	Melanoma	WM.266.4	>2500
27	Pancreatic Carcinoma	HPAC	73
29	Pancreatic Carcinoma	HPAF II	47
41	Pancreatic Carcinoma	CAPAN-1	>2500
60	Pancreatic Carcinoma	Panc-1	>2500
30	Ovarian Carcinoma	ES2	322
31	Ovarian Carcinoma	IGROV1	199
32	Ovarian Carcinoma	MDAH2774	314
33	Ovarian Carcinoma	SK-OV-3	86
34	Ovarian Carcinoma	OVCAR-3	126
36	Ovarian Carcinoma	OVCAR-5	336
37	Ovarian Carcinoma	OVCAR-8	36

TABLE 17

	Cell Line	MCP-1 (pg/mL)	
1	Colon Carcinoma	COLO-205	<10
2	Colon Carcinoma	HCT-15	60
3	Colon Carcinoma	HCT-116	122
4	Colon Carcinoma	HT-29	102
5	Cervical Cancer	HT-3	127

TABLE 17-continued

	Cell Line	MCP-1 (pg/mL)	
38	Prostate Carcinoma	22Rv1	55
39	Prostate Carcinoma	LNCaP	>2500
40	Prostate Carcinoma	DU150	>2500
42	Prostate Carcinoma	PC-3	163
28	Prostate Carcinoma	DU145	68
43	Renal Carcinoma	A498	>2500
44	Renal Carcinoma	786-0(35h)	>2500
45	Renal Carcinoma	SK-RC-01	>2500
46	Renal Carcinoma	SK-RC-10	>2500
47	Renal Carcinoma	Caki-1	115
48	Renal Carcinoma	Caki-2	>2500
49	Renal Carcinoma	RXF-393	>2500
50	Renal Carcinoma	SK-RC-52	>2500
51	Renal Carcinoma	SN12C	>2500
52	Renal Carcinoma	TK-10	533
62	Renal Carcinoma	769-P	512
23	Liver Carcinoma	C3A	0
59	Liver Carcinoma	HepG2	>2500
19	Cervical Cancer Epidermoid	MS 751	>2500
35	Cervical Cancer	Hela	>2501
	Cervical	C-33A	20
1	Cervical	Ca Ski	32
2	Cervical	ME-180	54
3	Uterus	KLE	>2500
4	Uterus	RL95-2	28
5	Uterus	HEC-1-A	47
		MCP-1	

Example 7

Effect of Anti-MCP-1 Antibodies in Mouse Tumor Model

[0220] To evaluate the effect of anti-MCP-1 antibodies on the growth of a subcutaneous tumor, exponentially growing Panc-1 cells were harvested and resuspended in 0.2 ml of Hank's Balanced Salt solution (HBSS). Tumors were produced following the injected of 5×10^6 Panc-1 cells admixed with Growth factor reduced Matrigel into the flanks of female BALB/c nude mice. Beginning on the day of implantation, animals were treated with 5 mg of anti-MCP-1 antibody 1.7.3, and antibody PK, which was directed to KLH or PBS at the times indicated on the graph. Tumor growth was monitored weekly and the results presented as mean \pm SD (FIG. 4). The difference between the control and treated animals was statistically significant when compared using the student T test ($P < 0.002$). Accordingly, anti-MCP-1 antibodies provide an effective treatment for reducing tumor growth in vivo.

Example 8

Software-assisted Analysis of MCP-1 Antibodies

[0221] The above-described calcium flux, chemotaxis and affinity data for the MCP-1 antibodies were analyzed using Guided Analytic software available from Spotfire, Inc., Somerville, Mass. The results are shown in FIGS. 5 and 6.

Example 9

Structural Analysis of Anti-MCP-1 Antibodies

[0222] The variable heavy chains and the variable light chains for the antibodies shown in Table 1 were sequenced to determine their DNA sequences. The complete sequence

information for all anti-MCP-1 antibodies are shown in the sequence listing with nucleotide and amino acid sequences for each gamma and kappa chain combination.

[0223] The variable heavy sequences were analyzed to determine the VH family, the D-region sequence and the J-region sequence. The sequences were then translated to determine the primary amino acid sequence and compared to the germline VH, D and J-region sequences to assess somatic hypermutations. FIG. 7 shows a Clustal W comparison of anti-MCP-1 sequences using VH1-24, indicating the CD, CDR1, CDR2, and CDR3 regions, and the associated dendrogram. FIG. 8 shows a Clustal W comparison of anti-MCP-1 sequences using VK-B3, indicating the CD, CDR1, CDR2, and CDR3 regions, and the associated dendrogram. FIG. 9 shows a Clustal W comparison of anti-MCP-1 sequences using VK-08, indicating the CD, CDR1, CDR2, and CDR3 regions, and the associated dendrogram. FIG. 10 shows a Clustal W comparison of anti-MCP-1 sequences using VH6-1, indicating the CD, CDR1, CDR2, and CDR3 regions, and the associated dendrogram.

Example 10

Use of Anti-MCP-1 Antibodies as a Diagnostic Agent

[0224] A. Detection of MCP-1 Antigen in a Sample

[0225] An Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of MCP-1 antigen in a sample is developed. In the assay, wells of a microtiter plate, such as a 96-well microtiter plate or a 384-well microtiter plate, are adsorbed for several hours with a first fully human monoclonal antibody directed against the antigen. The immobilized antibody serves as a capture antibody for any of the antigen that may be present in a test sample. The wells are rinsed and treated with a blocking agent such as milk protein or albumin to prevent nonspecific adsorption of the analyte.

[0226] Subsequently the wells are treated with a test sample suspected of containing the antigen, or with a solution containing a standard amount of the antigen. Such a sample may be, for example, a serum sample from a subject suspected of having levels of circulating antigen considered to be diagnostic of pathology.

[0227] After rinsing away the test sample or standard, the wells are treated with a second fully human monoclonal anti-MCP-1 antibody that is labeled by conjugation with biotin. The labeled anti-MCP-1 antibody serves as a detecting antibody. After rinsing away excess second antibody, the wells are treated with avidin-conjugated horseradish peroxidase (HRP) and a suitable chromogenic substrate. The concentration of the antigen in the test samples is determined by comparison with a standard curve developed from the standard samples.

[0228] This ELISA assay provides a highly specific and very sensitive assay for the detection of the MCP-1 antigen in a test sample.

[0229] B. Determination of MCP-1 Concentration in Patient Samples

[0230] A sandwich ELISA is developed to quantify MCP-1 levels in human serum. The two anti-MCP-1 antibodies used in the sandwich ELISA, preferably recognize

different epitopes on the MCP-1 molecule (data not shown). The ELISA is performed as follows: 50 μ l of capture anti-MCP-1 antibody in coating buffer (0.1 M NaHCO₃, pH 9.6) at a concentration of 2 μ g/mL is coated on ELISA plates (Fisher). After incubation at 4° C. overnight, the plates are treated with 200 μ l of blocking buffer (0.5% BSA, 0.1% Tween 20, 0.01% Thimerosal in PBS) for 1 hr at 25° C. The plates are washed (3 \times) using 0.05% Tween 20 in PBS (washing buffer, WB). Normal or patient sera (Clinomics, Bioreclamation) are diluted in blocking buffer containing 50% human serum. The plates are incubated with serum samples overnight at 4° C., washed with WB, and then incubated with 100 μ l/well of biotinylated detection anti-MCP-1 antibody for 1 hr at 25° C. After washing, the plates are incubated with HRP-Streptavidin for 15 min, washed as before, and then treated with 100 μ l/well of o-phenylenediamine in H₂O₂ (Sigma developing solution) for color generation. The reaction is stopped with 50 μ l/well of H₂SO₄ (2M) and analyzed using an ELISA plate reader at 492 nm. Concentration of PRO antigen in serum samples is calculated by comparison to dilutions of purified MCP-1 antigen using a four-parameter curve-fitting program.

[0231] C. Staging of Cancer in a Patient

[0232] It will be appreciated that based on the results set forth and discussed in Examples 10A-10B, through use of embodiments of the invention described herein, it is possible to stage a cancer in a subject based on expression levels of the MCP-1 antigen. For a given type of cancer, samples of blood are taken from subjects diagnosed as being at various stages in the progression of the disease, and/or at various points in the therapeutic treatment of the cancer. The concentration of the MCP-1 antigen present in the blood samples is determined using a method that specifically determines the amount of the antigen that is present. Such a method includes an ELISA method, such as the method described in Examples 10A-10B. Using a population of samples that provides statistically significant results for each stage of progression or therapy, a range of concentrations of the antigen that may be considered characteristic of each stage is designated.

[0233] In order to stage the progression of the cancer in a subject under study, or to characterize the response of the subject to a course of therapy, a sample of blood is taken from the subject and the concentration of the MCP-1 antigen present in the sample is determined. The concentration so obtained is used to identify in which range of concentrations

the value falls. The range so identified correlates with a stage of progression or a stage of therapy identified in the various populations of diagnosed subjects, thereby providing a stage in the subject under study.

Example 11

Uses of Anti-MCP-1 Antibodies for Tumor Treatment

[0234] To determine the in vivo effects of anti-MCP-1 antibody treatment in human patients with tumors, such human patients are injected over a certain amount of time with an effective amount of anti-MCP-1 antibody. At periodic times during the treatment, the human patients are monitored to determine whether their tumors progress, in particular, whether the tumors grow and metastasize.

[0235] A tumor patient treated with anti-MCP-1 antibodies has a lower level of tumor growth and metastasis compared to the level of tumor growth and metastasis of tumors in tumor patients treated with control antibodies. Control antibodies that may be used include antibodies of the same isotype as the anti-MCP-1 antibodies tested and further, may not have the ability to bind to MCP-1 tumor antigen.

[0236] The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The embodiments of the invention described herein are not to be limited in scope by the construct deposited, since the deposited embodiment is intended as a single illustration of certain aspects of the invention and any constructs that are functionally equivalent are within the scope of this invention.

[0237] All references cited herein, including patents, patent applications, papers, text books, and the like, and the references cited therein, to the extent that they are not already, are hereby incorporated herein by reference in their entirety.

[0238] The foregoing description and Examples detail certain preferred embodiments of the invention and describes the best mode contemplated by the inventors. It will be appreciated, however, that no matter how detailed the foregoing may appear in text, the invention may be practiced in many ways and the invention should be construed in accordance with the appended claims and any equivalents thereof.

SEQUENCE LISTING

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<211> LENGTH: 1335

<212> TYPE: DNA

<213> ORGANISM: Homosapien

<400> SEQUENCE: 1

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tcttgcaagg tttccggata caccctcact gaattatcca tgcaactgggt ggcacaggct 120

-continued

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ttttggagtg gttattttga ctactggggc cagggaaacc tggtcaccgt ctectcagcc 360
tccaccaagg gcccatcggt cttccccctg gcgccctgct ccaggagcac ctccgagagc 420
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aactcaggcg ctctgaccag cggcgtgcac accttcccag ctgtcctaca gtcctcagga 540
ctctactccc tcagcagcgt ggtgaccgtg ccctccagca acttcggcac ccagacctac 600
acctgcaacg tagatcacia gccccagcaac accaagggtg acaagacagt tgagcgcaaa 660
tgttggtgctg agtgcaccac gtgcccagca ccacctgtgg caggaccgtc agtcttctc 720
ttcccccaaa aaccaagga caccctcatg atctcccgga ccctgaggt cacgtgcgtg 780
gtggtggacg tgagccacga agaccccag gtccagtcca actggtacgt ggacggcgtg 840
gagggtgata atgccaagc aaagccacgg gaggagcagt tcaacagcac gttccgtgtg 900
gtcagcgtcc tcaccgttgt gcaccaggac tggtgaacg gcaaggagta caagtgcaag 960
gttccaaca aagcctccc agccccatc gagaaaacca tctccaaaac caaaggcag 1020
ccccgagaac cacaggtgta caccctgccc ccatcccggg aggagatgac caagaaccag 1080
gtcagcctga cctgcctggt caaaggcttc taccacagc acatgccctg ggagtgggag 1140
agcaatgggc agccggagaa caactacaag accacacctc ccatgctgga ctccgacggc 1200
tccttcttcc tctacagaa gctcaccgtg gacaagagca ggtggcagca ggggaacgtc 1260
ttctcatgct ccgtgatgca tgaggctctg cacaaccact acacgcagaa gagcctctcc 1320
ctgtctccgg gtaaa 1335

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<210> SEQ ID NO 2

<211> LENGTH: 445

<212> TYPE: PRT

<213> ORGANISM: Homosapien

<400> SEQUENCE: 2

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

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Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
 145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
 165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
 180 185 190

Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro
 195 200 205

Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu
 210 215 220

Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
 225 230 235 240

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
 245 250 255

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln
 260 265 270

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
 275 280 285

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu
 290 295 300

Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
 305 310 315 320

Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
 325 330 335

Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
 340 345 350

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
 355 360 365

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
 370 375 380

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly
 385 390 395 400

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
 405 410 415

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
 420 425 430

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 435 440 445

<210> SEQ ID NO 3
 <211> LENGTH: 660
 <212> TYPE: DNA
 <213> ORGANISM: Homosapien
 <400> SEQUENCE: 3

gacatcgtga tgaccagtc tccagactcc ctggctatgt ctctgggcga gagggccacc 60
 atcaactgta agtccagcca gagtgtttta tacagctcca acaataagaa ctacttagtt 120
 tggtagcagc agaaaccagg acagcctoct aaactgctca tttactgggc atctatccgg 180
 gaatccgggg tcctgaccg attcagttcc agcgggtctg agacagattt cactctcacc 240
 atcagcagcc tgcaggctga agatgtggca gtttattact gtcagcaata ttttagtagt 300

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ccgtggacgt tcggccaag gaccaagtg gaaatcaaac gaactgtggc tgcaccatct 360
gtcttcatct tcccgccatc tgatgagcag ttgaaatctg gaactgcctc tgttgtgtgc 420
ctgctgaata acttctatcc cagagaggcc aaagtacagt ggaaggtgga taacgccctc 480
caatcgggta actcccagga gagtgtcaca gacgaggaca gcaaggacag cacctacagc 540
ctcagcagca ccttgacgct gagcaaagca gactacgaga aacacaaagt ctacgcctgc 600
gaagtcaccc atcagggcct gagctcgccc gtcacaaaga gottcaacag gggagagtgt 660

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<210> SEQ ID NO 4
<211> LENGTH: 220
<212> TYPE: PRT
<213> ORGANISM: Homosapien

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<400> SEQUENCE: 4

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

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<210> SEQ ID NO 5
<211> LENGTH: 475
<212> TYPE: DNA
<213> ORGANISM: Homosapien

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<400> SEQUENCE: 5

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cagggtccagc tggtagcagtc tggggctgag gtgaagaagc ctggggcctc agtgaaggtc 60
tcctgcaagg tttccggata caccctcact gaattatcca tgcactgggt ggcacaggct 120
cctggaaaag ggcttgagtg gatgggaggt tttgatcctg aagatggtga aacaatctac 180

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gcacagaagt tccagggcag agtcaccatg accgaggaca catctacaga cacagcctac 240
atggagctga gcagcctgag atctgaggac acggccgtgt attattgtgc aaccaacgaa 300
ttttgagtg gttattttga ctactggggc cagggaaacc tggtcaccgt ctctcagcc 360
tccaccaagg gcccatcggg etttcccctg gcgcctgct ccaggagcac tacttcccc 420
ggcgtgcaca ctttcccagc tgtcctacag tcctcaggac tctactccct cagca 475

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<210> SEQ ID NO 6
<211> LENGTH: 158
<212> TYPE: PRT
<213> ORGANISM: Homosapien

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<400> SEQUENCE: 6

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

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<210> SEQ ID NO 7
<211> LENGTH: 477
<212> TYPE: DNA
<213> ORGANISM: Homosapien

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<400> SEQUENCE: 7

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gacatcgtga tgaccacagtc tccagcctcc ctggctgagt ctctgggcga gagggccacc 60
atcaattgca agtccagcca gagtgtttta tatagctcca acaataagaa ctacttagtt 120
tggtagcagc agaaactagg acagcccctt aagctgctca tttactgggc atctaccggg 180
gaatccgggg tcctgaccg attcagtggc agcgggtctg ggacagattt cactctcacc 240
atcagcagcc tgcaggctga agatgtggca gtttattact gtcaacaata ttatcgtagt 300
ccgtggacgt tcggccaagg gaccaaggtg gaaatcaaac gaactgtggc tgcaccatct 360
gtcttcatct tcccgcacc tgatgagcag ttgaaatctg gaactgcctc tgttgtgtgc 420
ctgctgaata acttctatcc cagagaggcc aaagtacagt ggaaggtgga taacgcc 477

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<210> SEQ ID NO 8
<211> LENGTH: 159
<212> TYPE: PRT

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-continued

<213> ORGANISM: Homosapien

<400> SEQUENCE: 8

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

<210> SEQ ID NO 9

<211> LENGTH: 556

<212> TYPE: PRT

<213> ORGANISM: Homosapien

<400> SEQUENCE: 9

Cys Ala Gly Gly Thr Cys Cys Ala Gly Cys Thr Gly Gly Thr Ala Cys
 1 5 10 15
 Ala Gly Thr Cys Thr Gly Gly Gly Gly Cys Thr Gly Ala Gly Gly Thr
 20 25 30
 Gly Ala Ala Gly Ala Ala Gly Cys Cys Thr Gly Gly Gly Gly Cys Cys
 35 40 45
 Thr Cys Ala Gly Thr Gly Ala Ala Gly Gly Thr Cys Thr Cys Cys Thr
 50 55 60
 Gly Cys Ala Ala Gly Gly Thr Thr Thr Cys Cys Gly Gly Ala Thr Ala
 65 70 75 80
 Cys Ala Cys Cys Cys Thr Cys Ala Cys Thr Gly Ala Ala Thr Thr Ala
 85 90 95
 Thr Cys Cys Ala Thr Gly Cys Ala Cys Thr Gly Gly Gly Thr Gly Cys
 100 105 110
 Gly Ala Cys Ala Gly Gly Cys Thr Cys Cys Thr Gly Gly Ala Ala Ala
 115 120 125
 Ala Gly Gly Gly Cys Thr Thr Gly Ala Gly Thr Gly Gly Ala Thr Gly
 130 135 140
 Gly Gly Ala Gly Gly Thr Thr Thr Thr Gly Ala Thr Cys Cys Thr Gly
 145 150 155 160
 Ala Ala Gly Ala Thr Gly Gly Thr Gly Ala Ala Ala Cys Ala Ala Thr
 165 170 175
 Cys Thr Ala Cys Gly Cys Ala Cys Ala Gly Ala Ala Gly Thr Thr Cys

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180				185				190							
Cys	Ala	Gly	Gly	Gly	Cys	Ala	Gly	Ala	Gly	Thr	Cys	Ala	Cys	Cys	Ala
	195						200					205			
Thr	Gly	Ala	Cys	Cys	Gly	Ala	Gly	Gly	Ala	Cys	Ala	Cys	Ala	Thr	Cys
	210					215					220				
Thr	Ala	Cys	Ala	Gly	Ala	Cys	Ala	Cys	Ala	Gly	Cys	Cys	Thr	Ala	Cys
	225				230					235					240
Ala	Thr	Gly	Gly	Ala	Gly	Cys	Thr	Gly	Ala	Gly	Cys	Ala	Gly	Cys	Cys
			245						250					255	
Thr	Gly	Ala	Gly	Ala	Thr	Cys	Thr	Gly	Ala	Gly	Gly	Ala	Cys	Ala	Cys
		260							265					270	
Gly	Gly	Cys	Cys	Gly	Thr	Gly	Thr	Ala	Thr	Thr	Ala	Cys	Thr	Gly	Thr
		275					280					285			
Gly	Cys	Ala	Ala	Cys	Ala	Ala	Ala	Cys	Gly	Ala	Thr	Thr	Thr	Thr	Thr
	290					295						300			
Gly	Gly	Ala	Gly	Thr	Gly	Gly	Thr	Thr	Ala	Thr	Thr	Ala	Thr	Ala	Ala
	305				310					315					320
Cys	Thr	Ala	Cys	Thr	Gly	Gly	Gly	Gly	Cys	Cys	Ala	Gly	Gly	Gly	Ala
			325						330					335	
Ala	Cys	Cys	Cys	Thr	Gly	Gly	Thr	Cys	Ala	Cys	Cys	Gly	Thr	Cys	Thr
			340						345				350		
Cys	Cys	Thr	Cys	Ala	Gly	Cys	Cys	Thr	Cys	Cys	Ala	Cys	Cys	Ala	Ala
		355					360					365			
Gly	Gly	Gly	Cys	Cys	Cys	Ala	Thr	Cys	Gly	Gly	Thr	Cys	Thr	Thr	Cys
	370					375					380				
Cys	Cys	Cys	Cys	Thr	Gly	Gly	Cys	Gly	Cys	Cys	Cys	Thr	Gly	Cys	Thr
	385				390					395					400
Cys	Cys	Ala	Gly	Gly	Ala	Gly	Cys	Ala	Cys	Cys	Thr	Cys	Cys	Gly	Ala
			405						410					415	
Gly	Ala	Gly	Cys	Ala	Cys	Ala	Gly	Cys	Gly	Gly	Cys	Cys	Cys	Thr	Gly
		420							425				430		
Gly	Gly	Cys	Thr	Gly	Cys	Cys	Thr	Gly	Gly	Thr	Cys	Ala	Ala	Gly	Gly
		435					440					445			
Ala	Cys	Thr	Ala	Cys	Thr	Thr	Cys	Cys	Cys	Cys	Gly	Ala	Ala	Cys	Cys
	450					455					460				
Gly	Gly	Thr	Gly	Ala	Cys	Gly	Gly	Thr	Gly	Thr	Cys	Gly	Thr	Gly	Gly
	465				470					475					480
Ala	Ala	Cys	Thr	Cys	Ala	Gly	Gly	Cys	Gly	Cys	Thr	Cys	Thr	Gly	Ala
			485						490					495	
Cys	Cys	Ala	Gly	Cys	Gly	Gly	Cys	Gly	Thr	Gly	Cys	Ala	Cys	Ala	Cys
		500							505				510		
Cys	Thr	Thr	Cys	Cys	Cys	Ala	Gly	Cys	Thr	Gly	Thr	Cys	Cys	Thr	Ala
		515					520					525			
Cys	Ala	Gly	Thr	Cys	Cys	Thr	Cys	Ala	Gly	Gly	Ala	Cys	Thr	Cys	Thr
	530					535					540				
Ala	Cys	Thr	Cys	Cys	Cys	Thr	Cys	Ala	Gly	Cys	Ala				
	545				550					555					

<210> SEQ ID NO 10

<211> LENGTH: 185

<212> TYPE: PRT

<213> ORGANISM: Homosapien

-continued

<400> SEQUENCE: 10

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

<210> SEQ ID NO 11

<211> LENGTH: 490

<212> TYPE: DNA

<213> ORGANISM: Homosapien

<400> SEQUENCE: 11

gacatcgtga tgaccagtc tccagactcc ctggctgtgt ctctgggcca gagggccacc 60
 atcaactgca agtccagcca gagtgtttta tacagctcca acaataagaa ctacttagtt 120
 tggtagcaac agaaccagg acagcctoct aaactgctca tttactgggc atctatccgg 180
 gaatccgggg tcctgaccg attcagtggc agcgggtctg ggacagattt cactctcacc 240
 atcaacagcc tgcaggctga agatgtggca gtttattact gtcagcagta tttttatagt 300
 ccgtggacgt tcggccaagg gaccaagggtg gaaatcaaac gaactgtggc tgcaccatct 360
 gtcttcatct tcccgccatc tgatgagcag ttgaaatctg gaactgcctc tgttgtgtgc 420
 ctgctgaata acttctatcc cagagaggcc aaagtacagt ggaaggtgga taacgccttc 480
 caatcgggta 490

<210> SEQ ID NO 12

<211> LENGTH: 163

<212> TYPE: PRT

<213> ORGANISM: Homosapien

<400> SEQUENCE: 12

Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
 1 5 10 15

-continued

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser
 20 25 30

Ser Asn Asn Lys Asn Tyr Leu Val Trp Tyr Gln Gln Lys Pro Gly Gln
 35 40 45

Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Ile Arg Glu Ser Gly Val
 50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
 65 70 75 80

Ile Asn Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
 85 90 95

Tyr Phe Tyr Ser Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
 100 105 110

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
 115 120 125

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
 130 135 140

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
 145 150 155 160

Gln Ser Gly

<210> SEQ ID NO 13
 <211> LENGTH: 543
 <212> TYPE: DNA
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 13

cagggtccagc tgggtacagtc tggggctgag gtgaagaagc ctggggcctc agtgaaggtc 60

tcctgcaagg tttccggaca caccctcact gaattatcca tgcactgggt gcgacaggct 120

cctggaaaag ggcttgagtg gatgggaggt tttgatcctg aagatgatga aacaatctac 180

gcacagaagt tccaggacag agtcaccatg accgaggaca catctacaga cacagcctac 240

atggagctga gcagcctaag atctgaggac acggccgtgt attactgtgc aaccaacgat 300

ttttgagtg gttatatttg atgctggggc cagggaaacc tggtcaccgt ctctcagcc 360

tccaccaagg gcccatcggt cttccccctg gcgccctgct ccaggagcac ctccgagagc 420

acagcggccc tgggctgcct ggtaaggac tacttccccg aaccggtgac ggtgtcgtgg 480

aactcaggcg ctctgaccag cggcgtgcac accttcccag ctgtctaca gtctcagga 540

ctt 543

<210> SEQ ID NO 14
 <211> LENGTH: 181
 <212> TYPE: PRT
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 14

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15

Ser Val Lys Val Ser Cys Lys Val Ser Gly His Thr Leu Thr Glu Leu
 20 25 30

Ser Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
 35 40 45

Gly Gly Phe Asp Pro Glu Asp Asp Glu Thr Ile Tyr Ala Gln Lys Phe
 50 55 60

-continued

Gln Asp Arg Val Thr Met Thr Glu Asp Thr Ser Thr Asp Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Thr Asn Asp Phe Trp Ser Gly Tyr Phe Asp Cys Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115 120 125

Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165 170 175

Gln Ser Ser Gly Leu
180

<210> SEQ ID NO 15
 <211> LENGTH: 490
 <212> TYPE: DNA
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 15

gacatcgtgc tgaccagtc tccagactcc ctggctgtgt gtctgggcga gaggccacc 60
 atcaactgca agtccagcca gagtgtttta tatagtccca acaataagaa cttcttagtt 120
 tggtagcagc agagaccagg acagcctoct aagctgctca tttactgggc atctaccgg 180
 gaatccgggg tccttgaccg attcagtgcc agcgggtctg ggacagattt cactctcacc 240
 atcagcagcc tgcaggctga agatgtggca gtttattact gtcagcaata ttatagtagt 300
 ccgtggacgt tcggccaagg gaccaaggtg gaaatcaaac gaactgtggc tgcaccatct 360
 gtcttcatct tcccgccatc tgatgagcag ttgaaatctg gaactgcctc tgttgtgtgc 420
 ctgctgaata acttctatcc cagagaggcc aaagtacagt ggaaggtgga taacgcctc 480
 caatcgggta 490

<210> SEQ ID NO 16
 <211> LENGTH: 163
 <212> TYPE: PRT
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 16

Asp Ile Val Leu Thr Gln Ser Pro Asp Ser Leu Ala Val Cys Leu Gly
1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser
20 25 30

Pro Asn Asn Lys Asn Phe Leu Val Trp Tyr Gln Gln Arg Pro Gly Gln
35 40 45

Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
85 90 95

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Tyr Tyr Ser Ser Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
 100 105 110

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
 115 120 125

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
 130 135 140

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
 145 150 155 160

Gln Ser Gly

<210> SEQ ID NO 17
 <211> LENGTH: 1335
 <212> TYPE: DNA
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 17

```

caggtccagc tggtagcagc tggggctgag gtgaagaagc ctggggcctc agtgaaggct 60
tcctgcaagg tttccggata caccctcact gaattatcca tgcactgggt gcgacaggct 120
cctggaaaag ggttgtagtg gatgggaggt tttgatcctg aagatggtga aacaatctac 180
gcacagaagt tccagggcag agtcacatg accgaggaca catctacaga cacagtctac 240
atggagctga gcagcctgag atctgaggac acggccatgt attactgtgc aacacgggag 300
ttttgactg gttattttga ccactggggc cagggaaccc tggtcaccgt ctctcagcc 360
tccaccaagg gcccatcggg cttccccctg gcgccctgct ccaggagcac ctccgagagc 420
acagcggccc tgggctgcct ggtcaaggac tacttccccg aaccgggtgac ggtgtcgtgg 480
aactcaggcg ctctgaccag cggcgtgcac accttcccag ctgtcctaca gtcctcagga 540
ctctactccc tcagcagcgt ggtgaccgtg ccctccagca acttoggcac ccagacctac 600
acctgcaacg tagatcacia gccagcaac accaagggtg acaagacagt tgagcgcaaa 660
tgttgtgtcg agtcccacc gtgcccagca ccacctgtgg caggaccgtc agtcttctc 720
ttcccccaa aaccaagga caccctcatg atctcccgga cccctgaggt cacgtgcgtg 780
gtggtggacg tgagccacga agaccccag gtccagtcca actggtacgt ggacggcgtg 840
gaggtgcata atgccaagc aaagccacgg gaggagcagt tcaacagcac gttccgtgtg 900
gtcagcgtcc tcaccgttgt gcaccaggac tggctgaacg gcaaggagta caagtgcaag 960
gttccaaca aagcctccc agccccatc gagaaaacca tctccaaaac caaagggcag 1020
ccccgagaac cacaggtgta caccctgccc ccatcccggg aggagatgac caagaaccag 1080
gtcagcctga cctgcctggt caaaggcttc taccacagc acatgccctg ggagtgggag 1140
agcaatgggc agccggagaa caactacaag accacacctc ccatgctgga ctccgacggc 1200
tccttcttcc tctacagaa gctcaccgtg gacaagagca ggtggcagca ggggaacgtc 1260
ttctcatgct ccgtgatgca tgaggctctg cacaaccact acacgcagaa gagcctctcc 1320
ctgtctccgg gtaaa 1335

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<210> SEQ ID NO 18
 <211> LENGTH: 445
 <212> TYPE: PRT
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 18

-continued

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15
 Ser Val Lys Val Ser Cys Lys Val Ser Gly Tyr Thr Leu Thr Glu Leu
 20 25 30
 Ser Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
 35 40 45
 Gly Gly Phe Asp Pro Glu Asp Gly Glu Thr Ile Tyr Ala Gln Lys Phe
 50 55 60
 Gln Gly Arg Val Thr Met Thr Glu Asp Thr Ser Thr Asp Thr Val Tyr
 65 70 75 80
 Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys
 85 90 95
 Ala Thr Arg Glu Phe Trp Thr Gly Tyr Phe Asp His Trp Gly Gln Gly
 100 105 110
 Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
 115 120 125
 Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
 130 135 140
 Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
 145 150 155 160
 Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
 165 170 175
 Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
 180 185 190
 Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro
 195 200 205
 Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu
 210 215 220
 Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
 225 230 235 240
 Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
 245 250 255
 Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln
 260 265 270
 Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
 275 280 285
 Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu
 290 295 300
 Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
 305 310 315 320
 Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
 325 330 335
 Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
 340 345 350
 Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
 355 360 365
 Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
 370 375 380
 Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly
 385 390 395 400

-continued

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
 165 170 175

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
 180 185 190

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
 195 200 205

Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 210 215 220

<210> SEQ ID NO 21
 <211> LENGTH: 543
 <212> TYPE: DNA
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 21

caggtccagc tggtagcagtc tggggctgag gtgaagaagc ctggggcctc agtgaaggtc 60
 tcctgcaagg tttccggata cacttttact gaattatcca tgcactgggt ggcacaggct 120
 cctggaaaag ggcttgagtg gatgggaggt tttgatcctg aagatggtga aacaagctac 180
 gcacagaagt tccggggcag agtcaccatg accgaggaca catctacaga cacagcccac 240
 atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc aaccaacgat 300
 ttttgagtg gttattttga ctattggggc cagggaaacc tggtcaccgt ctctcagcc 360
 tccaccaagg gcccatcggc etttcccctg gcgcctgct ccaggagcac ctccgagagc 420
 acagcggccc tgggctgcct ggtcaaggac tacttcccgc aaccggtgac ggtgctgctgg 480
 aactcaggcg ctctgaccag cggcgtgcac accttcccag ctgtctaca gtctcagga 540
 ctt 543

<210> SEQ ID NO 22
 <211> LENGTH: 181
 <212> TYPE: PRT
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 22

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15

Ser Val Lys Val Ser Cys Lys Val Ser Gly Tyr Thr Phe Thr Glu Leu
 20 25 30

Ser Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
 35 40 45

Gly Gly Phe Asp Pro Glu Asp Gly Glu Thr Ser Tyr Ala Gln Lys Phe
 50 55 60

Arg Gly Arg Val Thr Met Thr Glu Asp Thr Ser Thr Asp Thr Ala His
 65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Thr Asn Asp Phe Trp Ser Gly Tyr Phe Asp Tyr Trp Gly Gln Gly
 100 105 110

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
 115 120 125

Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
 130 135 140

-continued

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
 145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
 165 170 175

Gln Ser Ser Gly Leu
 180

<210> SEQ ID NO 23
 <211> LENGTH: 460
 <212> TYPE: DNA
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 23

gacatccaga tgacccagtc tccatcttcc gtgtctgcat ctgtaggaga cagagtcacc 60
 atcacttgtc gggcgagtca gggattgac atctacttag cctggtatca gcagaaacca 120
 gggaaagccc ctaagctcct gatcaatgct gcatccagtt tgcaaaacgg ggtcccctca 180
 aggttcggcg gcagtgatc tgggacagat ttcactctca ccatcagcgg cctgcagcct 240
 gaagattttg caacttacta ttgtcaactg acttactttt tcccgtggac gttcggccaa 300
 gggaccaagg tggaaatcaa acgaaactgtg gctgcaccat ctgtttcat cttcccgcca 360
 tctgatgagc agttgaaatc tggaactgcc tctgttgtgt gcctgctgaa taacttctat 420
 cccagagagg ccaaagtaca gtggaagggt gataacgccc 460

<210> SEQ ID NO 24
 <211> LENGTH: 153
 <212> TYPE: PRT
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 24

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Asp Ile Tyr
 20 25 30

Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
 35 40 45

Asn Ala Ala Ser Ser Leu Gln Asn Gly Val Pro Ser Arg Phe Gly Gly
 50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Gly Leu Gln Pro
 65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Leu Thr Tyr Phe Phe Pro Trp
 85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
 100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
 115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
 130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala
 145 150

<210> SEQ ID NO 25
 <211> LENGTH: 543
 <212> TYPE: DNA
 <213> ORGANISM: Homosapien

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<400> SEQUENCE: 25

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caggtccagc tggtagctc tggggctgag gtgaagaagc ctggggcctc agtgaaggtc      60
tcctgcaagg ttcccgata caccctcact gaattatcca tgcactgggt gcgacgaatt      120
cctggaaaag ggcttgagtg gatgggaggt ttgaccctg aagatggtga aacaatctac      180
gcacagaagt tccagggcag agtcaccatg accgaggaca catctacaga cacagcctac      240
atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc acaaacgat      300
ttttggagtg gctattgggg ccaactggggc cagggaaacc tggtcaccgt ctctcagcc      360
tccaccaagg gcccatcggg cttccccctg gcgccctgct ccaggagcac ctccgagagc      420
acagcggccc tgggctgcct ggtaaggac tacttccccg aaccgggtgac ggtgtcgtgg      480
aactcaggcg ctctgaccag cggcgtgcac accttcccag ctgtctaca gtctcagga      540
ctt                                                                                   543

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<210> SEQ ID NO 26

<211> LENGTH: 181

<212> TYPE: PRT

<213> ORGANISM: Homosapien

<400> SEQUENCE: 26

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Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1             5             10             15
Ser Val Lys Val Ser Cys Lys Val Ser Gly Tyr Thr Leu Thr Glu Leu
 20             25             30
Ser Met His Trp Val Arg Arg Ile Pro Gly Lys Gly Leu Glu Trp Met
 35             40             45
Gly Gly Phe Asp Pro Glu Asp Gly Glu Thr Ile Tyr Ala Gln Lys Phe
 50             55             60
Gln Gly Arg Val Thr Met Thr Glu Asp Thr Ser Thr Asp Thr Ala Tyr
 65             70             75             80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85             90             95
Ala Thr Asn Asp Phe Trp Ser Gly Tyr Trp Gly His Trp Gly Gln Gly
100             105             110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
115             120             125
Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
130             135             140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
145             150             155             160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
165             170             175
Gln Ser Ser Gly Leu
180

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<210> SEQ ID NO 27

<211> LENGTH: 459

<212> TYPE: DNA

<213> ORGANISM: Homosapien

<400> SEQUENCE: 27

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gacatcgtga tgaccacgtc tccagactcc ctggctgtgt ctctgggcga gagggccacc      60

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atcaactgca agtccagcca gagtgtttta tacagctcca acaataagaa ctacctagct 120
tggtagcaag ctgctcattt actggacata tatccgggaa tccgggggtcc ctgaccgatt 180
cagtggcagc gggctctggga cagatttccac tctcaccatc agcagcctgc aggctgaaga 240
tgtggcagtt tattactgtc aggaacatta tagtattccg tggacgttcg gccaaaggac 300
caaggtggaa atcaaacgaa ctgtggctgc accatctgtc ttcactctcc cgccatctga 360
tgagcagttg aactgcctct gttgtgtgcc tgctgaataa cttctatccc agagaggcca 420
aagtacagtg gaaggtggat aacgccctcc aatcgggta 459

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<210> SEQ ID NO 28
<211> LENGTH: 149
<212> TYPE: PRT
<213> ORGANISM: Homosapien

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<400> SEQUENCE: 28

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

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<210> SEQ ID NO 29
<211> LENGTH: 524
<212> TYPE: DNA
<213> ORGANISM: Homosapien

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<400> SEQUENCE: 29

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caggtccagc tggtagcagc tggggctgag gtgaagaagc ctggggcctc agtgaaggtc 60
tcctgcaagg tttccggata caccctcact gaattatcca tgcactgggt gcgacaggct 120
cctggaaaag ggcttgagtg gatgggaggt tttgatcctg aagatgatga aacaatctac 180
gcacagaagt tccagggcag agtcaccatg accgaggaca catctacaga cacggcctac 240
atggagctga gcagcctgag atctgaggac acggccgtgt atttctgtgc aaccaacgat 300
ttttgagtg gttattttga ctgctgggac cagggaaacc tggtcaccgt ctctcagcc 360
tccaccaagg gcccatcggg cttccccctg gcgccctgct ccaggaacac ctccgagagc 420
acagcgccc tgggtgcct ggtaaggac tacttccccg aaccggtgac ggtgtcgtgg 480

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-continued

aactcaggcg ctctgaccag cggcgtgcac accttcccag ctgt 524

<210> SEQ ID NO 30
 <211> LENGTH: 174
 <212> TYPE: PRT
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 30

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15
 Ser Val Lys Val Ser Cys Lys Val Ser Gly Tyr Thr Leu Thr Glu Leu
 20 25 30
 Ser Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
 35 40 45
 Gly Gly Phe Asp Pro Glu Asp Asp Glu Thr Ile Tyr Ala Gln Lys Phe
 50 55 60
 Gln Gly Arg Val Thr Met Thr Glu Asp Thr Ser Thr Asp Thr Ala Tyr
 65 70 75 80
 Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe Cys
 85 90 95
 Ala Thr Asn Asp Phe Trp Ser Gly Tyr Phe Asp Cys Trp Asp Gln Gly
 100 105 110
 Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
 115 120 125
 Pro Leu Ala Pro Cys Ser Arg Asn Thr Ser Glu Ser Thr Ala Ala Leu
 130 135 140
 Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
 145 150 155 160
 Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
 165 170

<210> SEQ ID NO 31
 <211> LENGTH: 490
 <212> TYPE: DNA
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 31

gacatcgtga tgaccagtc tccagactcc ctggctgcgt ctctgggcca gagggccacc 60
 atcaactgca agtccagtc gagtgtttta tacaggtcca acaataagaa ttatttagtt 120
 tggtagcagc aaaaaccagg acagcctoct aagctgctca tttactgggc atctatccgg 180
 gaatccgggg tccctgaccg attcagtgcc agcgggtctg ggacagattt cactctcacc 240
 atcagcagcc tgcaggctga agatgtggca gttttattct gtcagcaata ttatagttct 300
 ccgtggacgt ttggccaagg gaccaagggtg gaaatcaaac gaactgtggc tgcaccatct 360
 gtcttcatct tccgcgcatc tgatgagcag ttgaaatctg gaactgcctc tgttgtgtgc 420
 ctgctgaata acttctatcc cagagaggcc aaagtacagt ggaaggtgga taacgccctc 480
 caatcgggta 490

<210> SEQ ID NO 32
 <211> LENGTH: 163
 <212> TYPE: PRT
 <213> ORGANISM: Homosapien

-continued

<400> SEQUENCE: 32

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

<210> SEQ ID NO 33

<211> LENGTH: 545

<212> TYPE: DNA

<213> ORGANISM: Homosapien

<400> SEQUENCE: 33

cagggtccagc tggtagcagtc tggggctgag gtgaagaagc ctggggcctc agtgaaggtc 60
 tcctgcaagg tttccggata caccctcact gaattatcca tgcactgggt gcgacaggct 120
 cctgaaaaag ggcttgagtg gatgggaggt tttgatcctg aagatggtga aacaatctac 180
 gcacagaagt tccagggcag agtcaccatg accgaggaca catctacaga cacagcctac 240
 atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc aacctggtat 300
 agtgggatct acttagcttt tgatatctgg ggccaaggga caatggtcac cgtctcttca 360
 gcctccacca agggcccatc ggtcttcccc ctggcgccct gotccaggag cacctccgag 420
 agcacagcgg ccttgggctg cctgggcaag gactacttcc ccgaaccggt gacggtgtcg 480
 tggaaactcag gcgctctgac cagcggcgtg cacaccttcc cagctgtcct acagtcctca 540
 ggatt 545

<210> SEQ ID NO 34

<211> LENGTH: 181

<212> TYPE: PRT

<213> ORGANISM: Homosapien

<400> SEQUENCE: 34

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15
 Ser Val Lys Val Ser Cys Lys Val Ser Gly Tyr Thr Leu Thr Glu Leu
 20 25 30

-continued

Ser Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
 35 40 45

Gly Gly Phe Asp Pro Glu Asp Gly Glu Thr Ile Tyr Ala Gln Lys Phe
 50 55 60

Gln Gly Arg Val Thr Met Thr Glu Asp Thr Ser Thr Asp Thr Ala Tyr
 65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Thr Trp Tyr Ser Gly Ile Tyr Leu Ala Phe Asp Ile Trp Gly Gln
 100 105 110

Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
 115 120 125

Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala
 130 135 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
 145 150 155 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
 165 170 175

Leu Gln Ser Ser Gly
 180

<210> SEQ ID NO 35
 <211> LENGTH: 472
 <212> TYPE: DNA
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 35

gaaattgtgc tgactcagtc tccagacttt cagtctgtga ctccaaagga gaaagtcacc 60
 atcacctgcc gggccagtc gagcattggt agtagcttac actggtacca gcagaaacca 120
 gatcagcttc caaagctcct catcaagtat gcttcccagt cttctcagg ggtcccctcg 180
 aggttcagtg gcagtgatc tgggacagat ttcaccctca coactaatag cctggaagct 240
 gaagatgctg caacgtatta ctgtcatcag agtagtagtt tacctcacac ttcggcgga 300
 gggaccaagg tggagatcaa acgaaactgtg gctgcacat ctgtcttcat cttcccgcga 360
 tctgatgagc agttgaaatc tggaactgcc tctgttggtg gctgctgaa taacttctat 420
 cccagagagg ccaaagtaca gtggaagtg gataacgccc tccaatcggg ta 472

<210> SEQ ID NO 36
 <211> LENGTH: 157
 <212> TYPE: PRT
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 36

Glu Ile Val Leu Thr Gln Ser Pro Asp Phe Gln Ser Val Thr Pro Lys
 1 5 10 15

Glu Lys Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Gly Ser Ser
 20 25 30

Leu His Trp Tyr Gln Gln Lys Pro Asp Gln Ser Pro Lys Leu Leu Ile
 35 40 45

Lys Tyr Ala Ser Gln Ser Phe Ser Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Asn Ser Leu Glu Ala
 65 70 75 80

-continued

Glu Asp Ala Ala Thr Tyr Tyr Cys His Gln Ser Ser Ser Leu Pro His
 85 90 95
 Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
 100 105 110
 Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
 115 120 125
 Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
 130 135 140
 Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
 145 150 155

<210> SEQ ID NO 37
 <211> LENGTH: 1335
 <212> TYPE: DNA
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 37

cagggtccagt tggtagcagtc tggggctgag gtgaagaagc ctggggcctc agtgaaggtc 60
 tcctgcaagg tttccggata caccctcact gaattatcca tgcactgggt ggcacaggct 120
 cctggaaaag ggcttgagtg gatgggaggt tttgatcctg aagatggtga aacaatctac 180
 gcacagaagt tccagggcag agtcagtatg accgaggaca catccacaga cacagcctac 240
 atggagctga gcagcctgag atctgaggac acggccgtgt atttctgtgc aaccaacgaa 300
 ttttgagtg gttattttga ctactggggc cagggaaccc tggtcaccgt ctctcagcc 360
 tccaccaagg gcccatcggt cttcccctg gcgccctgct ccaggagcac ctccgagagc 420
 acagcgccc tgggctgctt ggtcaaggac tacttcccgc aaccgggtgac ggtgtcgtgg 480
 aactcaggcg ctctgaccag cggcgtgcac accttcccag ctgtcttaca gtctctcagga 540
 ctctactccc tcagcagcgt ggtgaccgtg ccctccagca acttcggcac ccagacctac 600
 acctgcaacg tagatcacia gccacgcaac accaagggtg acaagacagt tgagcgaaa 660
 tgttggtgag agtgcccacc gtgcccagca ccacctgtgg caggaccgtc agtcttcttc 720
 tccccccaa aaccaagga caccctcatg atctcccga cccctgaggt cagtgctgtg 780
 gtggtggagc tgagccacga agaccccag gtccagtcca actggtactg ggacggcgtg 840
 gaggtgcata atgccaagac aaagccacgg gaggagcagt tcaacagcac gttccgtgtg 900
 gtcagcgtcc tcaccgttgt gcaccaggac tggctgaacg gcaaggagta caagtgcaag 960
 gtctccaaca aaggcctccc agccccatc gagaaaacca tctccaaaac caaagggcag 1020
 ccccgagaac cacaggtgta caccctgccc ccatcccggg aggagatgac caagaaccag 1080
 gtcagcctga cctgctgtgt caaaggcttc taccacagcg acatgcccgt ggagtgggag 1140
 agcaatgggc agccggagaa caactacaag accacacctc ccatgctgga ctccgacggc 1200
 tccttcttcc tctacagcaa gctcaccgtg gacaagagca ggtggcagca ggggaacgtc 1260
 ttctcatgct ccgtgatgca tgaggctctg cacaaccact acacgcagaa gagcctctcc 1320
 ctgtctccgg gtaaa 1335

<210> SEQ ID NO 38
 <211> LENGTH: 445
 <212> TYPE: PRT
 <213> ORGANISM: Homosapien

-continued

<400> SEQUENCE: 38

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15
 Ser Val Lys Val Ser Cys Lys Val Ser Gly Tyr Thr Leu Thr Glu Leu
 20 25 30
 Ser Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
 35 40 45
 Gly Gly Phe Asp Pro Glu Asp Gly Glu Thr Ile Tyr Ala Gln Lys Phe
 50 55 60
 Gln Gly Arg Val Ser Met Thr Glu Asp Thr Ser Thr Asp Thr Ala Tyr
 65 70 75 80
 Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe Cys
 85 90 95
 Ala Thr Asn Glu Phe Trp Ser Gly Tyr Phe Asp Tyr Trp Gly Gln Gly
 100 105 110
 Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
 115 120 125
 Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
 130 135 140
 Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
 145 150 155 160
 Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
 165 170 175
 Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
 180 185 190
 Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro
 195 200 205
 Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu
 210 215 220
 Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
 225 230 235 240
 Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
 245 250 255
 Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln
 260 265 270
 Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
 275 280 285
 Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu
 290 295 300
 Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
 305 310 315 320
 Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
 325 330 335
 Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
 340 345 350
 Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
 355 360 365
 Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
 370 375 380
 Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly
 385 390 395 400

-continued

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
 405 410 415

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
 420 425 430

His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 435 440 445

<210> SEQ ID NO 39
 <211> LENGTH: 660
 <212> TYPE: DNA
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 39

gacatcgtga tgaccagtc tccagactcc ctggctgtgt ctctgggcca gagggccacc 60
 atcaactgca agtccagcca gagtgtttta tacagctcca acaataagaa ctatttagtt 120
 tggtagcagc agagaccagg acagcctcct aagctgctca tttactgggc atctaccggg 180
 gaatccgggg tcctgaccg attcagtggc agcgggtctg ggacagattt cactctcacc 240
 atcagcagcc tgcaggctga agatgtggca gtttattact gtcagcaata tttttattct 300
 ccgtggacgt tcggccaagg gaccaaggta gaaatcaaac gaactgtggc tgcaccatct 360
 gtcttcatct tcccgccatc tgatgagcag ttgaaatctg gaactgcctc tgttgtgtgc 420
 ctgctgaata acttctatcc cagagaggcc aaagtacagt ggaaggtgga taacgcctc 480
 caatcgggta actcccagga gagtgtcaca gagcaggaca gcaaggacag cacctacagc 540
 ctgagcagca cctgacgct gagcaaagca gactacgaga aacacaaagt ctacgcctgc 600
 gaagtcaccc atcagggcct gagctcgccc gtcacaaaga gttcaacag gggagagtgt 660

<210> SEQ ID NO 40
 <211> LENGTH: 220
 <212> TYPE: PRT
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 40

Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
 1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser
 20 25 30

Ser Asn Asn Lys Asn Tyr Leu Val Trp Tyr Gln Gln Arg Pro Gly Gln
 35 40 45

Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
 50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
 65 70 75 80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
 85 90 95

Tyr Phe Tyr Ser Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
 100 105 110

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
 115 120 125

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
 130 135 140

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu

-continued

145	150	155	160
Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp	165	170	175
Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr	180	185	190
Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser	195	200	205
Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys	210	215	220

<210> SEQ ID NO 41
 <211> LENGTH: 556
 <212> TYPE: DNA
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 41

```

cagggtccagc tggtagcagtc tggggctgag gtgaagaagc ctggggcctc agtgaaggtc      60
tcctgcaagg tttccggaca cattttcact gaattatcca tacactgggt gcgacaggct      120
cctggaaaag ggctcgagtg gatgggaggt tttgatcctg aagatggtga aacaatctac      180
gcacagaagt tccagggcag agtcaccatg accgaggaca catctacaga cacagtctac      240
atggagctga gcagcctgag atctgaggac acggccctgt attactgtgc aaccaacgat      300
ttttggagtg gttattttga ctactggggc caggaaccc tggtcaccgt ctctcagcc      360
tccaccaagg gccatcgggt cttccccctg gcgccctgct ccaggagcac ctccgagagc      420
acagcggccc tgggctgcct ggtaaggac tacttccccg aaccggtgac ggtgtcgtgg      480
aactcaggcg ctctgaccag cggcgtgcac accttcccag ctgtcttaca gtctctcagga      540
ctctactccc tcagca
    
```

<210> SEQ ID NO 42
 <211> LENGTH: 185
 <212> TYPE: PRT
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 42

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

-continued

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
 145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
 165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser
 180 185

<210> SEQ ID NO 43
 <211> LENGTH: 490
 <212> TYPE: DNA
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 43

gacatcgtga tgaccacgctc tccaggtctc ctggctgtgt ctctgggcga gagggccacc 60
 atcaactgca agtccagcca gagtatttta ttcaggtcca acaataagaa ctatttaact 120
 tggtagcagc agaaccagg acagcctcct aaactgctca tttactgggc atctatccgg 180
 gaatccgggg tcctgatcg attcagtgcc agcgggtctg ggtcaaattt cactctcacc 240
 atcaccagcc tgcaggctga agatgtggca atttattact gtcagcaata ttatagtagt 300
 ccgtggacgt tcggccaagg gaccaaggtg gaaatcaaac gaactgtggc tgcaccatct 360
 gtcttcatct tcccgccatc tgatgagcag ttgaaatctg gaactgcctc tgttgtgtgc 420
 ctgctgaata acttctatcc cagagaggcc aaagtacagt ggaaggtgga taacgcctc 480
 caatcgggta 490

<210> SEQ ID NO 44
 <211> LENGTH: 163
 <212> TYPE: PRT
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 44

Asp Ile Val Met Thr Gln Ser Pro Gly Ser Leu Ala Val Ser Leu Gly
 1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Ile Leu Phe Arg
 20 25 30

Ser Asn Asn Lys Asn Tyr Leu Thr Trp Tyr Gln Gln Lys Pro Gly Gln
 35 40 45

Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Ile Arg Glu Ser Gly Val
 50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Ser Asn Phe Thr Leu Thr
 65 70 75 80

Ile Thr Ser Leu Gln Ala Glu Asp Val Ala Ile Tyr Tyr Cys Gln Gln
 85 90 95

Tyr Tyr Ser Ser Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
 100 105 110

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
 115 120 125

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
 130 135 140

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
 145 150 155 160

Gln Ser Gly

-continued

<210> SEQ ID NO 45

<211> LENGTH: 559

<212> TYPE: DNA

<213> ORGANISM: Homosapien

<400> SEQUENCE: 45

```

caggtccagc tggtagctc tggggctgag gtgaagaagc ctggggcctc agtgaaggtc      60
tcctgcaagg ttccggata caccctcact gaattatcca tgcactgggt gcgacaggct      120
cctgaaaag ggcttgagtg gatgggaggt ttgatcctg aagatggtga aacaatcaac      180
gcacagaagt tccagggcag agtcaccatg accgaggaca catctacaga cacaggctac      240
atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc aacagatcct      300
ggtggatata gtggctactt tgaccactgg ggccagggaa ccctggteac cgtctcctca      360
gcctccacca agggcccatc ggtcttcccc ctggcgcctt gctccaggag cacctccgag      420
agcacagcgg ccctgggctg cctgggtcaag gactacttcc ccgaaccggt gacggtgtcg      480
tggaaactcag gcgctctgac cagcggcgtg cacaccttcc cagctgtcct acagtcctca      540
ggactctact ccttcagca                                     559

```

<210> SEQ ID NO 46

<211> LENGTH: 186

<212> TYPE: PRT

<213> ORGANISM: Homosapien

<400> SEQUENCE: 46

```

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

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<210> SEQ ID NO 47

<211> LENGTH: 464

<212> TYPE: DNA

-continued

<213> ORGANISM: Homosapien

<400> SEQUENCE: 47

```

gacatcgtga tgaccagtc tccagatttc ctggctgtgt ctctgggcga gaggcccacc    60
atcaactgca agtccagcca gagtgttttt tacagctcca acaataagaa ctacttagtt    120
tggtagccagc agaaacccgg acagcctcct aagctgctcc tttactgggc atctaccggg    180
gaatccgggg tcctgaccg attcagtggc agcgggtctg ggacagattt cactctcacc    240
atcagcagcc tgcaggctga agatgtggca gtttattact gtcagcaata ttatagttct    300
ccgtggacgt tcggccaagg gaccaagggt gaaatcaaac gaactgtggc tgcaccatct    360
gtttctatct tcccgcctc tgatgagcag ttgaaatctg gaactgcctc tgttgtgtgc    420
ctgctgaata actttatcc cagagaggcc aaagtacagt ggaa                        464

```

<210> SEQ ID NO 48

<211> LENGTH: 154

<212> TYPE: PRT

<213> ORGANISM: Homosapien

<400> SEQUENCE: 48

```

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

```

<210> SEQ ID NO 49

<211> LENGTH: 476

<212> TYPE: DNA

<213> ORGANISM: Homosapien

<400> SEQUENCE: 49

```

caggtccagc tggtagcagtc tggggctgag gtgaagaagc ctggggcctc agtgaaggtc    60
tcctgcaagg tttccggata caccctcact gaattatcca tgcactgggt gcgacaggct    120
cctggaaaag ggcttgagtg gatgggaggt tttgatcctg aagatgatga aacaatctac    180
gcacagaagt tccagggcag agtcaccatg accgaggaca catctacaca cacagcctac    240
atggaactga gcagcctgag atctgaggac acggccgtgt attactgtgc aacacacgat    300

```

-continued

```

ttttggagtg cttattttta ctactggggc caggggaacc tggtaccgt ctctcagct 360
tccaccaagg gccatccgt cttcccctg gcgccctgct ccaggagcac ctccgagagc 420
acagccgccc tgggctgctt ggtaaggac tacttcccgc aaccgggtgac ggtgtc 476

```

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<210> SEQ ID NO 50
<211> LENGTH: 158
<212> TYPE: PRT
<213> ORGANISM: Homosapien

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<400> SEQUENCE: 50

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```

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

```

```

<210> SEQ ID NO 51
<211> LENGTH: 490
<212> TYPE: DNA
<213> ORGANISM: Homosapien

```

```

<400> SEQUENCE: 51

```

```

gacatcgtga tgaccagtc tccagactcc ctggctgtgt ctctgggcca gagggccacc 60
atcaactgca agtccagcca gagtgtttta tacggctcca acaataagag ctacttagct 120
tggtagcagc agaaccagg acagcctcct aagctgctca tttactgggc atctaccggg 180
gaatccgggg tccctgaccg attcagtgcc agcgggtctg ggacagattt cactctcacc 240
atcagcagcc tgcaggctgc agatgtggca gtttattact gtcagcaaca ttatagtact 300
ccgtgcagtt ttggccaggg gaccaaaactg gagatcaaac gaactgtggc tgcaccatct 360
gtcttcatct tccgcgcatc tgatgagcag ttgaaatctg gaactgcctc tgttgtgtgc 420
ctgctgaata acttctatcc cagagaggcc aaagtacagt ggaaggtgga taacgccctc 480
caatcgggta 490

```

```

<210> SEQ ID NO 52
<211> LENGTH: 163
<212> TYPE: PRT
<213> ORGANISM: Homosapien

```

-continued

<400> SEQUENCE: 52

```

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

<210> SEQ ID NO 53

<211> LENGTH: 550

<212> TYPE: DNA

<213> ORGANISM: Homosapien

<400> SEQUENCE: 53

```

cagggtgcagc tgggtgcagtc tggggctgag gtgaagaagc ctggggcctc agtgaaggtc      60
tcctgcaagg cttctggata caccttcacc ggctactatc tgcaactgggt gcgacaggcc      120
cctggacaag ggcttgagtg gatgggatgg atcaaccctt acaatgatgg cacaaactat      180
gcacagaagt ttcagggcag ggtcaccatg accagggaca cgtccatcag cacagcctac      240
atggagctga gcaggctgag atctgacgac acggccgctt attactgtgc gagagatata      300
gccgcagctg gagccgtcta ctttgactac tggggccagg gaaccctggt caccgtctcc      360
tcagcttcca ccaagggcc atccgtcttc ccctggcgc cctgetccag gacacctcc      420
gagagcacag ccgccctggg ctgcctggtc aaggactact ttcccgaac cggtgacggt      480
gtcgtggaac tcaggcggcc tgaccagcgg cgtgcacacc ttcccgctg tcctacagtc      540
ctcaggactt                                     550
    
```

<210> SEQ ID NO 54

<211> LENGTH: 183

<212> TYPE: PRT

<213> ORGANISM: Homosapien

<400> SEQUENCE: 54

```

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1           5           10           15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
          20           25           30
    
```

-continued

Tyr Leu His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
 35 40 45

Gly Trp Ile Asn Pro Tyr Asn Asp Gly Thr Asn Tyr Ala Gln Lys Phe
 50 55 60

Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
 65 70 75 80

Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg Asp Ile Ala Ala Ala Gly Ala Val Tyr Phe Asp Tyr Trp Gly
 100 105 110

Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
 115 120 125

Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala
 130 135 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Arg Thr Gly Asp Gly
 145 150 155 160

Val Val Glu Leu Arg Arg Pro Asp Gln Arg Arg Ala His Leu Pro Gly
 165 170 175

Cys Pro Thr Val Leu Arg Thr
 180

<210> SEQ ID NO 55
 <211> LENGTH: 458
 <212> TYPE: DNA
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 55

gacatccaga tgaccacgct tccatcctcc ctgtctgcat ctgtaggaga cagagtcacc 60
 atcacttgcc aggcgagatc ggacattacc acctatttaa attggtatca gcagaaacca 120
 gggaaagccc ctaagctcct gatctacgat gcaccaatt tggaaacagg ggtcccatca 180
 aggttcagtg gaagtggatc tgggacagat tttactttca ccacagcag cctgcagcct 240
 gaagatattg caacatatta ctgtcaacaa tatgataatc tcccgatcac cttcggccaa 300
 gggacacgac tggagattaa acgaaactgtg gctgcacat ctgtcttcat cttcccgcga 360
 tctgatgagc agttgaaatc tggaaactgcc tctgttgtgt gctgtctgaa taacttctat 420
 cccagagagg ccaaagtaca ggaaggtgg ataacgcc 458

<210> SEQ ID NO 56
 <211> LENGTH: 152
 <212> TYPE: PRT
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 56

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Thr Thr Tyr
 20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
 35 40 45

Tyr Asp Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro
 65 70 75 80

-continued

Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu
 130 135 140

Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
 145 150 155 160

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
 165 170 175

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser
 180 185 190

<210> SEQ ID NO 59
 <211> LENGTH: 458
 <212> TYPE: DNA
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 59

gacatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtaggaga cagagtcacc 60
 atcacttgcc gggcaagtca gggcattaga aatgatttag gctggatca gcagaaacca 120
 gggaaagccc ctaagcgctt gatctatgct acatccagtt tgcaaagtgg ggtcccatca 180
 aggttcagcg gcagtggttc tgggacagaa ttcactctca caatcagcag cctgcagcct 240
 gaagattttg caacttatta ctgtctacag cataatactt acccattcac tttcggccct 300
 gggaccaaag tggatatcaa acgaaactgtg gctgcacat ctgttttcat cttcccgcga 360
 tctgatgagc agttgaaatc tggaactgcc tctgttgtgt gctgtgtgaa taacttctat 420
 cccagagagg ccaaagtaca gtggaagggtg gataacgc 458

<210> SEQ ID NO 60
 <211> LENGTH: 152
 <212> TYPE: PRT
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 60

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Arg Asn Asp
 20 25 30

Leu Gly Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Arg Leu Ile
 35 40 45

Tyr Ala Thr Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln His Asn Thr Tyr Pro Phe
 85 90 95

Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys Arg Thr Val Ala Ala
 100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
 115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
 130 135 140

Lys Val Gln Trp Lys Val Asp Asn
 145 150

<210> SEQ ID NO 61

-continued

<211> LENGTH: 1338

<212> TYPE: DNA

<213> ORGANISM: Homosapien

<400> SEQUENCE: 61

```

cagggtgcagc tgcaggagtc gggcccagga ctggtgaagc cttcacagac cctgtccctc   60
acctgcactg tctcagggtg ctccatcagc agtgggtgta actactggaa ctggatccgc   120
cagcaccagc ggaagggcct ggagtggatt gggtagatct attacagtgg aaacacctac   180
tacaaccctg cctcaagag tcgaattacc atatcaatag acacgtctaa gaaccagttc   240
tccctgacct tgagctctgt gactgccgcg gacacggccg tgtattactg tgcgagagat   300
gggtggagac atgcttttga tatctggggc caagggacaa tggtcaccgt ctcttcagct   360
tccaccaagg gccatccgt cttcccctg gcgccctgct ccaggagcac ctccgagagc   420
acagccgcc tgggctgcct ggtcaaggac tacttcccgc aaccggtgac ggtgtcgtgg   480
aactcaggcg cctgaccag cggcgtgcac accttcccgc ctgtcctaca gtcctcagga   540
ctctactccc tcagcagcgt ggtgaccgtg ccctccagca gcttgggcac gaagacctac   600
acctgcaacg tagatcacia gccagcaac accaagggtg acaagagagt tgagtccaaa   660
tatggtcccc catgccatc atgccagca cctgagttcc tggggggacc atcagtcttc   720
ctgttcccc caaaaccaa ggacactctc atgatctccc ggaccctga ggtcacgtgc   780
gtggtggtgg acgtgagcca ggaagacccc gaggtccagt tcaactggta cgtggatggc   840
gtggaggtgc ataatgccaa gacaaagccc cgggaggagc agtcaacag cacgtaccgt   900
gtggtcagcg tcctcaccgt cctgcaccag gactggctga acggcaagga gtacaagtgc   960
aaggtctcca acaaaggcct cccgtcctcc atcgagaaaa ccatctcaa agccaaaggg  1020
cagccccgag agccacaggt gtacaccctg ccccatccc aggaggagat gaccaagaac  1080
caggtcagcc tgacctgcct ggtcaaaggc ttctacccca ggcacatcgc cgtggagtgg  1140
gagagcaatg ggcagccgga gaacaactac aagaccacgc ctcccgctgt ggactccgac  1200
ggctccttct tcctctacag caggctaacc gtggacaaga gcagggtgca ggaggggaat  1260
gtcttctcat gctccgtgat gcatgaggct ctgcacaacc actacacaca gaagagcctc  1320
tcctgtctc tgggtaaa                                     1338

```

<210> SEQ ID NO 62

<211> LENGTH: 446

<212> TYPE: PRT

<213> ORGANISM: Homosapien

<400> SEQUENCE: 62

```

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

```

-continued

85				90				95							
Cys	Ala	Arg	Asp	Gly	Gly	Asp	Asp	Ala	Phe	Asp	Ile	Trp	Gly	Gln	Gly
			100							105				110	
Thr	Met	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe
		115					120					125			
Pro	Leu	Ala	Pro	Cys	Ser	Arg	Ser	Thr	Ser	Glu	Ser	Thr	Ala	Ala	Leu
		130				135						140			
Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp
		145			150					155					160
Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu
			165						170					175	
Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser
			180						185				190		
Ser	Ser	Leu	Gly	Thr	Lys	Thr	Tyr	Thr	Cys	Asn	Val	Asp	His	Lys	Pro
		195					200					205			
Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Ser	Lys	Tyr	Gly	Pro	Pro
		210				215					220				
Cys	Pro	Ser	Cys	Pro	Ala	Pro	Glu	Phe	Leu	Gly	Gly	Pro	Ser	Val	Phe
		225			230					235					240
Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro
			245						250					255	
Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	Gln	Glu	Asp	Pro	Glu	Val
			260						265				270		
Gln	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr
		275					280					285			
Lys	Pro	Arg	Glu	Glu	Gln	Phe	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val
		290				295					300				
Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys
		305			310					315					320
Lys	Val	Ser	Asn	Lys	Gly	Leu	Pro	Ser	Ser	Ile	Glu	Lys	Thr	Ile	Ser
			325						330					335	
Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro
			340						345					350	
Ser	Gln	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val
		355					360					365			
Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly
		370				375					380				
Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp
		385			390					395					400
Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Arg	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp
			405						410					415	
Gln	Glu	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His
			420						425				430		
Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Leu	Gly	Lys		
		435					440					445			

<210> SEQ ID NO 63

<211> LENGTH: 642

<212> TYPE: DNA

<213> ORGANISM: Homosapien

<400> SEQUENCE: 63

-continued

```

gacatccaga tgaccagtc tccatcctcc ctgtctgcat ctgtaggaga cagagtcacc 60
atcacttgcc aggcgagtc gagacattagc aactatttaa attggtatca gcagaaacca 120
gggaaagccc ctaaactcct gatctacgat gcatccaatt tggaacacag ggtcccatca 180
aggttcagtg gaagtggatc tgggacagat ttactttca ccatcaacag cctgcagcct 240
gaagatattg caacatatta ctgtcaagaa tataataatc tcccgtacag ttttgccag 300
gggaccaagt tggagatcaa acgaaactgtg gctgcacat ctgtottcat cttcccacca 360
tctgatgagc agttgaaatc tggaactgcc tctgttgtgt gcctgctgaa taacttctat 420
cccagagagg ccaaagtaca gtggaagggtg gataacgccc tccaatcggg taactcccag 480
gagagtgtca cagagcagga cagcaaggac agcacctaca gcctcagcag caccctgacg 540
ctgagcaaag cagactacga gaaacacaaa gtctacgcct gcgaagtcac ccatcagggc 600
ctgagctcgc ccgtcacaaa gagcttcaac aggggagagt gt 642

```

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<210> SEQ ID NO 64
<211> LENGTH: 214
<212> TYPE: PRT
<213> ORGANISM: Homosapien

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<400> SEQUENCE: 64

```

```

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

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<210> SEQ ID NO 65
<211> LENGTH: 1341
<212> TYPE: DNA

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-continued

<213> ORGANISM: Homosapien

<400> SEQUENCE: 65

```

caggtccagc tggtagcagc tggggctgag gtgaagaagc ctggggcctc agtgcaggtc      60
tcctgcaagg ttcccgaga caccctcact gaattatcca tgcactgggt ggcacaggct      120
cctggaaaag ggcttgagtg gatgggaggt ttgatcctg aagatgggta aacaatctac      180
gcacggaagt tccaggcag agtcaccatg accgaggaca catctacaga cacagtttac      240
atggagctga gcagcctgag atctgaggac acggccgtgt atttctgtgc aacagattca      300
cgtggatata tgggctactt tgacaactgg ggccagggaa ccctggtcac cgtctcctca      360
gcttccacca agggcccatc cgtcttcccc ctggcgccct gctccaggag cacctccgag      420
agcacagccg ccctgggctg cctgggtcaag gactacttcc ccgaaccggg gacgggtgctg      480
tggaaactcag gcgcccctgac cagcggcgctg cacaccttcc cggtctcct acagtcctca      540
ggactctact cctcagcag cgtgggtgacc gtgccctcca gcagcttggg cacgaagacc      600
tacacctgca acgtagatca caagcccagc aacaccaagg tggacaagag agttgagtcc      660
aaatatggtc ccccatgccc atcatgccc gcacctgagt tcctgggggg accatcagtc      720
ttcctgttcc ccccaaaacc caaggacct ctcatgatet cccggacccc tgaggtcacg      780
tgcgtggtgg tggacgtgag ccaggaagac cccgaggtcc agttcaactg gtacgtggat      840
ggcgtggagg tgcataatgc caagacaaag ccgcgggagg agcagttcaa cagcacgtac      900
cgtgtggtca gcgtcctcac cgtcctgcac caggactggc tgaacggcaa ggagtacaag      960
tgcaaggtct ccaacaaagg cctcccgtcc tccatcgaga aaaccatctc caaagccaaa     1020
gggcagcccc gagagccaca ggtgtacacc ctgcccccat cccaggagga gatgaccaag     1080
aaccaggtca gcctgacctg cctgggtcaaa ggcttctacc ccagcgacat cgcctgggag     1140
tgggagagca atgggcagcc ggagaacaac tacaagacca cgcctcccgt gctggactcc     1200
gacggctcct tcttctctta cagcaggcta accgtggaca agagcaggtg gcaggagggg     1260
aatgtcttct catgctccgt gatgcatgag gctctgcaca accactacac acagaagagc     1320
ctctccctgt ctctgggtaa a                                             1341

```

<210> SEQ ID NO 66

<211> LENGTH: 447

<212> TYPE: PRT

<213> ORGANISM: Homosapien

<400> SEQUENCE: 66

```

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1           5           10          15
Ser Val Gln Val Ser Cys Lys Val Ser Gly Asp Thr Leu Thr Glu Leu
 20          25          30
Ser Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
 35          40          45
Gly Gly Phe Asp Pro Glu Asp Gly Glu Thr Ile Tyr Ala Arg Lys Phe
 50          55          60
Gln Gly Arg Val Thr Met Thr Glu Asp Thr Ser Thr Asp Thr Val Tyr
 65          70          75          80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe Cys
 85          90          95

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Ala Thr Asp Ser Arg Gly Tyr Ser Gly Tyr Phe Asp Asn Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
115 120 125

Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala
130 135 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
180 185 190

Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys
195 200 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro
210 215 220

Pro Cys Pro Ser Cys Pro Ala Pro Glu Phe Leu Gly Gly Pro Ser Val
225 230 235 240

Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr
245 250 255

Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu
260 265 270

Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys
275 280 285

Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser
290 295 300

Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys
305 310 315 320

Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile
325 330 335

Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro
340 345 350

Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu
355 360 365

Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn
370 375 380

Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser
385 390 395 400

Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg
405 410 415

Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu
420 425 430

His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
435 440 445

<210> SEQ ID NO 67

<211> LENGTH: 660

<212> TYPE: DNA

<213> ORGANISM: Homosapien

<400> SEQUENCE: 67

gacatcgtga tgaccagctc tccagactcc ctggctgtgt ctctgggcga gagggccacc

60

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```

atcaactgca agtccagcca gagtgtttta tacagctcca acaataacaa ctacttagtt 120
tggtagcagc agaaccagg acagcctoct aaattgctca tttactgggc atctaccgg 180
gaattcgggg ttctgaccg attcagtggc agcgggtctg ggacagattt cactctcacc 240
atcagcagcc tgcaggctga agatgtggca gtttattact gtcagcaata ttatttttct 300
ccgtggacgt tcggccaagg gaccaagggtg gaaatcaaac gaactgtggc tgcaccatct 360
gtcttcatct tccgcctatc tgatgagcag ttgaaatctg gaactgcctc tgttgtgtgc 420
ctgctgaata acttctatcc cagagaggcc aaagtacagt ggaaggtgga taacgccctc 480
caatcgggta actcccagga gagtgtcaca gagcaggaca gcaaggacag cacctacagc 540
ctcagcagca cctgacgct gagcaaagca gactacgaga aacacaaagt ctacgcctgc 600
gaagtcaccc atcagggcct gagctcgccc gtcacaaaga gcttcaacag gggagagtgt 660

```

<210> SEQ ID NO 68

<211> LENGTH: 220

<212> TYPE: PRT

<213> ORGANISM: Homosapien

<400> SEQUENCE: 68

```

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

```

<210> SEQ ID NO 69

<211> LENGTH: 556

<212> TYPE: DNA

<213> ORGANISM: Homosapien

-continued

<400> SEQUENCE: 69

```

cagggtccagc tggtagcagtc tggggctgag gtgaagaagc ctggggcctc agtgaaggtc      60
tcctgcaagg tttccggata caccctcact gatttatcca tgcactgggt gcgacaggct      120
cctggaaaag ggottgagtg gatgggaggt tttgatcctg aagatgggta aacaatctac      180
gcacagaagt tccagggcag agtcaccatg accgaggaca catcttcaga cacagcctac      240
atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc aacccacgaa      300
ttttggagtg gttattttga ctactggggc cagggaaacc tggtcaccgt ctctcagct      360
tccaccaagg gccatccgt cttcccctg gcgccctgct ccaggagcac ctccgagagc      420
acagccgccc tgggtgcct ggtaaggac tacttcccgc aaccgggtgac ggtgtcgtgg      480
aactcaggcg cctgaccag cggcgtgcac accttcccgg ctgtctaca gtctcagga      540
ctctactccc tcagca                                          556
    
```

<210> SEQ ID NO 70

<211> LENGTH: 185

<212> TYPE: PRT

<213> ORGANISM: Homosapien

<400> SEQUENCE: 70

```

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1           5           10           15
Ser Val Lys Val Ser Cys Lys Val Ser Gly Tyr Thr Leu Thr Asp Leu
 20           25           30
Ser Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
 35           40           45
Gly Gly Phe Asp Pro Glu Asp Gly Glu Thr Ile Tyr Ala Gln Lys Phe
 50           55           60
Gln Gly Arg Val Thr Met Thr Glu Asp Thr Ser Ser Asp Thr Ala Tyr
 65           70           75           80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85           90           95
Ala Thr His Glu Phe Trp Ser Gly Tyr Phe Asp Tyr Trp Gly Gln Gly
 100          105          110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
 115          120          125
Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
 130          135          140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
 145          150          155          160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
 165          170          175
Gln Ser Ser Gly Leu Tyr Ser Leu Ser
 180          185
    
```

<210> SEQ ID NO 71

<211> LENGTH: 476

<212> TYPE: DNA

<213> ORGANISM: Homosapien

<400> SEQUENCE: 71

```

gacatcgtga tgaccagtc tccagactcc ctggctgtgt ctctgggcga gagggccacc      60
    
```

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```

atcaactgca agtccagcca gagtgtttta ttcagctcca acaataagag ctacttaact 120
tggtagcagc agaaaccagg acagcctcct aaattactca tttctgggc atctatccgg 180
gaatccgggg tccctgaccg aatcagtgcc agcgggtctg ggacagatct cactctcacc 240
atcagcagcc tgcaggctga agatgcggca gtttattact gtcagcaata ttatagtagt 300
ccgtggacgt tcggccaagg gaccaagggt gaaatcaaac gaactgtggc tgcaccatct 360
gtcttcatct tccgcctc tgatgagcag ttgaaatctg gaactgcctc tgttgtgtgc 420
ctgctgaata acttctatcc cagagaggcc aaagtacagt ggaaggtgga taacgc 476

```

```

<210> SEQ ID NO 72
<211> LENGTH: 158
<212> TYPE: PRT
<213> ORGANISM: Homosapien

```

```

<400> SEQUENCE: 72

```

```

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

```

```

<210> SEQ ID NO 73
<211> LENGTH: 546
<212> TYPE: DNA
<213> ORGANISM: Homosapien

```

```

<400> SEQUENCE: 73

```

```

cagggtccagc tggtagcagtc tggggctgag gtgaagaagc ctggggcctc agtgaaggtc 60
tcctgcaagg tttccggata caccctcagt gaattatcca tgcactgggt ggcacaggct 120
cctgaaaaag ggcttgagtg gatgggaggt tttgatcctg aagatggtga aataatccac 180
gcacagaagt tccagggcag agtcacatg accgaggaca catctacaga cacagcctac 240
atggagctga gcagcctgag atctgaggac acggcctgtg attactgtgc aacaggcgat 300
ttttggagtg gttattacct tgactggtgg ggccaggaa ccttggtcac cgtctcctca 360
gcttccacca agggccctc cgtcttcccc ctggcgcctt gctccaggag cacctccgag 420
agcacagccg ccttgggctg cctggtcaag gactacttcc ccgaaccggt gacggtgtcg 480

```

-continued

 tggaaactcag gcgccctgac cagcggcgtg cacaccttcc cggtgtcct acagtcctca 540

ggactt 546

<210> SEQ ID NO 74

<211> LENGTH: 182

<212> TYPE: PRT

<213> ORGANISM: Homosapien

<400> SEQUENCE: 74

 Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15

 Ser Val Lys Val Ser Cys Lys Val Ser Gly Tyr Thr Leu Ser Glu Leu
 20 25 30

 Ser Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
 35 40 45

 Gly Gly Phe Asp Pro Glu Asp Gly Glu Ile Ile His Ala Gln Lys Phe
 50 55 60

 Gln Gly Arg Val Thr Met Thr Glu Asp Thr Ser Thr Asp Thr Ala Tyr
 65 70 75 80

 Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

 Ala Thr Gly Asp Phe Trp Ser Gly Tyr Tyr Leu Asp Trp Trp Gly Gln
 100 105 110

 Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
 115 120 125

 Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala
 130 135 140

 Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
 145 150 155 160

 Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
 165 170 175

 Leu Gln Ser Ser Gly Leu
 180

<210> SEQ ID NO 75

<211> LENGTH: 457

<212> TYPE: DNA

<213> ORGANISM: Homosapien

<400> SEQUENCE: 75

gaaatagtga tgatgcagtc tccagccacc ctgtctgtgt ctccagggga aagagccacc 60

ctctcctgca gggccagtca gagtgttaac agcaacttag cctggtacca gcagaaacct 120

ggccaggctc ccaggctcct catcaacggt gcatccacca gggccaactgg catcccagcc 180

aggttcagtg gcagtggttc tgggacagag ttcaccctca ccatcagcag cctgcagtct 240

gaagattttg caatttatta ctgtcagcag tataatgact ggcctacggt cactttcggc 300

ggagggacca aggtggagat caatcgaact gtggctgcac catctgtctt catcttcccg 360

ccatctgatg agcagttgaa atctggaact gcctctgttg tgtgcctgct gaataacttc 420

tatcccagag aggcctaaagt acagtgggaa ggtggat 457

<210> SEQ ID NO 76

<211> LENGTH: 152

<212> TYPE: PRT

-continued

<213> ORGANISM: Homosapien

<400> SEQUENCE: 76

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

<210> SEQ ID NO 77

<211> LENGTH: 470

<212> TYPE: DNA

<213> ORGANISM: Homosapien

<400> SEQUENCE: 77

cagggtccagc tggtagcagtc tggggctgag gtgaagaagc ctggggcctc agtgaaggtc 60
 tcctgcaagg tttccggata caccctcact gaattatcca tgcactgggt gcgacaggct 120
 cctgaaaaag ggcttgagtg gatgggaggt tttgatcctg aagatggtga aacaatgtac 180
 gcacagaagt tccagggcag agtcaccatg accgaggaca catctacaga cacagcctac 240
 atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc aaccgacgat 300
 ttttgagtg gttattttga ctactggggc caggaaccc tggtcaccgt ctctcagcc 360
 tccaccaagg gcccatcggt cttccccctg gcgccctgct ccaggagcac ctccgagagc 420
 acagcggccc tgggctgcct ggtcaaggac tacttccccg aaccggcagg 470

<210> SEQ ID NO 78

<211> LENGTH: 156

<212> TYPE: PRT

<213> ORGANISM: Homosapien

<400> SEQUENCE: 78

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15
 Ser Val Lys Val Ser Cys Lys Val Ser Gly Tyr Thr Leu Thr Glu Leu
 20 25 30
 Ser Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
 35 40 45
 Gly Gly Phe Asp Pro Glu Asp Gly Glu Thr Met Tyr Ala Gln Lys Phe

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50	55	60
Gln Gly Arg Val Thr Met Thr Glu Asp Thr Ser Thr Asp Thr Ala Tyr 65 70 75 80		
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys 85 90 95		
Ala Thr Asp Asp Phe Trp Ser Gly Tyr Phe Asp Tyr Trp Gly Gln Gly 100 105 110		
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe 115 120 125		
Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu 130 135 140		
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Ala 145 150 155		
 <210> SEQ ID NO 79 <211> LENGTH: 490 <212> TYPE: DNA <213> ORGANISM: Homosapien <400> SEQUENCE: 79		
gacatcgtga tgaccagtc tccagactcc ctggctgtgt ctctggacga gagggccacc	60	
atcaactgca agtccagcca gagtgtttta tacagtccca accaaaagaa ctacttagtt	120	
tggtatcagc agaagccagg acagcctcct aagctgctcc tttactgggc atctatccgg	180	
gaatccgggg tcctcgaccg attcagtggc agcgggtctg ggacagattt cactctcacc	240	
atcagcagcc tgcaggctga agatgtggca gtttattact gtcaacaaag ttattttact	300	
ccgtggacgt tcggccaagg gaccaaggtg gaaatcaaac gaactgtggc tgcaccatct	360	
gtcttcatct tcccgccatc tgatgagcag ttgaaatctg gaactgcctc tgttgtgtgc	420	
ctgctgaata acttctatcc cagagaggcc aaagtacagt ggaaggtgga taacgcctc	480	
caatcgggta	490	
 <210> SEQ ID NO 80 <211> LENGTH: 163 <212> TYPE: PRT <213> ORGANISM: Homosapien <400> SEQUENCE: 80		
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Asp 1 5 10 15		
Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser 20 25 30		
Pro Asn Gln Lys Asn Tyr Leu Val Trp Tyr Gln Gln Lys Pro Gly Gln 35 40 45		
Pro Pro Lys Leu Leu Leu Tyr Trp Ala Ser Ile Arg Glu Ser Gly Val 50 55 60		
Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr 65 70 75 80		
Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln 85 90 95		
Ser Tyr Phe Thr Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile 100 105 110		
Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp		

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115	120	125	
Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn			
130	135	140	
Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu			
145	150	155	160
Gln Ser Gly			
<210> SEQ ID NO 81			
<211> LENGTH: 556			
<212> TYPE: DNA			
<213> ORGANISM: Homosapien			
<400> SEQUENCE: 81			
caggtccagc tggtagctc tggggctgag gtgaagaagc ctggggcctc agtgaaggtc	60		
tcctgcaagg tttccggata caccctcagt gaattatcca tgcactgggt gcgacaggct	120		
cctggaagag ggcttgagtg gatgggaggt tttgatcctg aagatgatga aacaatctac	180		
gcacagaagt tccagggcag agtcaccatg accgaggaca catctacaga cacagccttc	240		
atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc aaccacgat	300		
ttttggagtg gttattttca ctactggggc cagggaaacc tggtcaccgt ctctcagct	360		
tccaccaagg gcccatccgt cttccccctg gcgcccctgt ccaggagcac ctccgagagc	420		
acagccgcc tgggtgcct ggtcaaggac tacttccccg aaccggtgac ggtgtcgtgg	480		
aactcaggcg cctgaccag cggcgtgcac acctccccg ctgtctaca gtctcagga	540		
ctctactccc tcagca	556		

<210> SEQ ID NO 82
 <211> LENGTH: 185
 <212> TYPE: PRT
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 82

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

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Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
 165 170 175

Gln Ser Ser Gly Leu Tyr Ser Leu Ser
 180 185

<210> SEQ ID NO 83
 <211> LENGTH: 476
 <212> TYPE: DNA
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 83

gacatcgtga tgaccagtc tccagactcc ctggctgtgt ctctgggcca gagggccacc 60
 atcaactgca agtccagcca gagtgtttta tacagctccg acaataagag ctacttagtt 120
 tggtagcagc agaaaccagg acagcctcct aaggtgctca tttactgggc atctattcgg 180
 gaatccgggg tcctcgaccg attcagtggc agcgggtctg ggacagattt cactctcacc 240
 atcagcagcc tgcaggctga agatgtggca gtttattact gtcagcaata ttatactagt 300
 ccgtggacgt tcggccaagg gaccaaggtg gaaatcaaac gaactgtggc tgcaccatct 360
 gtcttcatct tcccgccatc tgatgagcag ttgaaatctg gaactgcctc tgttgtgtgc 420
 ctgctgaata acttctatcc cagagaggcc aaagtacagt ggaaggtgga taacgc 476

<210> SEQ ID NO 84
 <211> LENGTH: 158
 <212> TYPE: PRT
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 84

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

<210> SEQ ID NO 85
 <211> LENGTH: 543
 <212> TYPE: DNA
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 85

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caggtccagc tggtagctc tggggctgag gtgaagaagc ctggggcctc agtgaaggtc   60
tcctgtaagg ttccggata caccctcact gaattatcca tgcactgggt gcgacaggct   120
cctggaaaag ggcttgagtg gatgggaggt ttgatcctg aagatggtga aacaatctac   180
gcacagaagt tccagggcag agtcaccatg accgaggaca catctacaga cacagcctac   240
atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc aatccacgag   300
ttttggagtg gttattttga ctactggggc cagggaaacc tggtoaccgt ctcttcagct   360
tccaccaagg gccatccgt cttccccctg gcgccctgct ccaggagcac ctccgagagc   420
acagccgccc tgggctgctt ggtaaggac tacttccccg aaccgggtgac ggtgtcgtgg   480
aactcaggcg cctgaccag cggcgtgac accttccccg ctgtcctaca gtctcagga   540
ctt                                                                    543

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<210> SEQ ID NO 86
<211> LENGTH: 181
<212> TYPE: PRT
<213> ORGANISM: Homosapien

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<400> SEQUENCE: 86

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Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1          5          10          15
Ser Val Lys Val Ser Cys Lys Val Ser Gly Tyr Thr Leu Thr Glu Leu
 20          25          30
Ser Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
 35          40          45
Gly Gly Phe Asp Pro Glu Asp Gly Glu Thr Ile Tyr Ala Gln Lys Phe
 50          55          60
Gln Gly Arg Val Thr Met Thr Glu Asp Thr Ser Thr Asp Thr Ala Tyr
 65          70          75          80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85          90          95
Ala Ile His Glu Phe Trp Ser Gly Tyr Phe Asp Tyr Trp Gly Gln Gly
 100          105          110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
 115          120          125
Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
 130          135          140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
 145          150          155          160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
 165          170          175
Gln Ser Ser Gly Leu
 180

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<210> SEQ ID NO 87
<211> LENGTH: 477
<212> TYPE: DNA
<213> ORGANISM: Homosapien

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<400> SEQUENCE: 87

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gacatcgtga tgaccagtc tccagactcc ctggctgtgt ctctgggcca gagggccacc   60
atcaactgca agtccagcct gagtgtttta tacagctcca acaataagaa ctatttagtt   120

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tggtagcttc agaaaccagc acagcctcct aagttgctca tttactgggc atctaccgg 180
gaatccgggg tcctgaccg attcagtgcc agcgggtctg ggacagattt cactctcacc 240
atcagcagcc tgcaggccga agatgtggca gtttattact gtcagcaata ttatagttct 300
ccgtggacgt tcggccaagg gaccaaggtg gaaatcaaac gaactgtggc tgcaccatct 360
gtcttcatct tcccgccatc tgatgagcag ttgaaatctg gaactgcctc tgttgtgtgc 420
ctgctgaata acttctatcc cagagaggcc aaagtacagt ggaaggtgga taacgcc 477

```

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<210> SEQ ID NO 88
<211> LENGTH: 159
<212> TYPE: PRT
<213> ORGANISM: Homosapien

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<400> SEQUENCE: 88

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```

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

```

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<210> SEQ ID NO 89
<211> LENGTH: 1335
<212> TYPE: DNA
<213> ORGANISM: Homosapien

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<400> SEQUENCE: 89

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```

caggtccagc tggtagctc tggggctgag gtgaagaagc ctggggcctc agtgaaggtc 60
tcctgcaagg tttccggata caccctcact gaattatcca tgcactgggt ggcacagact 120
cctggaaaag ggcttgagtg gatgggaggt tttgatcctg aagatggtga aacaatctac 180
gcacagaagt tccaggacag agtcaccatg accgaggaca catctacaga cacagcctac 240
atggaactga gcagcctgag atctgaggac acggccgtgt attactgtgc acaaacgat 300
ttttgactg gttattatga ctactggggc cagggaaacc tggtcaccgt ctctcagcc 360
tccaccaagg gcccatcgtt cttcccctg gcgcctgct ccaggagcac ctccgagagc 420
acagggccc tgggtgcct ggtcaaggac tacttcccg aaccggtgac ggtgtcgtgg 480
aactcaggcg ctctgaccag cggcgtgcac accttcccag ctgtctaca gtcctcagga 540

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ctctactccc tcagcagcgt ggtgaccgtg ccctccagca acttoggcac ccagacctac 600
acctgcaacg tagatcaciaa gccccagcaac accaaggtgg acaagacagt tgagcgcaaa 660
tgttgtgtcg agtgcccacc gtgcccagca ccacctgtgg caggaccgtc agtcttcttc 720
ttcccccaaa aaccaagga caccctcatg atctcccga cccctgaggt cacgtgcgtg 780
gtggtggacg tgagccacga agaccccag gtccagtcca actggtacgt ggacggcgtg 840
gaggtgcata atgccaagc aaagccacgg gaggagcagt tcaacagcac gttccgtgtg 900
gtcagcgtcc tcaccgttgt gcaccaggac tggctgaacg gcaaggagta caagtgcaag 960
gtctccaaca aaggcctccc agcccccatc gagaaaacca tctccaaaac caaagggcag 1020
ccccgagaac cacaggtgta caccctgccc ccatcccggg aggagatgac caagaaccag 1080
gtcagcctga cctgcctggt caaaggcttc taccacagcg acatcgccgt ggagtgggag 1140
agcaatgggc agccggagaa caactacaag accacacctc ccatgctgga ctccgacggc 1200
tccttcttcc tctacagcaa gctcaccgtg gacaagagca ggtggcagca ggggaacgtc 1260
ttctcatgct ccgtgatgca tgaggctctg cacaaccact acacgcagaa gagcctctcc 1320
ctgtctccgg gtaaa 1335

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<210> SEQ ID NO 90

<211> LENGTH: 445

<212> TYPE: PRT

<213> ORGANISM: Homosapien

<400> SEQUENCE: 90

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

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210	215	220
Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu 225 230 235 240		
Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu 245 250 255		
Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln 260 265 270		
Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys 275 280 285		
Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu 290 295 300		
Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys 305 310 315 320		
Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys 325 330 335		
Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser 340 345 350		
Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys 355 360 365		
Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln 370 375 380		
Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly 385 390 395 400		
Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln 405 410 415		
Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn 420 425 430		
His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys 435 440 445		

<210> SEQ ID NO 91
 <211> LENGTH: 660
 <212> TYPE: DNA
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 91

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gacatcgtga tgaccagtc tccagactcc ctggctgtgt ctctgggcga gagggccacc 60
atcaactgca agtccagcca gagtgtttta tacagctcca acaataagaa ctacttagtt 120
tggtagcagc agaaccagg acagcctoct aagacgctca tttactgggc atctaccggg 180
gaatccgggg tccctgaccg attcagtggc agcgggtctg ggacagattt cactctcacc 240
atcagcagcc tgcaggctga agatgtggga gtttattact gtcaacaata ttatactagt 300
ccgtggacgt tcggccaagg gaccaagggtg gaaatcaagc gaactgtggc tgcaccatct 360
gtcttcatct tccgcgcatc tgatgagcag ttgaaatctg gaactgcctc tgttgtgtgc 420
ctgctgaata acttctatcc cagagaggcc aaagtacagt ggaaggtgga taacgccctc 480
caatcgggta actcccagga gagtgtcaca gagcaggaca gcaaggacag cacctacagc 540
ctcagcagca cctgacgct gagcaaagca gactacgaga aacacaaagt ctacgcctgc 600
gaagtcaccc atcagggcct gagctcgccc gtcacaaaga gcttcaacag gggagagtgt 660
    
```

<210> SEQ ID NO 92

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<211> LENGTH: 220
<212> TYPE: PRT
<213> ORGANISM: Homosapien

<400> SEQUENCE: 92

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

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<210> SEQ ID NO 93
<211> LENGTH: 560
<212> TYPE: DNA
<213> ORGANISM: Homosapien

<400> SEQUENCE: 93

cagggtgcagc tgcaggagtc gggcccagga ctggtgaagc cgtcacagac cctgtccctc      60
acctgcactg tctctggtgg ctccatcagc agtgggtggt actactggag ctggatccgc      120
cagcaccagc ggaaggcctt ggagtggatt gggtacatct attacagtgg gagcacctac      180
tacaaccctg ccctcaagag tcgagttatc atatcagtag acacgtctaa gaaccagttc      240
tccctgaagc tgacctctgt gactgccgag gacacggccg tgtattactg tgcgagatca      300
tatagcagct cgtccccact ggttcgacct ctggggccag ggaaccctgg tcaccgtctc      360
ctcagcttcc accaagggcc catccgtctt ccccctggcg ccctgctcca ggagcacctc      420
cgagagcaca gccgccttgg gctgcctggt caaggactac ttccccgaac cggtgacggt      480
gtcgtggaac tcaggcggcc tgaccagcgg cgtgcacacc ttcccggctg tcctacagtc      540
ctcaggactc tactccctca

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<210> SEQ ID NO 94

<211> LENGTH: 186

<212> TYPE: PRT

<213> ORGANISM: Homosapien

<400> SEQUENCE: 94

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
 1 5 10 15
 Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Gly
 20 25 30
 Gly Tyr Tyr Trp Ser Trp Ile Arg Gln His Pro Gly Lys Gly Leu Glu
 35 40 45
 Trp Ile Gly Tyr Ile Tyr Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser
 50 55 60
 Leu Lys Ser Arg Val Ile Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
 65 70 75 80
 Ser Leu Lys Leu Thr Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
 85 90 95
 Cys Ala Arg Ser Tyr Ser Ser Ser Ser Pro Leu Val Arg Pro Leu Gly
 100 105 110
 Pro Gly Asn Pro Gly His Arg Leu Leu Ser Phe His Gln Gly Pro Ile
 115 120 125
 Arg Leu Pro Pro Gly Ala Leu Leu Gln Glu His Leu Arg Glu His Ser
 130 135 140
 Arg Pro Gly Leu Pro Gly Gln Gly Leu Leu Pro Arg Thr Gly Asp Gly
 145 150 155 160
 Val Val Glu Leu Arg Arg Pro Asp Gln Arg Arg Ala His Leu Pro Gly
 165 170 175
 Cys Pro Thr Val Leu Arg Thr Leu Leu Pro
 180 185

<210> SEQ ID NO 95

<211> LENGTH: 458

<212> TYPE: DNA

<213> ORGANISM: Homosapien

<400> SEQUENCE: 95

gacatccaga tgaccacgctc tccatcctcc ctgtctgcat ctgtaggaga cagagtcacc 60
 atcacttgcc gggcaagtca gggcattaga aatgatttag gotggatca gcagaaacca 120
 gggaaagccc ctaagcgcct gatctatgct gcatccagtt tgcaaagtgg ggtcccatca 180
 aggttcagcg gcagtggatc tgggacagaa ttcactctca caatcagcag cctgcagcct 240
 gaagattttg caacttatta ctgtctacag cataatagtt acccattcac tttcggcct 300
 gggaccaaag tggatatcaa acgaactgtg gctgcacat ctgtttcat cttcccgcca 360
 tctgatgagc agttgaaatc tggaactgcc tctgttgtgt gctgctgaa taacttctat 420
 cccagagagg ccaaagtaca gtggaagtg gataacgc 458

<210> SEQ ID NO 96

<211> LENGTH: 152

<212> TYPE: PRT

<213> ORGANISM: Homosapien

<400> SEQUENCE: 96

-continued

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

<210> SEQ ID NO 97
 <211> LENGTH: 559
 <212> TYPE: DNA
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 97

```

caggtccagc tggtagcgtc tggggctgag gtgaagaagc ctggggcctc agtgaaggtc    60
tcctgcaagg tttccggata caccctcact gaattatcca tgcactgggt ggcacaggct    120
cctggaaaag ggcttgagtg gatgggaggt tttgatcctg aagatggtga aacaatctac    180
gcacagaagt tccaggcagc agtcaccatg accgaggaca catctacaga cacagcctac    240
atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc aacagatcgc    300
gagttttgga gtggttattt ctaccactgg ggccaggaa ccttggtcac cgtctcctca    360
gcctccacca agggcccatc ggttctcccc ctggcgccct gctccaggag cacctccgag    420
agcacagcgg ccctgggctg cctgggtcaag gactacttcc ccgaaccggt gacggtgtcg    480
tggaactcag gcgctctgac cagcggcgtg cacaccttcc cagctgtcct acagtcctca    540
ggactctact ccctcagca                                     559
  
```

<210> SEQ ID NO 98
 <211> LENGTH: 186
 <212> TYPE: PRT
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 98

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15
 Ser Val Lys Val Ser Cys Lys Val Ser Gly Tyr Thr Leu Thr Glu Leu
 20 25 30
 Ser Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
 35 40 45
 Gly Gly Phe Asp Pro Glu Asp Gly Glu Thr Ile Tyr Ala Gln Lys Phe

-continued

Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
 130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
 145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
 165 170 175

Gln Ser Ser Gly Leu
 180

<210> SEQ ID NO 103
 <211> LENGTH: 491
 <212> TYPE: DNA
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 103

gacatcgtga tgaccagtc tccagactcc ctggctgtgt ctctgggcga gagggccacc 60
 atcaactgca agtccagtc gagtgtttta tacaggtcta acaataagag ctacttagtt 120
 tggtaaccagc agaaactagg acagtctcct aagctgctca tttactgggc atctaccgg 180
 gaatccgggg tcctgaccg attcagtggc agcgggtctg ggacagattt cactctcacc 240
 atcagcagcc tgcaggctga agatgtggca gtttattatt gtcaacaata ttatagtact 300
 ccgtggacgt tcggccaagg gaccaagggt gaaatcaaac gaactgtggc tgcaccatct 360
 gtcttcatct tcccgccatc tgatgagcag ttgaaatctg gaactgcctc tgttgtgtgc 420
 ctgctgaata acttctatcc cagagagcc aaagtacagt ggaaggtgga taacgcctc 480
 ccaatcgggt a 491

<210> SEQ ID NO 104
 <211> LENGTH: 163
 <212> TYPE: PRT
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 104

Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
 1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Leu Tyr Arg
 20 25 30

Ser Asn Asn Lys Ser Tyr Leu Val Trp Tyr Gln Gln Lys Leu Gly Gln
 35 40 45

Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
 50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
 65 70 75 80

Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
 85 90 95

Tyr Tyr Ser Thr Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
 100 105 110

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
 115 120 125

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
 130 135 140

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
 145 150 155 160

-continued

Pro Ile Gly

<210> SEQ ID NO 105
 <211> LENGTH: 499
 <212> TYPE: DNA
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 105

```

cagggtccagc tggtagcagtc tggggctgag gtgaagaagc ctggggcctc agtgaaggtc      60
tcctgcaagg tttccggata caccctcact gaattatcca tgcactgggt gcgacaggct      120
cctggaaaag ggcttgagtg gatgggaggt tttgatcctg aagatggtga aacaatctac      180
gcacagaagt tccagggcag agtcaccatg accgaggaca catctacaga cacagcctac      240
atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc aacagacgat      300
ttttgagtg gttattttga ctactggggc cagggaaccc tggtcaccgt ctctcagcc      360
tccaccaagg gcccatcggt cttccccctg gcgccctgct ccaggagcac ctccgagagc      420
acagcggccc tgggctgcct ggtaaggac tacttccccg aaccgggtgac ggtgtcgtgg      480
aactcaggcg ctctgacca                                          499
  
```

<210> SEQ ID NO 106
 <211> LENGTH: 166
 <212> TYPE: PRT
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 106

```

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1           5           10          15
Ser Val Lys Val Ser Cys Lys Val Ser Gly Tyr Thr Leu Thr Glu Leu
 20          25          30
Ser Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
 35          40          45
Gly Gly Phe Asp Pro Glu Asp Gly Glu Thr Ile Tyr Ala Gln Lys Phe
 50          55          60
Gln Gly Arg Val Thr Met Thr Glu Asp Thr Ser Thr Asp Thr Ala Tyr
 65          70          75          80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85          90          95
Ala Thr Asp Asp Phe Trp Ser Gly Tyr Phe Asp Tyr Trp Gly Gln Gly
 100         105         110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
 115         120         125
Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
 130         135         140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
 145         150         155         160
Asn Ser Gly Ala Leu Thr
 165
  
```

<210> SEQ ID NO 107
 <211> LENGTH: 448
 <212> TYPE: DNA
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 107

-continued

```

gacatcgtga tgaccacgtc tccagactcc ctggctgtgt ctctgggcga gagggccacc 60
atcaactgca agtccagcca gagtgtttta tacagctcca acaataagaa ctacttagtt 120
tggtagcagc agaaaccagg acagcctcct aagctgctca tttactgggc atctaccgg 180
gaatccgggg tcctgaccg attcagtggc agcgggtctg ggacagattt cactctcacc 240
atcagcagcc tgcaggctga agatgtggca gtttattact gtcagcaata ttatagtcct 300
acgtggacgt tcggccaagg gaccaagggt gaaatcaaac gaactgtggc tgcaccatct 360
gtcttcatct tcccgccatc tgatgagcag ttgaaatctg gaactgcctc tgttgtgtgc 420
ctgctgaata acttctatcc cagagagg 448

```

```

<210> SEQ ID NO 108
<211> LENGTH: 149
<212> TYPE: PRT
<213> ORGANISM: Homosapien

```

```

<400> SEQUENCE: 108

```

```

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

```

```

<210> SEQ ID NO 109
<211> LENGTH: 540
<212> TYPE: DNA
<213> ORGANISM: Homosapien

```

```

<400> SEQUENCE: 109

```

```

caggtccagc tggtagcagtc tggggctgag gtgaagaagc ctggggcctc agtgaaggtc 60
tcctgcaagg tttccggata caccctcact gaattatcca tgcactgggt ggcacaggct 120
cctggaaaag ggcttgagtg gatgggaggt tttgatcctg aagatggtga aacaatctac 180
gcacagaagt tccagggcag agtcaccatg accgaggaca catctacaga cacagcctac 240
atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc aacggacgat 300
ttttgagtg gttattttga ctactggggc cagggaaacc tggtcaccgt ctctcagcc 360
tccaccaagg gcccatcggg cttcccctg gcgcctgct ccaggagcac ctccgagagc 420

```

-continued

```
acagcggccc tgggctgcct ggtcaaggac tacttccccg aaccggtgac ggtgtcgtgg 480
aactcaggcg ctctgaccag cggcgtgcac accttcccag ctgtctaca gtcctcagga 540
```

```
<210> SEQ ID NO 110
<211> LENGTH: 180
<212> TYPE: PRT
<213> ORGANISM: Homosapien
```

```
<400> SEQUENCE: 110
```

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1           5           10           15
Ser Val Lys Val Ser Cys Lys Val Ser Gly Tyr Thr Leu Thr Glu Leu
          20           25           30
Ser Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
          35           40           45
Gly Gly Phe Asp Pro Glu Asp Gly Glu Thr Ile Tyr Ala Gln Lys Phe
 50           55           60
Gln Gly Arg Val Thr Met Thr Glu Asp Thr Ser Thr Asp Thr Ala Tyr
 65           70           75           80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
          85           90           95
Ala Thr Asp Asp Phe Trp Ser Gly Tyr Phe Asp Tyr Trp Gly Gln Gly
          100          105          110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
          115          120          125
Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
          130          135          140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
          145          150          155          160
Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
          165          170          175
Gln Ser Ser Gly
          180
```

```
<210> SEQ ID NO 111
<211> LENGTH: 478
<212> TYPE: DNA
<213> ORGANISM: Homosapien
```

```
<400> SEQUENCE: 111
```

```
gacatcgtga tgaccagtc tccagactcc ctggctgtgt ctctgggcga gagggccacc 60
atcaactgca agtccagcca gagtgtttta tacagctcca acaataagaa ctacttagct 120
tggtaccagc agaaaccagg acagcctcct aagctgctca tttactggac atctaccggg 180
gaatccgggg tccctgaccg attcagtgcc agcgggtctg tgacagattt cactctcacc 240
atcagcagcc tgcaggctga agatgtggca gtttattact gtcagcaata ttatagttct 300
ccgtggacgt tcggccaagg gaccaaggtg gaaatcaaac gaactgtggc tgcaccatct 360
gtcttcatct tcccgccatc tgatgagcag ttgaaatctg gaactgcctc tgttgtgtgc 420
ctgctgaata acttctatcc cagagaggcc aaagtacagt ggaaggtgga taacgcct 478
```

```
<210> SEQ ID NO 112
<211> LENGTH: 159
```

-continued

```

<212> TYPE: PRT
<213> ORGANISM: Homosapien
<400> SEQUENCE: 112
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
 1             5             10             15
Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser
 20             25             30
Ser Asn Asn Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
 35             40             45
Pro Pro Lys Leu Leu Ile Tyr Trp Thr Ser Thr Arg Glu Ser Gly Val
 50             55             60
Pro Asp Arg Phe Ser Gly Ser Gly Ser Val Thr Asp Phe Thr Leu Thr
 65             70             75             80
Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
 85             90             95
Tyr Tyr Ser Ser Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
 100            105            110
Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
 115            120            125
Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
 130            135            140
Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala
 145            150            155

```

```

<210> SEQ ID NO 113
<211> LENGTH: 542
<212> TYPE: DNA
<213> ORGANISM: Homosapien
<400> SEQUENCE: 113
cagggtccagc tggtagcagtc tggggctgag gtgaagaagc ctggggcctc agtgaaggtc      60
tcctgcaagg tttccggata caccctcagt gaattatcca tgcactgggt gcgacaggct      120
cctggaaaag ggcttgagtg gatgggaggt tttgatcctg aagatggtga aacaatctac      180
gcacagaagt tccagggcag agtcacatg accgaggaca catctacaga cacagcctac      240
atggagctga gcagcctgag atctgaggac acggccgtgt tttactgtgc aacaaagagg      300
gaatatagtg gctactttga ctactggggc cagggaaacc tggtcaccgt ctctcagcc      360
tccaccaagg gccatcgggt cttccccctg gcgccctgct ccaggagcac ctccgagagc      420
acagcggccc tgggctgcct ggtcaaggac tacttccccg aaccggtgac ggtgtcgtgg      480
aactcaggcg ctctgaccag cggcgtgcac accttcccag ctgtctaca gtcctcagga      540
ct                                                                                   542

```

```

<210> SEQ ID NO 114
<211> LENGTH: 180
<212> TYPE: PRT
<213> ORGANISM: Homosapien
<400> SEQUENCE: 114
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1             5             10             15
Ser Val Lys Val Ser Cys Lys Val Ser Gly Tyr Thr Leu Ser Glu Leu
 20             25             30

```

-continued

Ser Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
 35 40 45

Gly Gly Phe Asp Pro Glu Asp Gly Glu Thr Ile Tyr Ala Gln Lys Phe
 50 55 60

Gln Gly Arg Val Thr Met Thr Glu Asp Thr Ser Thr Asp Thr Ala Tyr
 65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Phe Tyr Cys
 85 90 95

Ala Thr Lys Arg Glu Tyr Ser Gly Tyr Phe Asp Tyr Trp Gly Gln Gly
 100 105 110

Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
 115 120 125

Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
 130 135 140

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
 145 150 155 160

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
 165 170 175

Gln Ser Ser Gly
 180

<210> SEQ ID NO 115
 <211> LENGTH: 477
 <212> TYPE: DNA
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 115

gacatcgtga tgaccagtc tccagactcc ctggctgtgt ctctgggcca gagggccacc 60
 atcaactgca agtccagcca gagtgtttta tacagctcca acagtaagaa ctacttagct 120
 tggttccagc agaaaccagg acagcctcct aagctgctca tttactgggc atctaccggg 180
 gaatccgggg tcctgaccg attcagtggc agcgggtctg ggacagattt cactctcacc 240
 atcagccgcc tgcaggtgca agatgtggca gttttattcct gtcagcaata ttttattact 300
 ccgtggagct tcggccaagg gaccaaggtg gaactcaaac gaactgtggc tgcaccatct 360
 gtcttcatct tcccgccatc tgatgagcag ttgaaatctg gaactgcctc tgttgtgtgc 420
 ctgctgaata acttctatcc cagagaggcc aaagtacagt ggaaggtgga taacgcc 477

<210> SEQ ID NO 116
 <211> LENGTH: 159
 <212> TYPE: PRT
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 116

Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
 1 5 10 15

Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser
 20 25 30

Ser Asn Ser Lys Asn Tyr Leu Ala Trp Phe Gln Gln Lys Pro Gly Gln
 35 40 45

Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
 50 55 60

Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr

-continued

65		70		75		80
Ile Ser Arg Leu	Gln Ala Glu Asp Val	Ala Val Tyr Ser Cys Gln Gln				
	85		90		95	
Tyr Phe Ile Thr	Pro Trp Thr Phe Gly Gln Gly Thr	Lys Val Glu Leu				
	100		105		110	
Lys Arg Thr Val	Ala Ala Pro Ser Val Phe Ile Phe	Pro Pro Ser Asp				
	115		120		125	
Glu Gln Leu Lys	Ser Gly Thr Ala Ser Val Val Cys	Leu Leu Asn Asn				
	130		135		140	
Phe Tyr Pro Arg	Glu Ala Lys Val Gln Trp Lys Val	Asp Asn Ala				
	145		150		155	

<210> SEQ ID NO 117

<211> LENGTH: 459

<212> TYPE: DNA

<213> ORGANISM: Homosapien

<400> SEQUENCE: 117

```

caggtgcagc ctgagcagtc gggccagga ctggtgaagc cctcgagac cctctcactc      60
acctgtgccca tctccgggga cagtgtctct agcaacagtg ctgcttgaa ctggatcagg      120
cagtcacctt cgagaggcct tgagtggctg ggaaggacat actacaggtc caagtgggat      180
agtgatcatg cagtatctgt gagaagtoga ataaccatct acccagacac atccaagaac      240
cagttctccc tgcagctgaa ctctgtgact cccgaggaca cggtgtgta ttactgtgca      300
agagatcgga ttagtgggac ctatgtcggg atggacgtct gggccaagg gaccacggtc      360
accgtctcct cagcctccca caagggccca tcggtcttcc cctggcgcc cctgctccag      420
gagcacctcc gagagcacag cggccctggg ctgcctggc                               459

```

<210> SEQ ID NO 118

<211> LENGTH: 153

<212> TYPE: PRT

<213> ORGANISM: Homosapien

<400> SEQUENCE: 118

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

-continued

Glu His Ser Gly Pro Gly Leu Pro Gly
145 150

<210> SEQ ID NO 119
<211> LENGTH: 526
<212> TYPE: DNA
<213> ORGANISM: Homosapien

<400> SEQUENCE: 119

```
ccagctcagc tcttgggct gctaagtctc tgggtccctg gatccaatga ggatattgtg 60
atgaccacaga ctccactctc cctgcccgctc acccctggag agccggcctc catctcctgc 120
aggtctagtc agagcctctt ggatagtgat gatggaaaca cctatttggga ctggtacctg 180
cagaagccag ggcagtctcc acagctcctg atctatacgc tttcctttcg ggcctctgga 240
gtcccagaca ggttcagtgg cagtgggtca ggcactgatt tcacactgac aatcagcagg 300
gtggaggctg aggatgttgg agtttattac tgcattgcaac gtatagagtt tcctctcact 360
ttcggcggag ggaccaaggt ggagatcaaa cgaactgtgg ctgcaccatc tgtcttcatc 420
ttcccgccat ctgatgagca gttgaaatct ggaactgcct ctgttgtgtg cctgctgaat 480
aacttctatc ccagagaggc caaagtacag tggaaggtgg ataacg 526
```

<210> SEQ ID NO 120
<211> LENGTH: 175
<212> TYPE: PRT
<213> ORGANISM: Homosapien

<400> SEQUENCE: 120

```
Pro Ala Gln Leu Leu Gly Leu Leu Met Leu Trp Val Pro Gly Ser Asn
 1 5 10 15
Glu Asp Ile Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro
 20 25 30
Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu Asp
 35 40 45
Ser Asp Asp Gly Asn Thr Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly
 50 55 60
Gln Ser Pro Gln Leu Leu Ile Tyr Thr Leu Ser Phe Arg Ala Ser Gly
 65 70 75 80
Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu
 85 90 95
Thr Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met
 100 105 110
Gln Arg Ile Glu Phe Pro Leu Thr Phe Gly Gly Gly Thr Lys Val Glu
 115 120 125
Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser
 130 135 140
Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn
 145 150 155 160
Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn
 165 170 175
```

<210> SEQ ID NO 121
<211> LENGTH: 499
<212> TYPE: DNA
<213> ORGANISM: Homosapien

-continued

<400> SEQUENCE: 121

```

cagggtccagg tggtagactc tggggctgag gtgaagaacc ctggggcctc agtgaaggtc      60
tcctgcaagg tttccggatc caccctcact gaattatcca tgcactgggt gcgacaggct      120
cctggaaaag ggttgtagtg gatgggaggt tttgatcctg aagatgggta aacaatctac      180
gcacagaagt tccagggcag agtcacatg accgaggaca catctacaga cacagtctac      240
atggagctga gcagcctgag atctgaggac acggccgtgt attactgtgc aaccaacgat      300
ttttggagtg gttattttga ctactggggc cagggaaacc tggtcaccgt ctctcagcc      360
tccaccaagg gccatcgggt cttcccctg gcgccctgct ccaggagcac ctccgagagc      420
acagcggccc tgggtgcctt ggtcaaggac tacttcccgc aaccgggtgac ggtgtcgtgg      480
aactcaggcg ctctgacca                                     499

```

<210> SEQ ID NO 122

<211> LENGTH: 166

<212> TYPE: PRT

<213> ORGANISM: Homosapien

<400> SEQUENCE: 122

```

Gln Val Gln Val Val Gln Ser Gly Ala Glu Val Lys Asn Pro Gly Ala
 1           5           10           15
Ser Val Lys Val Ser Cys Lys Val Ser Gly Ser Thr Leu Thr Glu Leu
 20           25           30
Ser Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
 35           40           45
Gly Gly Phe Asp Pro Glu Asp Gly Glu Thr Ile Tyr Ala Gln Lys Phe
 50           55           60
Gln Gly Arg Val Thr Met Thr Glu Asp Thr Ser Thr Asp Thr Val Tyr
 65           70           75           80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85           90           95
Ala Thr Asn Asp Phe Trp Ser Gly Tyr Phe Asp Tyr Trp Gly Gln Gly
 100          105          110
Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
 115          120          125
Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
 130          135          140
Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
 145          150          155          160
Asn Ser Gly Ala Leu Thr
 165

```

<210> SEQ ID NO 123

<211> LENGTH: 536

<212> TYPE: DNA

<213> ORGANISM: Homosapien

<400> SEQUENCE: 123

```

cagggtcttca tttctctgtt gctctggatc tctgatgtct atggggacat cgtgatgacc      60
cagtctccag actccctggc tgtgtctctg ggcgagaggg ccaccatcac ctgcaagtcc      120
agccagactg ttttatacag ctccaacaat aagaactact tagtttggtg tcagcagaaa      180
tcaggacagc ctctaagct gctcattcac tgggcateta tccgggaatc cggggtccct      240

```

-continued

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gaccgattca gtggcagcgg gtctgggaca gatttcacgc tcaccatcag cagcctgcag 300
gctgaagatg tggcagttta ttactgtcag caatattata gtagtccgtg gacgttcggc 360
caagggacca aggtggaaat caaacgaact gtggctgcac catctgtctt catcttcccg 420
ccatctgatg agcagttgaa atctggaact gcctctgttg tgtgcctgct gaataacttc 480
tatcccagag aggccaaagt acagtggaag gtggataacg cccttccaat cgggta 536

```

```

<210> SEQ ID NO 124
<211> LENGTH: 178
<212> TYPE: PRT
<213> ORGANISM: Homosapien

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```

<400> SEQUENCE: 124

```

```

Gln Val Phe Ile Ser Leu Leu Leu Trp Ile Ser Asp Val Tyr Gly Asp
 1           5           10           15
Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly Glu
 20           25           30
Arg Ala Thr Ile Thr Cys Lys Ser Ser Gln Thr Val Leu Tyr Ser Ser
 35           40           45
Asn Asn Lys Asn Tyr Leu Val Trp Tyr Gln Gln Lys Ser Gly Gln Pro
 50           55           60
Pro Lys Leu Leu Ile His Trp Ala Ser Ile Arg Glu Ser Gly Val Pro
 65           70           75           80
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
 85           90           95
Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Tyr
 100          105          110
Tyr Ser Ser Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
 115          120          125
Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
 130          135          140
Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
 145          150          155          160
Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Pro
 165          170          175

```

```

Ile Gly

```

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<210> SEQ ID NO 125
<211> LENGTH: 414
<212> TYPE: DNA
<213> ORGANISM: Homosapien

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```

<400> SEQUENCE: 125

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```

caggtgcagg ctgagcagtc gggccagga ctggtgaagc cctcgcagac cctctcactc 60
acctgtgcc a tctccgggga cagtgtctct agctacagtg ctgcttgaa ctggatcagg 120
cagtcccctt cgagaggcct tgagtggctg ggaaggacat actacaggtc caagtggtat 180
agtgatcatg cagtatctgt gagaagtoga ataaccatct acccagacac atccaagaac 240
cagttctccc tgcagctgaa ctctgtgact cccgaggaca cggtgtgta ttactgtgca 300
agagatcgg a ttagtgggaa ctatgtcggg atggacgtct ggggccaagg gaccacggtc 360
accgtctcct cagcctccac caagggcccc atcggctcttc ccctggcccc cctc 414

```

-continued

<210> SEQ ID NO 126
 <211> LENGTH: 138
 <212> TYPE: PRT
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 126

```

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

```

<210> SEQ ID NO 127
 <211> LENGTH: 514
 <212> TYPE: DNA
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 127

```

gttttcattt ctctgttgc ctggatctct ggtgcctacg gggacatcgt gatgaccag    60
tctccagact ccttggctgt gtctctgggc gagagggcca ccatcaactg caagtccagc    120
cagagtgttt tatacagttc caacaataag aactacatag tttggtacca gcagaaacca    180
gggcgcctc ctaagttgct catttactgg acatctaccc gggaatccgg ggtccctgac    240
cgattcagtg gcagcgggct tggaacagat ttcactctca ctatcagtag cctgcaggct    300
gaagatgtgg cagtttatta ctgtcagcaa tatttttagt ctccgtggac gttcggccaa    360
gggaccaaag tggacatcaa acgaactgtg gctgcacat ctgtttcat cttcccgcga    420
tctgatgagc agttgaaatc tggaactgcc tctgttgtgt gcctgctgaa taacttctat    480
cccagagagg ccaaagtaca gtggaaggtg gata                                514

```

<210> SEQ ID NO 128
 <211> LENGTH: 171
 <212> TYPE: PRT
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 128

```

Val Phe Ile Ser Leu Leu Leu Trp Ile Ser Gly Ala Tyr Gly Asp Ile
 1          5          10          15
Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly Glu Arg
 20          25          30
Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser Ser Asn

```


-continued

Asp Arg Ile Ser Gly Thr Tyr Val Gly Met Asp Val Trp Gly Gln Gly
 100 105 110
 Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
 115 120 125
 Pro Leu Ala Pro Leu Leu Gln Glu His Leu Arg Glu His Ser Gly Pro
 130 135 140
 Gly Leu Pro Gly
 145

<210> SEQ ID NO 131
 <211> LENGTH: 505
 <212> TYPE: DNA
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 131
 gggctgctaa tgctctggat acctggatcc agtgcagata ttgggatgac ccagactcca 60
 ctctctctgt ccgtcaccoc tggacagccg gcctccatct cctgtaagtc tagtcagagc 120
 ctctctgtata gtgatggaaa gacctatttg tattggtacc tgcagaagcc aggccagcct 180
 ccacaacacc tgatctatga agtttccaac cggttctctg gagtgccaga taggttcagt 240
 ggcagcgggt ctgggacaga tttcacactg aaaatcagcc ggggtggaggc tgatgatgtt 300
 ggggtttatt actgcatgca aactatacac cttccgctca ctttcggcgg agggaccaag 360
 gtggagatcc aacgaactgt ggctgcacca tctgtcttca tottcccgcc atctgatgag 420
 cagttgaaat ctggaactgc ctctgttggtg tgctgctga ataactteta tcccagagag 480
 gccaaagtac agtgaaggt ggata 505

<210> SEQ ID NO 132
 <211> LENGTH: 168
 <212> TYPE: PRT
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 132
 Gly Leu Leu Met Leu Trp Ile Pro Gly Ser Ser Ala Asp Ile Gly Met
 1 5 10 15
 Thr Gln Thr Pro Leu Ser Leu Ser Val Thr Pro Gly Gln Pro Ala Ser
 20 25 30
 Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser Asp Gly Lys Thr
 35 40 45
 Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Pro Pro Gln His Leu
 50 55 60
 Ile Tyr Glu Val Ser Asn Arg Phe Ser Gly Val Pro Asp Arg Phe Ser
 65 70 75 80
 Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu
 85 90 95
 Ala Asp Asp Val Gly Val Tyr Tyr Cys Met Gln Thr Ile His Leu Pro
 100 105 110
 Leu Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Gln Arg Thr Val Ala
 115 120 125
 Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
 130 135 140
 Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu
 145 150 155 160

-continued

Ala Lys Val Gln Trp Lys Val Asp
165

<210> SEQ ID NO 133
<211> LENGTH: 447
<212> TYPE: DNA
<213> ORGANISM: Homosapien

<400> SEQUENCE: 133

gagcagtcgg gtccaggact ggtgaagccc tcgcagaccc tctcactcac ctgtgccatc 60
tccggggaca gtgtctctag caacagtgtct gcttggaaact ggatcaggca gtccccttcg 120
agaggccttg agtggctggg aaggacatac tacaggtcca agtggtatag tgatcatgca 180
gtatctgtga gaagtcgaat aaccatctac ccagacacat ccaagaacca gttctccctg 240
cagctgaact ctgtgactcc cgaggacacg gctgtgtatt actgtgcaag agatcggatt 300
agtgggacct atgtcgggat ggacgtctgg ggccaagga ccacggtcac cgtctcctca 360
gcctccacca agggcccacc ggtcttcccc ctggcgcccc tgtccagga gcacctccga 420
gagcacagcg gccttgggct gcctggc 447

<210> SEQ ID NO 134
<211> LENGTH: 149
<212> TYPE: PRT
<213> ORGANISM: Homosapien

<400> SEQUENCE: 134

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

<210> SEQ ID NO 135
<211> LENGTH: 520
<212> TYPE: DNA
<213> ORGANISM: Homosapien

<400> SEQUENCE: 135

caggctctca tttctctgtt gctctggatc tctggtgcct acggggacat cgtgatgacc 60
cagtctccag actcctcggc tgtgtctctg ggcgagaggg ccgccatcaa ctgcaagtcc 120

-continued

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agccagactg ttttatacag ctccaacaat aagaactact tggtttgta ccagcagaaa 180
ccaggacagc ctccaagct gctcatttac tgggcatcta cccgggaatc cggggtccct 240
gaccgattca gtggcagcgg gtctgggaca gatttcactc tcaccatcag cagcctgcag 300
gctgaagatg tggcagttta ttactgtcaa caatattata aaagtccgtg gacgttcggc 360
caagggacca aggtggaaat caaacgaact gtggctgcac catctgtctt catcttcccg 420
ccatctgatg agcagttgaa atctggaact gcctctgttg tgtgctgct gaataacttc 480
tatccagag aggcctaaat acagtggaag gtggataacg 520

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<210> SEQ ID NO 136
<211> LENGTH: 173
<212> TYPE: PRT
<213> ORGANISM: Homosapien

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<400> SEQUENCE: 136

```

```

Gln Val Phe Ile Ser Leu Leu Leu Trp Ile Ser Gly Ala Tyr Gly Asp
 1           5           10          15
Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly Glu
          20           25           30
Arg Ala Ala Ile Asn Cys Lys Ser Ser Gln Thr Val Leu Tyr Ser Ser
      35           40           45
Asn Asn Lys Asn Tyr Leu Val Trp Tyr Gln Gln Lys Pro Gly Gln Pro
 50           55           60
Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val Pro
 65           70           75           80
Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
          85           90           95
Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln Tyr
          100          105          110
Tyr Lys Ser Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
          115          120          125
Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
          130          135          140
Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
          145          150          155          160
Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn
          165          170

```

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<210> SEQ ID NO 137
<211> LENGTH: 490
<212> TYPE: DNA
<213> ORGANISM: Homosapien

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<400> SEQUENCE: 137

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```

caggtccagc tggtagctg tggggctgag gtgaagaagc ctggggcctc agtgaaggtc 60
tcctgcaagg tttccggata caccctcact gaattatcca tgcaactgggt gcgacaggct 120
cctggaaaag ggcttgagtg gatgggaggt ttgatcctg aaaatggtga aacaatccac 180
gcacagaagt tccagggcag agtcatcatg accgaggaca catctacaga cacagcctac 240
atggagetga gcagcctgag atctgaggac acggccgtgt attactgtgc aacagatcag 300
ggtgatata gtggctactt tgactgctgg ggccagggaa ccttggtcac cgtctcctca 360

```

-continued

```

gcttccacca agggcccatc cgtcttcccc ctggcgccct gotccaggag cacctccgag 420
agcacagccg ccttgggctg cctgggtcaag gactacttcc ccgaaccggt gacgggtgctg 480
tggaactcag 490

```

```

<210> SEQ ID NO 138
<211> LENGTH: 163
<212> TYPE: PRT
<213> ORGANISM: Homosapien

```

```

<400> SEQUENCE: 138

```

```

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

```

```

<210> SEQ ID NO 139
<211> LENGTH: 540
<212> TYPE: DNA
<213> ORGANISM: Homosapien

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```

<400> SEQUENCE: 139

```

```

agaccaggt cttcatttct ctgttgctct ggatctctgg tgcctacggg gacatcgtga 60
tgaccagtc tccagactcc ctggctgtgt ctctgggcga gagggccacc atcaactgca 120
agtccagcca gagtatttta tacagctcca ataataagaa ttatttagtt tggtagcagc 180
agaaaccagg acagcctcct aagttgctca ttactgggc atctaccggg gaatccgggg 240
tccctgaccg attcagtggc agcgggtctg ggacagattt cactctcacc atcagcagcc 300
tgcaggctga agatgtggca gtttattact gtcagcaata ttatagtagt cctccgacgt 360
tcggccaagg gaccaaggtg gaaatcaaac gaactgtggc tgcaccatct gtcttcatct 420
tcccgccatc tgatgagcag ttgaaatctg gaactgcctc tgttgtgtgc ctgctgaata 480
acttctatcc cagagaggcc aaagtacagt ggaagtgga taacgcctc caatcgggta 540

```

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<210> SEQ ID NO 140
<211> LENGTH: 179
<212> TYPE: PRT

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-continued

<213> ORGANISM: Homosapien

<400> SEQUENCE: 140

Thr Gln Val Phe Ile Ser Leu Leu Leu Trp Ile Ser Gly Ala Tyr Gly
 1 5 10 15
 Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
 20 25 30
 Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Ile Leu Tyr Ser
 35 40 45
 Ser Asn Asn Lys Asn Tyr Leu Val Trp Tyr Gln Gln Lys Pro Gly Gln
 50 55 60
 Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
 65 70 75 80
 Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
 85 90 95
 Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
 100 105 110
 Tyr Tyr Ser Ser Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
 115 120 125
 Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
 130 135 140
 Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
 145 150 155 160
 Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
 165 170 175
 Gln Ser Gly

<210> SEQ ID NO 141

<211> LENGTH: 518

<212> TYPE: DNA

<213> ORGANISM: Homosapien

<400> SEQUENCE: 141

accatggagt ggacctggag ggtcctcttc ttggtggcag cagctacagg caccacgccc 60
 caggctccagc tggtagcagtc tggggctgag gtgaagaagc ctggggcctc agtgaaggctc 120
 tcctgcaagg tttccggata caccctcact gaattatcca tgcactgggt gcgacaggct 180
 cctggaaaag ggcttgagtg gatgggaggt tttgatcctg aagatggtga aacaatctac 240
 gcacagaagt tccagggcag agtcaccatg accgaggaca catctacaga cacagcctac 300
 atggagctga gttagcctgag aactgaggac acggccctgt attactgtac aacggacgat 360
 ttttgagtg gttattttga ctactggggc caggaaccc tggtcaccgt ctctcagcc 420
 tccaccaagg gccatcggt cttccccctg gcgccctgct ccaggagcac ctccgagagc 480
 acagcggcct gggctgcctg gtcaaggact acttcccc 518

<210> SEQ ID NO 142

<211> LENGTH: 172

<212> TYPE: PRT

<213> ORGANISM: Homosapien

<400> SEQUENCE: 142

Thr Met Glu Trp Thr Trp Arg Val Leu Phe Leu Val Ala Ala Ala Thr
 1 5 10 15

-continued

Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys
 130 135 140

Leu
 145

<210> SEQ ID NO 147
 <211> LENGTH: 428
 <212> TYPE: DNA
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 147

gctccgctac ttctaccct cctcgtcac tgcacagggt cttgggcca ttttatgctg 60
 actcagcccc actctgtgtc ggagtctccg ggaagacgg taaccatctc ctgcacccgc 120
 agcagtgcca gcattgccag caactatgtg cagtggttcc agcagcgcgc ggcagttcc 180
 cccaccactg taatctatga ggatgaccaa agaccctctg gggtcctga teggttctgt 240
 ggtccatcg acagctcctc caactctgcc tcctcacca tctctggact gaggactgag 300
 gacgaggctg actactactg tcagtcttat gatagcagca atcatgtggt attcggcgga 360
 gggaccaagc tgaccgtcct aggtcagccc aaggctgccc cctcggtcac tctgttcccc 420
 ccctcctc 428

<210> SEQ ID NO 148
 <211> LENGTH: 142
 <212> TYPE: PRT
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 148

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

<210> SEQ ID NO 149
 <211> LENGTH: 76
 <212> TYPE: PRT
 <213> ORGANISM: Homosapien

<400> SEQUENCE: 149

Gln Pro Asp Ala Ile Asn Ala Pro Val Thr Cys Cys Tyr Asn Phe Thr
 1 5 10 15

-continued

Asn	Arg	Lys	Ile	Ser	Val	Gln	Arg	Leu	Ala	Ser	Tyr	Arg	Arg	Ile	Thr
			20					25					30		
Ser	Ser	Lys	Cys	Pro	Lys	Glu	Ala	Val	Ile	Phe	Lys	Thr	Ile	Val	Ala
		35					40					45			
Lys	Glu	Ile	Cys	Ala	Asp	Pro	Lys	Gln	Lys	Trp	Val	Gln	Asp	Ser	Met
	50					55					60				
Asp	His	Leu	Asp	Lys	Gln	Thr	Gln	Thr	Pro	Lys	Thr				
65					70					75					

What is claimed is:

1. A human monoclonal antibody that binds to MCP-1 and comprises a heavy chain amino acid having a sequence selected from the group consisting of SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, 46, 50, 54, 58, 62, 66, 70, 74, 78, 82, 86, 90, 94, 98, 102, 106, 110, 114, 118, 122, 126, 130, 134, 138, 142 and 146.

2. The antibody of claim 1, further comprising a light chain amino acid having a sequence selected from the group consisting of SEQ ID NOS: 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 52, 56, 60, 64, 68, 72, 76, 80, 84, 88, 92, 96, 100, 104, 108, 112, 116, 120, 124, 128, 132, 136, 140, 144 and 148.

3. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:2 and the light chain amino acid comprises the sequence of SEQ ID NO:4.

4. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:6 and the light chain amino acid comprises the sequence of SEQ ID NO:8.

5. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:10 and the light chain amino acid comprises the sequence of SEQ ID NO:12.

6. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:14 and the light chain amino acid comprises the sequence of SEQ ID NO:16.

7. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:18 and the light chain amino acid comprises the sequence of SEQ ID NO:20.

8. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:22 and the light chain amino acid comprises the sequence of SEQ ID NO:24.

9. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:26 and the light chain amino acid comprises the sequence of SEQ ID NO:28.

10. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:30 and the light chain amino acid comprises the sequence of SEQ ID NO:32.

11. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:34 and the light chain amino acid comprises the sequence of SEQ ID NO:36.

12. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:38 and the light chain amino acid comprises the sequence of SEQ ID NO:40.

13. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:42 and the light chain amino acid comprises the sequence of SEQ ID NO:44.

14. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:46 and the light chain amino acid comprises the sequence of SEQ ID NO:48.

15. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:50 and the light chain amino acid comprises the sequence of SEQ ID NO:52.

16. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:54 and the light chain amino acid comprises the sequence of SEQ ID NO:56.

17. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:58 and the light chain amino acid comprises the sequence of SEQ ID NO:60.

18. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:62 and the light chain amino acid comprises the sequence of SEQ ID NO:64.

19. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:66 and the light chain amino acid comprises the sequence of SEQ ID NO:68.

20. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:70 and the light chain amino acid comprises the sequence of SEQ ID NO:72.

21. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:74 and the light chain amino acid comprises the sequence of SEQ ID NO:76.

22. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:78 and the light chain amino acid comprises the sequence of SEQ ID NO:80.

23. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:82 and the light chain amino acid comprises the sequence of SEQ ID NO:84.

24. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:86 and the light chain amino acid comprises the sequence of SEQ ID NO:88.

25. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:90 and the light chain amino acid comprises the sequence of SEQ ID NO:92.

26. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:94 and the light chain amino acid comprises the sequence of SEQ ID NO:96.

27. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:98 and the light chain amino acid comprises the sequence of SEQ ID NO:100.

28. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:102 and the light chain amino acid comprises the sequence of SEQ ID NO:104.

29. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:106 and the light chain amino acid comprises the sequence of SEQ ID NO:108.

30. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:110 and the light chain amino acid comprises the sequence of SEQ ID NO:112.

31. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:114 and the light chain amino acid comprises the sequence of SEQ ID NO:116.

32. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:116 and the light chain amino acid comprises the sequence of SEQ ID NO:118.

33. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:118 and the light chain amino acid comprises the sequence of SEQ ID NO:120.

34. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:122 and the light chain amino acid comprises the sequence of SEQ ID NO:124.

35. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:126 and the light chain amino acid comprises the sequence of SEQ ID NO:128.

36. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:130 and the light chain amino acid comprises the sequence of SEQ ID NO:132.

37. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ

ID NO:134 and the light chain amino acid comprises the sequence of SEQ ID NO:136.

38. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:138 and the light chain amino acid comprises the sequence of SEQ ID NO:140.

39. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:142 and the light chain amino acid comprises the sequence of SEQ ID NO:144.

40. The human monoclonal antibody of claim 2, wherein the heavy chain amino acid comprises the sequence of SEQ ID NO:146 and the light chain amino acid comprises the sequence of SEQ ID NO:148.

41. An antibody immobilized on an insoluble matrix, wherein the antibody is the antibody of claim 2.

42. An improved method for assaying the level of monocyte chemo-attractant protein-1 (MCP-1) in a patient sample, wherein said improved method comprises the use of the anti-MCP-1 antibody of claim 2 for detection of the level of MCP-1 in the assay of a patient sample.

43. A method according to claim 42 wherein the patient sample is blood.

44. A composition, comprising the antibody or fragment thereof of claim 2, and a pharmaceutically acceptable carrier.

45. A method of effectively treating a neoplastic disease, comprising:

selecting an animal in need of treatment for a neoplastic disease;

administering to said animal a therapeutically effective dose of a fully human monoclonal antibody that specifically binds to monocyte chemo-attractant protein-1 (MCP-1).

46. The method of claim 45, wherein said neoplastic disease is selected from the group consisting of: breast cancer, ovarian cancer, bladder cancer, lung cancer, glioblastoma, stomach cancer, endometrial cancer, kidney cancer, colon cancer, pancreatic cancer, and prostate cancer.

47. A method of effectively treating inflammatory conditions, comprising:

selecting an animal in need of treatment for an inflammatory condition;

administering to said animal a therapeutically effective dose of a fully human monoclonal antibody that specifically binds to monocyte chemo-attractant protein-1 (MCP-1).

48. The method of claim 47, wherein said inflammatory condition is selected from the group consisting of: rheumatoid arthritis, glomerulonephritis, atherosclerosis, psoriasis, restenosis, autoimmune disease, and multiple sclerosis.

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专利名称(译)	针对单核细胞化学引诱蛋白-1 (MCP-1) 的抗体及其用途		
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[标]申请(专利权)人(译)	GUDAS JEAN中号 哈克FRENDSCHO MARY FOORD ORIT 梁美娜大号 AHLUWALIA KIRAN 巴克塔SUNIL		
申请(专利权)人(译)	GUDAS JEAN M. 哈克 - FRENDSCHO MARY FOORD ORIT 梁美娜L. AHLUWALIA KIRAN 巴克塔SUNIL		
当前申请(专利权)人(译)	GUDAS JEAN M. 哈克 - FRENDSCHO MARY FOORD ORIT 梁美娜L. AHLUWALIA KIRAN 巴克塔SUNIL		
[标]发明人	GUDAS JEAN M HAAK FRENDSCHO MARY FOORD ORIT LIANG MEINA L AHLUWALIA KIRAN BHAKTA SUNIL		
发明人	GUDAS, JEAN M. HAAK-FRENDSCHO, MARY FOORD, ORIT LIANG, MEINA L. AHLUWALIA, KIRAN BHAKTA, SUNIL		
IPC分类号	G01N33/53 A61K39/395 A61P9/10 A61P13/12 A61P17/06 A61P19/02 A61P25/00 A61P29/00 A61P35/00 A61P35/04 A61P37/00 A61P43/00 C07K16/00 C07K16/24 C07K17/00 C12N15/09 C12P21/08 C07K16/44		
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摘要(译)			

本文描述的本发明的实施方案涉及针对抗原单核细胞化学引诱蛋白-1 (MCP-1) 的抗体和这些抗体的用途。特别地, 根据一些实施方案, 提供了针对抗原MCP-1的完全人单克隆抗体。编码的核苷酸序列和包含重链和轻链免疫球蛋白分子的氨基酸序列, 特别是对应于跨越框架区和/或互补决定区 (CDR) 的连续重链和轻链序列的序列, 特别是来自FR1至FR4或CDR1至CDR3的序列。提供。还提供了表达此类免疫球蛋白分子和单克隆抗体的杂交瘤或其他细胞系。

Figure 1

