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(54) **ENHANCED T CELL RECEPTOR-MEDIATED TUMOR NECROSIS FACTOR SUPERFAMILY AND CHEMOKINE MRNA EXPRESSION IN PERIPHERAL BLOOD LEUKOCYTES IN PATIENTS WITH CROHN'S DISEASE**

ERHÖHTE EXPRESSION VON T-ZELLENREZEPTORVERMITTELTER TUMORNEKROSEFAKTORSUPERFAMILIE UND CHEMOKIN-MRNA IN PERIPHEREN BLUT LEUKOZYTEN BEI PATIENTEN MIT MORBUS& xA;CROHN

EXPRESSION AUGMENTÉE DE L'ARNM DE LA& xA;SUPERFAMILLE DES FACTEURS DE NÉCROSE& xA;TUMORALE ET DES CHIMIOKINES INDUITE PAR LE& xA;RÉCEPTEUR DES LYMPHOCYTES T DANS DES& xA;LEUCOCYTES DU SANG PÉRIPÉHRIQUE CHEZ DES& xA; PATIENTS ATTEINTS DE LA MALADIE DE CROHN

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(73) Proprietors:
- **Hitachi Chemical Company, Ltd.**
  Tokyo 163-0449 (JP)
- **Hitachi Chemical Research Center, Inc.**
  Irvine CA 92617 (US)
- **Cedars-Sinai Medical Center**
  Los Angeles, CA 90048 (US)

(72) Inventors:
- **MITSUHASHI, Masato**
  Irvine, CA 92614 (US)
- **TARGAN, Stephan, R.**
  Santa Monica, CA 90402 (US)

(74) Representative: **Vossius & Partner**
Siebertstrasse 4
81675 München (DE)

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Description

BACKGROUND

Field

[0001] The disclosure relates to a method for predicting patient responsiveness to Crohn's disease treatments involving antagonizing tumor necrosis factor-α ("TNF-α"), another tumor necrosis factor superfamily member, or a cytokine, and to a method of monitoring the effectiveness of such therapy. The disclosure also relates to a method for screening compounds for use in the treatment of Crohn's disease. The disclosure also relates to a method for monitoring the disease state in Crohn's disease patients.

Description of the Related Art

[0002] Autoimmune disease is characterized by production of either antibodies that react with host cells or immune effector T cells that are autoreactive. Autoantibodies are frequently identified in certain types of autoimmune disease, such as anti-acetylcholine receptor antibodies in myasthenia gravis and anti-DNA antibodies in systemic lupus erythematosus. However, such autoantibodies are not seen in many types of autoimmune disease. Moreover, autoantibodies are often detected among healthy individuals, but such antibodies do not induce autoimmune disease. Thus, beside autoantibodies, additional yet-to-be identified mechanisms are evidently involved in the pathogenesis of autoimmune disease.

[0003] Once autoantibodies bind to the target host cells, the complement cascade is thought to be activated to form the C5-9 membrane attack complex on the target cell membranes, which leads to the death of host cells (see Esser, Toxicology 87, 229 (1994)). By-product chemotactic factors, such as C3a, C4a, or C5a recruit more leukocytes to the lesion (see Hugli, Crit. Rev. Immunol. 1, 321 (1981)). Recruited leukocytes or naturally present leukocytes at the lesion recognize antibody-bound cells (immune complex) via Fc receptors ("FcR"). Once the FcR is cross-bridged by the immune complex, leukocytes release TNF-α (see Debets et al., J Immunol. 141, 1197 (1988)), which binds to specific receptors present on the surface of host cells, and induce apoptosis or cell damage (see Micheau et al., Cell 114, 181 (2003)). Activated FcR also initiates the release of chemotactic cytokines to recruit different subsets of leukocytes to the lesion (see Chantry et al., Eur. J. Immunol. 19, 189 (1989)). In addition to the FcR, T cell receptors ("TCR") on cytotoxic T cells may also recognize host cells, and an activated TCR functions in the same manner as cross-bridged FcR (see Brehm et al., J. Immunol. 175, 5043 (2005)). TCR function is well characterized in terms of antigen presentation with an interaction with major histocompatibility complex (MHC) molecules (see Isaacs et al., Inflamm. Bowel Dis. 11 Suppl 1, S3 (2005), and Garcia et al., Cell 122, 333 (2005)). Although the TCR-mediated cytotoxic function is not well characterized, it may be involved in cases where no autoantibody is identified, because immunoglobulins and the TCR are unique molecules which are capable of recognizing the specific structure of the target. This is an overall hypothesis of the molecular mechanism of autoimmune disease.

[0004] Crohn's disease ("CD") is an immune disease involving inflammation of the gastrointestinal tract. Although it is well characterized clinically, its pathogenesis is poorly understood. It is known, however, that the expression of TNF-α, also known as tumor necrosis factor superfamily member 2 ("TNFSF-2"), is increased in inflammatory bowel diseases such as CD. The role of TNF-α and mucosal T-helper-1 cytokines in the pathogenesis of Crohn's disease is described by Plevy et al., The Journal of Immunology 159 (1997), 6276-6282. Although mild to moderate CD may be treated with 5-ASA agents such as sulfasalazine, glucocorticoids, or purine analogs such as azathioprine or 6-mercaptopurine, therapeutic options for severe CD cases refractory to standard therapies, such as the administration of cyclosporine, tacrolimus, or anti-inflammatory cytokines, are limited and of varying effect. Pharmacogenetic investigations have not associated polymorphisms in the TNF gene or TNF receptor gene with responsiveness to e.g. infliximab (see Mascheretti et al., The Pharmacogenomics Journal 2 (2002), 127-136; Shetty and Forbes, Am. J. Pharmacogenomics 2 (2002), 215-221). Most CD patients will require at least one surgical intervention. Because the choice of therapeutic options depends on an assessment of the disease state in CD patients, it would be desirable to develop new methods of evaluating the disease state and monitoring the progression of the disease. Methods of diagnosing clinical subtypes of Crohn's disease with characteristic responsiveness to anti-Th1 cytokine therapy have been disclosed in WO 98/47004.

[0005] The development of infliximab (Remicade®), a chimeric mouse-human monoclonal antibody against TNF-α, has been a recent advance in the therapy of severe CD. However, only about 65% of patients will respond to this agent, and only about half of those patients will enter complete remission after repeated infusions of the antibody (typically, once every 8 weeks for 44 weeks). The prediction of response to infliximab in Crohn's disease is discussed by Chaudhary et al., Dig. Liver Dis. 37 (2005), 559-563. Because of the cost of the treatment, which can amount to tens of thousands of dollars per year, a method of quickly and easily assessing whether a CD patient would be a good candidate for infliximab therapy, and of assessing the effectiveness of infliximab therapy once treatment has begun, would be highly

desirable. Furthermore, a method of rapidly screening new agents that could be of use in treating CD would be of great benefit in developing new therapies to complement or supplant existing therapies.

SUMMARY

[0006] In an embodiment, a method of determining whether a human having Crohn's disease is likely to respond to a therapy is provided that comprises: stimulating leukocytes in vitro in a first sample that comprises leukocytes from the human; after the stimulation, measuring the amount of an mRNA selected from the group consisting of tumor necrosis factor subfamily ("TNFSF")-2, TNFSF-5, TNFSF-6, TNFSF-14, chemokine (C-C motif) ligand ("CCL")-2, CCL-3, CCL-4, and chemokine (C-X-C motif) ligand ("CXCL")-10 in the first sample; stimulating leukocytes in vitro in a second sample comprising leukocytes from the human with a control stimulus; measuring the amount of the mRNA in the second sample after stimulation; and determining a ratio of the amount of the mRNA in the first sample to the amount of the mRNA in the second sample, wherein the human is likely to respond to the therapy if the ratio is about 1.7:1 or greater.

[0007] In a further aspect, stimulating leukocytes in the first sample comprises intermixing an anti-T cell receptor antibody with the first sample.

[0008] In a further aspect, stimulating leukocytes in the first sample comprises intermixing with the first sample an agent selected from the group consisting of phorbol myristate acetate (PMA), phytohemagglutinin (PHA), wheat germ agglutinin (WGA), concanavalin-A (ConA), lipopolysaccharides (LPS), jacalin, fucoidan, heat-aggregated IgE, heat-aggregated IgA, and heat-aggregated IgM.

[0009] In a further aspect, at least one of the first and second samples comprises whole blood.

[0010] In a further aspect, the control stimulus comprises a purified control immunoglobulin.

[0011] In a further aspect, the therapy targets TNF-α activity.

[0012] In a further aspect, the therapy comprises administration of infliximab.

[0013] In a further aspect, the therapy comprises administration of an agent selected from the group consisting of cyclosporine A and tacrolimus.

[0014] In an embodiment, a method of evaluating the effectiveness of a Crohn's disease therapy in a human is provided that comprises: stimulating leukocytes in vitro in a first sample comprising leukocytes from the human; stimulating leukocytes in vitro in a second sample comprising leukocytes from the human with a control stimulus; measuring the amount of an mRNA selected from the group consisting of tumor necrosis factor subfamily ("TNFSF")-2, TNFSF-5, TNFSF-6, TNFSF-14, chemokine (C-C motif) ligand ("CCL")-2, CCL-3, CCL-4, and chemokine (C-X-C motif) ligand ("CXCL")-10 in the first and second samples after stimulation; calculating a first ratio of the amount of the mRNA in the first sample to the amount of the mRNA in the second sample; administering the therapy to the human; stimulating leukocytes in vitro in a third sample comprising leukocytes from the human obtained after the administration of therapy; stimulating leukocytes in vitro in a fourth sample comprising leukocytes from the human obtained after the administration of therapy with the control stimulus; measuring the level of the mRNA in the third and fourth samples after stimulation; calculating a second ratio of the amount of the mRNA in the third sample to the amount of the mRNA in the fourth sample; and comparing the first and second ratios, wherein a significant difference in the ratios is indicative of an effective therapy.

[0015] In a further aspect, stimulating leukocytes in the first and third samples comprises intermixing an anti-T cell receptor antibody with the sample.

[0016] In a further aspect, stimulating leukocytes in the first and third samples comprises intermixing with the sample an agent selected from the group consisting of phorbol myristate acetate (PMA), phytohemagglutinin (PHA), wheat germ agglutinin (WGA), concanavalin-A (ConA), lipopolysaccharides (LPS), jacalin, fucoidan, heat-aggregated IgE, heat-aggregated IgA, and heat-aggregated IgM.

[0017] In a further aspect, the control stimulus comprises a purified control immunoglobulin.

[0018] In a further aspect, at least one of the first, second, third and fourth samples comprises whole blood.

[0019] In a further aspect, the significant difference in the ratios is that the second ratio is greater than the first ratio, and the therapy comprises inactivation of tumor necrosis factor alpha.

[0020] In a further aspect, the therapy comprises administration of infliximab.

[0021] In a further aspect, the significant difference in the ratios is that the first ratio is greater than the second ratio.

[0022] In a further aspect, the therapy comprises administration of an agent selected from the group consisting of cyclosporine A and tacrolimus.

[0023] In an embodiment, a method of identifying a putative agent for treating Crohn's disease is provided that comprises: obtaining first, second, third, and fourth samples comprising leukocytes from a human whose leukocytes demonstrate at least a 1.7-fold increase in the transcription of an mRNA selected from the group consisting of tumor necrosis factor subfamily ("TNFSF")-2, TNFSF-5, TNFSF-6, TNFSF-14, chemokine (C-C motif) ligand ("CCL")-2, CCL-3, CCL-4, and chemokine (C-X-C motif) ligand ("CxCL")-10 when exposed to an anti-T cell receptor antibody; stimulating leukocytes in vitro in the first sample; stimulating leukocytes in vitro in the second sample with a control stimulus; measuring the amount of the mRNA in the first and second samples after stimulation; calculating a first ratio of the amount of the

mRNA in the first sample to the amount of the mRNA in the second sample; exposing the third and fourth samples to the agent; stimulating leukocytes in vitro in the third sample after the exposure; stimulating leukocytes in vitro in the fourth sample with the control stimulus after the exposure; measuring the level of the mRNA in the third and fourth samples after stimulation; calculating a second ratio of the amount of the mRNA in the third sample to the amount of the mRNA in the fourth sample; and comparing the first and second ratios, wherein a significant difference in the ratios is indicative of a putative agent.

[0024] In a further aspect, stimulating leukocytes in the first and third samples comprises intermixing an anti-T cell receptor antibody with the samples.

[0025] In a further aspect, stimulating leukocytes in the first and third samples comprises intermixing with the sample an agent selected from the group consisting of phorbol myristate acetate (PMA), phytohemagglutinin (PHA), wheat germ agglutinin (WGA), concanavalin-A (ConA), lipopolysaccharides (LPS), jacalin, fucoidan, heat-aggregated IgE, heat-aggregated IgA, and heat-aggregated IgM.

[0026] In a further aspect, the control stimulus comprises a purified control immunoglobulin.

[0027] In a further aspect, at least one of the first, second, third, and fourth samples comprises whole blood.

[0028] In a further aspect, the significant difference in the ratios is that the first ratio is greater than the second ratio.

[0029] In an embodiment, a method of evaluating the state of Crohn's disease in a human is provided that comprises: stimulating leukocytes in vitro in a first sample that comprises leukocytes and is obtained at a first time from the human; after the stimulation, measuring the amount of an mRNA selected from the group consisting of tumor necrosis factor subfamily ("TNFSF")-2, TNFSF-5, TNFSF-6, TNFSF-14, chemokine (C-C motif) ligand ("CCL")-2, CCL-3, CCL-4, and chemokine (C-X-C motif) ligand ("CXCL")-10 in the first sample; stimulating leukocytes in vitro with a control stimulus in a second sample comprising leukocytes obtained from the human at the first time; measuring the amount of the mRNA in the second sample after stimulation; determining a first ratio of the amount of the mRNA in the first sample to the amount of the mRNA in the second sample; stimulating leukocytes in vitro in a third sample that comprises leukocytes and is obtained from the human at a second time that is subsequent to the first time; after the stimulation, measuring the amount of the mRNA in the third sample; stimulating leukocytes in vitro with a control stimulus in a fourth sample comprising leukocytes obtained from the human at the second time; measuring the amount of the mRNA in the fourth sample after stimulation; determining a second ratio of the amount of the mRNA in the third sample to the amount of the mRNA in the fourth sample; and comparing the first and second ratios, wherein a significant difference in the first and second ratios is indicative of a change in the disease state.

[0030] In a further aspect, stimulating leukocytes in the first and third samples comprises intermixing an anti-T cell receptor antibody with the sample.

[0031] In a further aspect, stimulating leukocytes in the first and third samples comprises intermixing with the sample an agent selected from the group consisting of phorbol myristate acetate (PMA), phytohemagglutinin (PHA), wheat germ agglutinin (WGA), concanavalin-A (ConA), lipopolysaccharides (LPS), jacalin, fucoidan, heat-aggregated IgE, heat-aggregated IgA, and heat-aggregated IgM.

[0032] In a further aspect, the control stimulus comprises a purified control immunoglobulin.

[0033] In a further aspect, at least one of the first, second, third and fourth samples comprises whole blood.

[0034] In a further aspect, the significant difference in the ratios is that the second ratio is greater than the first ratio, and the change in disease state is a progression of the disease.

[0035] In a further aspect, the significant difference in the ratios is that the first ratio is greater than the second ratio, and the change in disease state is a regression of the disease.

BRIEF DESCRIPTION OF THE DRAWINGS

[0036]

Figure 1 shows the results of a quantification of FcR and TCR-mediated gene expression of TNFSF, CCL, CXCL, and interleukin mRNA in human leukocytes in peripheral whole blood from CD patients and controls.

Figure 2 shows the correlation among various HAG-induced mRNA responses.

Figure 3A - 3B shows the correlation among various anti-TCR-induced mRNA responses.

Figure 4 shows the effect of heat aggregated IgG (HAG) on TNFSF and chemokine mRNA expression in peripheral blood leukocytes.

Figure 5 shows the effect of anti-TCR antibodies on TNFSF and chemokine mRNA expression in peripheral blood leukocytes.

Figure 6 shows the results of stimulation of whole blood of healthy individuals with phorbol myristate acetate (PMA).

Figure 7 shows the results of stimulation of whole blood of healthy individuals with phytohemagglutinin (PHA).

Figure 8 shows the results of stimulation of whole blood of healthy individuals with wheat germ agglutinin (WGA)

Figure 9 shows the results of stimulation of whole blood of healthy individuals with concanavalin-A (ConA).

Figure 10 shows the results of stimulation of whole blood of healthy individuals with lipopolysaccharides (LPS).
Figure 11 shows the results of stimulation of whole blood of healthy individuals with jacalin.
Figure 12 shows the results of stimulation of whole blood of healthy individuals with fucoidan.
Figures 13A - 13D show the results of stimulation of whole blood of healthy individuals with heat-aggregated IgG.
Figures 14A - 14C shows the results of stimulation of whole blood of healthy individuals with heat-aggregated IgA.
Figures 15A - 15C shows the results of stimulation of whole blood of healthy individuals with heat-aggregated IgM.
Figures 16A - 16C shows the results of stimulation of whole blood of healthy individuals with heat-aggregated IgE.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0037] The present disclosure relates to the use of differential mRNA transcription patterns in leukocytes in response to specific cellular stimuli in assessing whether CD patients are good candidates for specific therapies, such as therapy with Remicade®. The present disclosure also relates to the use of such differential transcription patterns in assessing whether therapy administered to a CD patient, such as therapy with Remicade®, is effective. The present disclosure also relates to the use of such differential transcription patterns in screening candidate agents for use in treating CD. The present disclosure also relates to the use of such differential transcription patterns in evaluating the state of CD in patients over time and monitoring the progression of the disease.

[0038] As described above, the pathology of CD may be related to the functioning of the FcR or TCR in the immune cells of a CD patient. In order to further assess the possible role of the FcR or the TCR in the disease, it is useful to assess whether the function of the FcR or TCR in circulating leukocytes in peripheral blood is normal before the leukocytes migrate to the pathological sites in patients with autoimmune disease, or is already enhanced before this migration. In order to analyze whether the function of the FcR or TCR is normal or enhanced in peripheral blood leukocytes in patients with CD, heat aggregated human IgG ("HAG") or monoclonal antibody against α/β T cell receptor ("anti-TCR") was added directly into heparinized whole blood in order to stimulate the FcR and TCR, respectively. Although multiple FcRs exist for IgG (FcγR), such as FcγRI, IIa, IIb, and III (GeneBank UniGene database), HAG acts as a universal stimulus that can react with all FcR subtypes. The changes in the mRNA level of members of the TNF superfamily (TNFSF) mRNA (see, for example, the GeneBank UniGene database) and selected CCL and CXCL chemokine mRNAs and chemotactic interleukin mRNAs (IL-1β, IL-6, and IL-8) resulting from the stimulus with HAG and anti-TCR were quantified. Because γ/δ anti-TCR did not induce any of the TNFSF mRNAs when mixed with whole blood, the α/β anti-TCR was used for the stimulus.

[0039] The method employed was as follows. Nucleotide sequences for various TNFSF and chemokine genes were retrieved from the UniGene database in the GenBank. PCR primers for each gene were designed by Primer Express (Applied Biosystem, Foster City, CA) and HYBsimulator (RNAture, Irvine, CA) (see Mitsuhashi et al., Nature 367:759 (1994), and Hyndman et al., BioTechniques, 20:1090 (1996)) The sequences are summarized in Table I below. Oligonucleotides were synthesized by IDT (Coralville, IA).

Table 1: Primer sequences

Target mRNA	Forward	Reverse
TNFSF-1	CAGCTATCCACCCACACAGATG (SEQ ID NO: 1)	CGAAGGCTCCAAAGAAGACAGT (SEQ ID NO: 2)
TNFSF-2	TCAATCGGCCCGACTATCTC (SEQ ID NO: 3)	CAGGGCAATGATCCCAAAGT (SEQ ID NO: 4)
TNFSF-3	AGGGTGTACGTCAACATCAGTCA (SEQ ID NO: 5)	CACGGCCCCAAAGAAGGT (SEQ ID NO: 6)
TNFSF-4	GCCCCTCTTCCAACTGAAGAA (SEQ ID NO: 7)	GGTATTGTCAGTGGTCACATTCAAG (SEQ ID NO: 8)
TNFSF-5	CCACAGTTCCGCCAAACCT (SEQ ID NO: 9)	CACCTGGTTGCAATTCAAATACTC (SEQ ID NO: 10)
TNFSF-6	TGGCAGCATCTTCACTTCTAAATG (SEQ ID NO: 11)	GAAATGAGTCCCCAAAACATCTCT (SEQ ID NO: 12)
TNFSF-7	CACACTCTGCACCAACCTCACT (SEQ ID NO: 13)	TGCACTCCAAAGAAGGTCTCATC (SEQ ID NO: 14)
TNFSF-8	ACCACCATATCAGTCAATGTGGAT (SEQ ID NO: 15)	GAAGATGGACAACACATTCTCAAGA (SEQ ID NO: 16)
TNFSF-9	AGCTACAAAGAGGACACGAAGGA	CGCAGCTCTAGTTGAAAGAAGACA

(continued)

Target mRNA	Forward	Reverse
	(SEQ ID NO: 17)	(SEQ ID NO: 18)
TNFSF-10	GGGAATATTTPGAGCTTAAGGAAAATG	AAAAGGCCCCGAAAAAACTG
	(SEQ ID NO: 19)	(SEQ ID NO: 20)
TNFSF-11	GAGTATCTTCAACTAATGGTGTACGTCACT	TGGTGCTTCCTCCTTTCATCA
	(SEQ ID NO: 21)	(SEQ ID NO: 22)
TNFSF-12	TACTGTCAGGTGCACTTTGATGAG	GCAGTGGCTGAGAATTCCT
	(SEQ ID NO: 23)	(SEQ ID NO: 24)
TNFSF-13	ATATGGTGTCCGAATCCAGGAT	CCTGACCCATGGTGAAAGTCA
	(SEQ ID NO: 25)	(SEQ ID NO: 26)
TNFSF-13B	ATGCCTGAAACACTACCCAATAATT	GCAAGTTGGAGTTCATCTCCTTCT
	(SEQ ID NO: 27)	(SEQ ID NO: 28)
TNFSF-14	CGTCCGTGTGCTGGATGA	CATGAAAGCCCCGAAGTAAGAC
	(SEQ ID NO: 29)	(SEQ ID NO: 30)
TNFSF-15	TGCGAAGTAGGTAGCAACTGGTT	CCATTAGCTTGTCCCCTTCTTG
	(SEQ ID NO: 31)	(SEQ ID NO: 32)
TNFSF-18	CGGCTGTATAAAAACAAAGACATGAT	TCCCCAACATGCAATTCATAAG
	(SEQ ID NO: 33)	(SEQ ID NO: 34)
IL-1B	GAAGATGGAAAAGCGATTTGTCTT	GGGCATGTTTTCTGCTTGAGA
	(SEQ ID NO: 35)	(SEQ ID NO: 36)
IL-5	GCTCTTGGAGCTGCCTACGT	AAGGTCTCTTTCACCAATGCACTT
	(SEQ ID NO: 37)	(SEQ ID NO: 38)
IL-6	TCATCACTGGTCTTTTGGAGTTTG	TCTGCACAGCTCTGGCTTGT
	(SEQ ID NO: 39)	(SEQ ID NO: 40)
IL-8	TGCTAAAGAACTTAGATGTCAGTGCAT	TGGTCCACTCTCAATCACTCTCA
	(SEQ ID NO: 41)	(SEQ ID NO: 42)
IL-12A	GCAGGCCCTGAATTTCAACA	GAAGTATGCAGAGCTTGATTTTAGTTTTA
	(SEQ ID NO: 43)	(SEQ ID NO: 44)
IL-12B	GAAGTATGCAGAGCTTGATTTTAGTTTTA	CCCATTCGCT CCAAGATGAG
	(SEQ ID NO: 45)	(SEQ ID NO: 46)
IL-15	TGAAGTGCTTTCTCTTGGAGTTACA	CATTCCCATTAGAAGACAAACTGTTG
	(SEQ ID NO: 47)	(SEQ ID NO: 48)
IL-16C	AAAACCTCTTGGGAAGCATGAG	GGGACCCCGAGGACAGTACT
	(SEQ ID NO: 49)	(SEQ ID NO: 50)
CCL-2	CCATTGTGGCCAAGGAGATC	TGTCCAGGTGGTCCATGGA
	(SEQ ID NO: 51)	(SEQ ID NO: 52)
CCL-3	CACAGAATTTCATAGCTGACTACTTTGA	TCGCTTGGTTAGGAAGATGACA
	(SEQ ID NO: 53)	(SEQ ID NO: 54)
CCL-4	GGTATTCCAAACCAAAAGAAGCA	GTTCAGTTCCAGGTCATACACGTACT
	(SEQ ID NO: 55)	(SEQ ID NO: 56)
CCL-5	AGTCGTCTTTGTCACCCGAAA	AGCTCATCTCCAAAGAGTTGATGTAC
	(SEQ ID NO: 57)	(SEQ ID NO: 58)
CCL-7	TGTGCTGACCCCACACAGA	GCTTTGGAGTTTGGGTTTTCTTG
	(SEQ ID NO: 59)	(SEQ ID NO: 60)
CCL-8	AGAGCTACACAAGAATCACCAACATC	AGACCTCCTTGCCCCGTTT
	(SEQ ID NO: 61)	(SEQ ID NO: 62)
CCL-11	CCCAGAAAGCTGTGATCTTCAA	TCCTGCACCCACTTCTTCTTG
	(SEQ ID NO: 63)	(SEQ ID NO: 64)
CCL-13	CCAAACTGGGCAAGGAGATCT	GGCCCAGGTGTTTCATATAATTCT
	(SEQ ID NO: 65)	(SEQ ID NO: 66)
CCL-14	TGCTTCACCTACACTACCTACAAGATC	GACAATTCCGGGCTTGGA

(continued)

Target mRNA	Forward	Reverse
	(SEQ ID NO: 67)	(SEQ ID NO: 68)
CCL-18	CAGATTCCACAAAAGTTCATAGTTGAC	CCGGCCTCTCTTGGTTAGG
	(SEQ ID NO: 69)	(SEQ ID NO: 70)
CCL-19	CTGCTGTAGTGTTCACCACACTGA	CTGCTGTAGTGTTCACCACACTGA
	(SEQ ID NO: 71)	(SEQ ID NO: 72)
CCL-20	GATACACAGACCGTATTCTTCATCCTAA	TGAAAGATGATAGCATTGATGTCACA
	(SEQ ID NO: 73)	(SEQ ID NO: 74)
CCL-21	CGCTCTCAGGCAGAGCTATGT	CTTGTCCAGATGCTGCATCAG
	(SEQ ID NO: 75)	(SEQ ID NO: 76)
CCL-22	GCGCGTGGTGAAACACTTC	ATCGGCACAGATCTCCTTATCC
	(SEQ ID NO: 77)	(SEQ ID NO: 78)
CCL-23	CGAAGCATCCCGTGTTCACT	GATGACACCCGGCTTGGA
	(SEQ ID NO: 79)	(SEQ ID NO: 80)
CCL-24	CAGGAGTGATCTTCACCACCAA	GGCGTCCAGGTTCTTCATGT
	(SEQ ID NO: 81)	(SEQ ID NO: 82)
CCL-25	GGCGTCCAGGTTCTTCATGT	GTAGAATATCGCAGCAGGCAGAT
	(SEQ ID NO: 83)	(SEQ ID NO: 84)
CCL-26	CTGCTTCCAATACAGCCACAAG	GAGCAGCTGTTACTGGTGAATTCA
	(SEQ ID NO: 85)	(SEQ ID NO: 86)
CCL-27	CGTGCTTCACCTGGCTCAA	GGTGCTCAAACCACTGTGACA
	(SEQ ID NO: 87)	(SEQ ID NO: 88)
CCL-28	GGAAATGTTTGCCACAGGAAGA	TGTTTCGTGTTTCCCCTGATG
	(SEQ ID NO: 89)	(SEQ ID NO: 90)
CXCL-1	CCACTGCGCCCAAACC	GCAGGATTGAGGCAAGCTTT
	(SEQ ID NO: 91)	(SEQ ID NO: 92)
CXCL-2	CCCCTGGCCACTGAACTG	TGGATGTTCTTGAGGTGAATTCC
	(SEQ ID NO: 93)	(SEQ ID NO: 94)
CXCL-3	GGAATTCACCTCAAGAACATCCA	GTGGCTATGACTTCGGTTTGG
	(SEQ ID NO: 95)	(SEQ ID NO: 96)
CXCL-4	CCGTCCCAGGCACATCAC	CCGTCCCAGGCACATCAC
	(SEQ ID NO: 97)	(SEQ ID NO: 98)
CXCL-5	AGAGCTGCGTTGCGTTTGT	TGGCGAACACTTGCAGATTACT
	(SEQ ID NO:99)	(SEQ ID NO: 100)
CXCL-6	CAGAGCTGCGTTGCACTTGT	ACACCTGCAGTTTACCAATCGTT
	(SEQ ID NO: 101)	(SEQ ID NO: 102)
CXCL-7	TCTGGAATTCATCCCAAAAACA	TCTGGAATTCATCCCAAAAACA
	(SEQ ID NO: 103)	(SEQ ID NO: 104)
CXCL-9	CCACCTACAATCCTTGAAAGACCTT	CAGTGTAGCAATGATTTCAATTTTCTC
	(SEQ ID NO: 105)	(SEQ ID NO: 106)
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[0040] Heat aggregated IgG (HAG) was prepared by heating 20 mg/mL human IgG (Sigma, St. Louis) in PBS at 63˚C for 15 min (see Ostreiko et al., Immunol Lett. 15, 311 (1987)). In 8-well strip microtubes, 1.2 μl of HAG or anti-TCR antibody (1gG1 κ) or controls (phosphate buffered saline for HAG or mouse control IgG1 κ for anti-TCR) (BioLegend, San Diego) were added, and stored at -20˚C until use. Although mouse IgG1 κ was employed as a control for anti-TCR antibody stimulation here, other purified control immunoglobulins could also be employed. Sixty μl of fresh heparinized whole blood was added into each well in triplicate, and incubated at 37˚C for 2-8 hours with cap closed. After each

treatment, 50 μl of whole blood was transferred to filterplates as described below. Each blood sample was stored frozen at -80˚C until use.

[0041] The mRNA and cDNA were prepared from whole blood following the method set forth in Mitsuhashi et al., Clin. Chem. 52:4 (published as doi:10.1373/clinchem.2005.048983). The method disclosed in U.S. Patent Application, Serial No. 10/796,298, may also be employed. In brief, 96-well filterplates were placed over collection plates, and 150 μl 5 mM Tris, pH 7.4, was applied. Following centrifugation at 120 xg for 1 min at 4˚C, 50 μl of blood sample was applied to each well and immediately centrifuged at 120 xg for 2 min at 4˚C, followed by washing of each well with 300 μl PBS once with centrifugation at 2000 xg for 5 min at 4˚C. Then, 60 μl stock lysis buffer, supplemented with 1% 2-mercaptethanol (Bio Rad, Hercules, CA, USA), 0.5 mg/ml proteinase K (Pierce, Rockford, IL, USA), 0.1 mg/ml salmon sperm DNA (5 Prime Eppendorf/Brinkmann, Westbury, NY, USA), 0.1 mg/ml E. coli tRNA (Sigma), a cocktail of 10 mM each of specific reverse primers, and standard RNA34 oligonucleotides, were applied to the filterplates, followed by incubation at 37˚C for 10 min. The filterplates were then placed over oligo(dT)-immobilized microplates (GenePlate, RNAture) (see Mitsuhashi et al., Nature 357:519 (1992), and Hamaguchi et al., Clin. Chem. 44, 2256 (1998)), and centrifuged at 2000 xg for 5 min at 4˚C. Following overnight storage at 4˚C, the microplates were washed with 100 μl plain lysis buffer 3 times, followed by 150 μl wash buffer (0.5 M NaCl, 10 mM Tris, pH 7.4, 1 mM EDTA) 3 times at 4˚C. The cDNA was directly synthesized in each well by adding 30μ l buffer containing 1x RT-buffer, 1.25 mM each of dNTP, 4 units rRNasin, and 80 units of MMLV reverse transcriptase (Promega) (without primers), and incubation at 37˚C for 2 hours. The specific primer-primed cDNA existed in solution, and oligo(dT)-primed cDNA stayed immobilized in the microplate (see Hugli, Crit. Rev. Immunol. 1, 321 (1981)). For SYBR Green PCR (see Morrison et al., Biotechniques 24, 954 (198)), cDNA was diluted 4-fold in water, and 4 μl of cDNA solution was directly transferred to 384-well PCR plates, to which 5 μl iTaq SYBR master mix (BioRad, Hercules, CA) and 1 μl oligonucleotide cocktail (15 μM each of forward and reverse primer) were applied, and PCR was conducted in PRISM 7900HT (ABI), with one cycle of 95˚C for 10 min followed by 45 cycles of 95˚C for 30 sec and 60˚C for 1 min. TaqMan PCR could also be employed; in such a case, the cDNA solution is directly transferred to 384-well PCR plates, to which 5 μl of TaqMan universal master mix (ABI) and 1 μl oligonucleotide cocktail (15 μM each of forward and reverse primer, and 3-6 μM TaqMan probe) are applied, and PCR is conducted in PRISM 7900HT (ABI), with one cycle of 95˚C for 10 min followed by 45 cycles of 95˚C for 30 sec, 55˚C for 30 sec, and 60˚C for 1 min.

[0042] The 1 x RT buffer was used as a negative control to confirm that no primer dimer was generated under SYBR Green PCR conditions. Moreover, the melting curve was analyzed in each case to confirm that the PCR signals were derived from the single PCR product. The Ct was determined by analytical software (SDS, ABI). The ΔCt was calculated by subtracting the Ct values of appropriate control samples, and the fold increase was calculated as 2^(-ΔCt), by assuming that the efficiency of each PCR cycle was 100%.

[0043] Figure 1 shows the results of the analysis of the FcR and TCR-mediated gene expression of TNFSF, CCL, CXCL, and interleukin mRNA in human leukocytes in peripheral whole blood. The results are expressed in Figure 1 as a fold increase over the control values. Responders were defined to be individuals showing a greater than 1.7-fold increase in mRNA level in response to the stimulus. In other embodiments, responders may also be defined as individuals showing a greater than 1.7-fold decrease in mRNA level when compared to a control stimulus. Each datum (○ for healthy adults and Δ for CD patients) was the mean from triplicate aliquots of whole blood. Blanket areas are the mean ± 3 standard deviation of the values of external control RNA34 (R34). The statistical significance shown in Fig. 1B (*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$) was calculated by χ^2 test using the population of responders and non-responders, as described above. ++ indicates $p<0.01$ by the t-test using the log values of the fold increase of the responder population only.

[0044] As shown in Fig. 1A, HAG mainly induced TNFSF-2, 8, 15, 18, IL-1B, 8, CCL-2, 3, 4, 11, 20, and CXCL-1, 2, 3, whereas anti-TCR induced different members of the TNFSF (TNFSF-1, 2, 5, 6, 9, 10, 14) and chemokine (IL-6, CCL-2, 3, 4, 8, 20, and CXCL-10) mRNAs. Further data obtained using whole blood of healthy individuals stimulated with HAG and expressed in terms of the cycle threshold (Ct) (see below), are shown in Figure 13. Although CD patients did not show any enhancement in HAG-induced activities, the responder population (>1.7 fold increase) for anti-TCR-induced TNFSF-2, 5, 6, 14, and CCL-2, 3, and 4, was significantly larger in CD patients than in healthy controls. Interestingly, CD patients induced significantly ($p<0.001$) more TNFSF-2 (=TNFα) than TNFSF-14. These data suggest impairment of TCR function in peripheral blood leukocytes. This system will be useful in the analysis of cytotoxic functions of immune cells in CD and other autoimmune diseases.

[0045] Figure 2 shows the correlation among various HAG-induced mRNA responses. In Figure 2, the data from Fig. 1A were transformed to x-y plots. Figure 2A shows IL-1B vs IL-8; Figure 2B shows IL-1B vs CCL-2; Figure 2C shows IL-1B vs CXCL-1; Figure 2D shows CXCL-1 vs CXCL-2; Figure 2E shows IL-1B vs TNFSF-15; and Figure 2F shows TNFSF-15 vs TNFSF-8, respectively. In the Figure, ○: healthy adults, ▲: CD.

[0046] Figure 3 shows the correlation among various anti-TCR-induced mRNA responses. In Figure 3, the data from Fig. 1A were transformed to x-y plots. Figure 3A shows TNFSF-2 vs TNFSF-14; Figure 3B shows TNFSF-5 vs TNFSF-6; Figure 3C shows CCL-2 vs CCL-3; Figure 3D shows CCL-2 vs CCL-4; Figure 3E shows CCL-2 vs CCL-20; and Figure 3F shows CCL-8 vs CXCL-10, respectively. In the Figure, ○: healthy adults, ▲: CD. In Figure 3A, regression lines for

both control and CD patients were drawn.

[0047] Because of wide individual-to-individual variation, and the presence of the population of responders and non-responders, a standard Student's t-test is not appropriate for the analysis of these results. The responder populations (>1.7 fold increase, as noted above) for TNFSF-2, 5, 6, 14, CCL-2, 3, and 4 in CD patients were 92.9%, 66.7%, 75.0%, 82.1%, 75.0%, 75.0%, and 83.3%, respectively, which were significantly higher than that for healthy controls (38.9%, 28.6%, 42.9%, 44.4%, 33.3%, 20.0%, and 20.0%, respectively) by χ^2 test (see Fig. 1B: *). When the fold increase of the responder population was compared in log scale, CD patients showed significantly ($P<0.01$) higher values than control subjects (see Fig. 1B: ++). Moreover, similar to the results of HAG-induced gene expression (Fig. 2), responders for certain TNFSF members and chemokines were also responders for other TNFSF members and chemokines (Fig. 3), suggesting that the variation in the anti-TCR data is derived from individual responses and not assay techniques. Surprisingly, the correlation between anti-TCR-induced TNFSF-2 (=TNFα) and TNFSF-14 was significantly ($p<0.001$) different between healthy control and CD patients: CD patients induced more TNFSF-2 than TNFSF-14, although these 2 TNFSF responses were correlated with each other in both cases (Fig. 3A).

[0048] External control RNA34 was unchanged in all cases, suggesting that the assay was performed appropriately. Although the results showed wide individual-to-individual variation, responders to certain TNFSF and chemokines were also responders to other TNFSF and chemokines (see Fig. 2), suggesting that the variation of the data was derived from individual responses rather than the assay techniques. Interestingly, CD patients did not show any enhancement in HAG-induced activity, and some CD patients actually showed reduced activity in HAG-induced IL-1B, IL-8, CCL-20, and CXCL-3 mRNA expression (Fig. 1A). In contrast, anti-TCR induced different TNFSF (TNFSF-1, 2, 5, 6, 9, 10, 14) and chemokine (IL-6, CCL-2, 3, 4, 8, 20, and CXCL-10) mRNAs. IL-1B and IL-8 mRNA were not induced by anti-TCR.

[0049] Dose responses and kinetic studies for the HAG stimulation are shown in Figure 4. Figure 4 shows the effect of heat aggregated IgG (HAG) on TNFSF and chemokine mRNA expression in peripheral blood leukocytes. Figure 4A shows the kinetics of the reaction. Triplicate aliquots of 60 μl each of heparinized whole blood was mixed with PBS (○, Δ), or 200 μg/mL HAG (●, ▲) and incubated at 37˚C for 0-12 hours. TNFSF-15 (○, ●) and IL-8 (Δ, ▲) mRNA were then quantified as described above. The fold increase was calculated using the values at time=0 as a control. Figure 4B shows the dose response. Triplicate aliquots of 60 μl each of heparinized whole blood were mixed with various concentrations of HAG and incubated at 37˚C for 2 hours. TNFSF-2 (●), TNFSF-15 (▲), IL-8 (○), IL-1B (0), and CXCL-2 (Δ) mRNA were then quantified as described above. The fold increase was calculated using the values for the solvent (PBS) as a control. Each data point was the mean±standard deviation (A) or mean (B) from triplicate aliquots of whole blood.

[0050] Dose responses and kinetic studies for the anti-TCR stimulation are shown in Figure 5. Figure 5 shows the effect of anti-TCR antibody on TNFSF and chemokine mRNA expression in peripheral blood leukocytes. Figure 5A shows the dose response and kinetics of the stimulus. Triplicate aliquots of 60 μl each of heparinized whole blood were mixed with 10 (●), 1 (♦) or 0.1 (▲) μg/mL mouse anti-human α/β TCR IgG1κ, PBS (○) or 10 (□) μg/ml purified mouse IgG1κ, and incubated at 37˚C for 0-7 hours. Then TNFSF-2 mRNA was quantified as described above. Figure 5B shows the kinetics of the stimulus. Triplicate aliquots of 60 μl each of heparinized whole blood were mixed with 10 μg/ml mouse anti-human α/β TCR IgG1κ, or 10 μg/ml purified mouse IgG1κ, and incubated at 37˚C for 2 hours. TNFSF-2 (●), TNFSF-5 (□), TNFSF-6 (▲), TNFSF-9 (♦), TNFSF-14 (○), and CXCL-10 (Δ) mRNA were then quantified as described above. The fold increase was calculated by the values of mouse IgG1κ as control. Each data was the mean±standard deviation (A) or mean (B) from triplicate aliquots of whole blood.

[0051] In addition to the anti-TCR and HAG described above, other stimulating agents, such as phorbol myristate acetate (PMA), phytohemagglutinin (PHA), wheat germ agglutinin (WGA), concanavalin-A (Con-A), lipopolysaccharides (LPS), jacalin, fucoidan, heat-aggregated IgA, heat-aggregated IgE, and heat-aggregated IgM, also induce different subtypes of TNFSF and chemokines in whole blood taken from healthy individuals, as shown in Figures 6-12 and 14-16. The protocol followed in these assays was the same as that given above, with the exception of the different stimulus employed in each case. In Figures 6-16, data are expressed in terms of the cycle threshold (Ct), which is the number of cycles of PCR required to generate certain amounts of PCR products. The ΔCt values were obtained by subtracting Ct values of un-stimulated samples from stimulated samples. Since Ct is a log scale, 1 ΔCt unit indicates a change in quantity by a factor of 2. Because a higher expression level reduces the number of PCR cycles required to generate a standard amount of products, a negative ΔCt value indicates an increase in expression.

[0052] Many of these agents exhibit a stimulus pattern similar to that of anti-TCR. In particular, PMA and PHA stimulate the same TNFSF subtypes as does anti-TCR in CD patients (TNFSF-2, -5, -6, and -14), as shown in Figures 6 and 7. Furthermore, WGA, ConA, LPS, jacalin, and fucoidan all stimulate the TNFSF-2 subtype that is known to be linked to CD, as shown in Figures 8-12. As shown in Figure 16, heat-aggregated IgE stimulates TNFSF-2, as well as CCL-3 and -4 and CXCL-10. Finally, heat-aggregated IgA stimulates CCL-2, -3, and -4, as shown in Figure 14. Because of the similarity of the stimulus patterns to the anti-TCR pattern described above, these agents will also be useful in assessing the therapeutic options for CD patients and in screening for new drugs for treating CD.

[0053] Cytotoxic assays have generally been used to study actual cell death resulting from the activity of the immune system, such as that which is believed to occur in CD. Cytotoxic assays are generally conducted by incubating ^{51}Cr-

loaded target cells with effector cells at various ratios, and quantifying the amounts of ^{51}Cr radioactivity released from the dead or damaged cells (see Dunkley et al., J Immunol Methods 6, 39 (1974)). Radioactive materials have been replaced with non-radioactive materials, such as fluorometric materials, in some cases (see Kruger-Krasagakes et al., J Immunol Methods 156, 1 (1992)), but the basic principle is unchanged. The results of cytotoxic assays are thus reflective of actual cell death.

[0054] However, cytotoxic assays are performed under non-physiological experimental conditions, and complex cell-to-cell and cell-to-plasma interactions are difficult to assess in the course of such studies. Furthermore, cytotoxic assays do not indicate which TNFSF member is responsible for cell death. Once effector cells recognize the target cells, the effector cells' function is not only to kill the target, but also recruit other effector cells, because a single effector cell is not enough to kill many target cells. This recruitment function is thought to be represented by the release of chemotactic factors. The identity of such chemotactic factors released by effector cells would not be revealed by classic cytotoxic assays. The assay system set forth in this disclosure is, however, capable of identifying many classes of gene expression in effector cells simultaneously.

[0055] Identification of responsible TNFSF and chemokine subtypes is critically important, because these molecules react with specific receptors on the target cells or leukocytes. For example, according to UniGene's Expression Sequence Tag (EST) profile database, the receptor for TNFSF-2 (Tumor Necrosis Factor Receptor Super Family (TNFRSF)-1A) is present in the small intestine, but receptors for TNFSF-5, 6, and 14 (TNFSF-5, 6, and 14) are not. Thus, enhanced TNFSF-2 activity in CD patients (Fig. 1B, Fig. 3A) may be linked to damage to the small intestine, which is a major CD disease site. Furthermore, the receptor of CCL-2 (CCR-2) is known as the receptor of monocyte chemoattractant protein-1, and the receptor for CXCL-10 (CXCR-3) is responsible for the migration of NK cells and T cells (UniGene database). As noted above, the disclosed data indicate that the effector leukocytes of CD patients transcribe an increased amount of CCL-2 and CXCL-10 mRNA when stimulated, which may explain the various leukocyte infiltrations seen at CD disease sites.

[0056] The use of whole blood is preferable to using isolated leukocytes in culture media, because the former is more physiological than the latter, and whole populations of leukocytes can be screened. Longer incubation of whole blood may produce additional artifacts. Thus, the ideal way is to identify early signals of killer and recruitment signals in whole blood during a short period of incubation by switching *in vitro* to *ex vivo*. The transcription of mRNA is an earlier event than either protein synthesis or the final biological outcomes. Thus, mRNA is a logical target of this study. Among many TNFSF, CCL, CXCL, and interleukin mRNAs, the data in this disclosure show that FcR and TCR induce different subclasses of genes, as shown in Figure 1.

[0057] Recent studies have suggested that TNFSF-2 is one of the major factors in CD (see Isaacs et al., Inflamm. Bowel Dis. 11 Suppl 1, S3 (2005)). In fact, Remicade®, which is a monoclonal antibody against TNF-α, is used clinically for patients with severe CD and a poor history with other conventional treatments, as noted above. Although 1NF-α plays a crucial role in the pathogenesis of CD in lesions, the data set forth in the present disclosure indicate an underlying hyperfunction of TCR inducibility of several TNFSF members, including TNF-α (TNFSF-2) in circulating leukocytes in peripheral blood, which has not previously been observed. Since the present method uses whole blood, not intestinal tissues, it may be used as a diagnostic test for CD to evaluate possible responsiveness to TNF-α therapy, and to monitor the therapeutic response.

[0058] Specifically, in a preferred embodiment of a method for determining whether a human having CD is likely to respond to a therapy targeting TNF-α activity, such as therapy employing an anti-TNF-α monoclonal antibody, whole blood is obtained from a CD patient and samples of the blood are subjected to anti-TCR antibody stimulation and optionally to control stimulation (10 mg/ml purified mouse IgG1κ), as described above. The mRNA level of TNFSF-2 may be measured in the samples as described above. A CD patient having a significantly elevated level of TNFSF-2 mRNA after stimulation with anti-TCR antibody (as indicated, for example, by a fold change of greater than 1.7) is a good candidate for therapy targeting TNF-α.

[0059] Alternatively, the level of one or more other mRNAs that are differentially transcribed in response to a T-cell stimulus, such as TNFSF-5, TNFSF-6, TNFSF-14, CCL-2, CCL-3, CCL-4, or CXCL-10 may be measured. A CD patient having a significantly elevated (such as a 1.7-fold or greater change) post-stimulation level of one or more of these mRNAs may be a good candidate for a therapy that targets proteins associated with these mRNAs.

[0060] Furthermore, in a preferred embodiment of a method of evaluating the effectiveness of CD treatment targeting one or more of TNFSF-2, TNFSF-5, TNFSF-6, TNFSF-14, CCL-2, CCL-3, CCL-4, and CXCL-10 TNF-α in a patient, a first ratio of the amount of the mRNA in whole blood after T-cell stimulation using anti-TCR antibody or another stimulus in vitro to the amount after control stimulation in vitro is obtained prior to the initiation of the treatment. The course of treatment is then begun. At some point during or after the treatment, a second ratio of the amount of the mRNA in whole blood after anti-TCR antibody stimulation in vitro to the amount after control stimulation in vitro is obtained. A significant difference in the ratios can indicate the effectiveness of the therapy. For example, where the treatment is the administration of infliximab, the measured mRNA is TNFSF-2, and the second ratio is larger than the first ratio, this can indicate the effectiveness of the therapy. The reason that an increase in inducibility with respect to an mRNA associated with TNF-

α can indicate successful inactivation is that when secreted TNF-α is successfully inactivated by a therapeutic agent such as Remicade®, a feedback mechanism operates in the T-cells by which more TNFSF-2 mRNA is transcribed.

[0061] In other cases, a smaller second ratio with respect to a CD-associated mRNA may indicate a successful therapy. Such a result indicates that the T cells have become less inducible with respect to the measured mRNA after treatment. For example, therapy that reduced the inducibility of the leukocytes with respect to TNFSF-2, and thus the amount of TNF-α released, would presumably be of benefit in ameliorating the symptoms of CD. Similarly, reductions in the amount of CCL-2 and CXCL-10 transcribed would likely lead to reduced leukocyte infiltration and less severe symptoms.

[0062] Importantly, this *ex vivo* method can also be used for the screening of compounds which inhibit anti-TCR-mediated TNFα mRNA expression. Such compounds will be interesting drug targets, because a monoclonal antibody against TNF-α reacts with already-released TNF-α at the lesion, whereas these new drug candidates will block TNF-α production of leukocytes at the transcription level. This is a new strategy for drug development against autoimmune disease.

[0063] In an embodiment of a method of screening drug compounds using the disclosed system and thereby identifying a putative agent for treating CD, whole blood is obtained from CD patients that are responders, in that their leukocytes exhibit at least a 1.7-fold increase in the level of a CD-associated mRNA when exposed to a t-cell stimulation such as anti-TCR. A first ratio of the amount of the mRNA in whole blood after T-cell stimulus using anti-TCR antibody or another stimulus in vitro to the amount after control stimulation in vitro is calculated. Further whole blood samples from the subjects are exposed in vitro to the drug compound, and then differentially stimulated as described above. A second ratio of the amount of the mRNA in whole blood after the T-cell stimulus in vitro to the amount after control stimulation in vitro is then calculated. A significant difference in the ratios can indicate that the drug compound is a candidate for further investigation as a potential therapeutic for CD.

[0064] Additionally, in a preferred embodiment of a method of monitoring the state of the disease in a CD patient by measuring levels of one or more of TNFSF-2, TNFSF-5, TNFSF-6, TNFSF-14, CCL-2, CCL-3, CCL-4, and CXCL-10 mRNAs in samples comprising leukocytes obtained from the patient, a first ratio of the amount of the mRNA in whole blood after T-cell stimulus using anti-TCR antibody or other stimulus in vitro to the amount after control stimulation in vitro is obtained at a first time. At a second time subsequent to the first time, a second ratio of the amount of the mRNA in whole blood after the T-cell stimulus in vitro to the amount after control stimulation in vitro is obtained. A significant difference in the ratios can indicate a change in the disease state. For example, when the second ratio is larger than the first, this can indicate disease progression, while a larger first ratio can indicate that the disease has regressed.

SEQUENCE LISTING

[0065]

<110> Hitachi Chemical Co., Ltd; Hitachi Chemical Research Center, Inc.; Cedars-Sinai Medical Center; Mitsuhashi, Masato; Targan, Stephan R.

<120> ENHANCED T CELL RECEPTOR-MEDIATED TUMOR NECROSIS FACTOR SUPERFAMILY AND CHEMOKINE MRNA EXPRESSION IN PERIPHERAL BLOOD LEUKOCYTES IN PATIENTS WITH CROHN'S DISEASE

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Claims

1. A method of determining whether a human having Crohn's disease is likely to respond to a therapy which targets TNF- α, comprising:

   stimulating leukocytes in vitro in a first sample that comprises leukocytes from the human;
   after the stimulation, measuring the amount of an mRNA selected from the group consisting of tumor necrosis factor superfamily ("TNFSF")-2, TNFSF-5, TNFSF-6, TNFSF-14, chemokine (C-C motif) ligand ("CCL")-2, CCL-3, CCL-4, and chemokine (C-X-C motif) ligand ("CXCL")-10 in the first sample;
   stimulating leukocytes in vitro in a second sample comprising leukocytes from the human with a control stimulus;
   measuring the amount of the mRNA in the second sample after stimulation; and
   determining a ratio of the amount of the mRNA in the first sample to the amount of the mRNA in the second sample, wherein the human is likely to respond to the therapy if the ratio is about 1.7:1 or greater.

2. The method of claim 1, wherein stimulating leukocytes in the first sample comprises intermixing with the first sample an agent selected from the group consisting of an anti-T cell receptor antibody, phorbol myristate acetate (PMA), phytohemagglutinin (PHA), wheat germ agglutinin (WGA), concanavalin-A (ConA), lipopolysaccharides (LPS), jacalin, fucoidan, heat-aggregated IgE, heat-aggregated IgA, heat-aggregated IgG and heat-aggregated IgM.

3. The method of claim 1 or 2, wherein at least one of the first and second samples comprises whole blood.

4. A method of evaluating the effectiveness of a Crohn's disease therapy in a human, comprising:

stimulating leukocytes in vitro in a first sample comprising leukocytes from the human;
stimulating leukocytes in vitro in a second sample comprising leukocytes from the human with a control stimulus;
measuring the amount of an mRNA selected from the group consisting of tumor necrosis factor superfamily ("TNFSF")-2, TNFSF-5, TNFSF-6, TNFSF-14,
chemokine (C-C motif) ligand ("CCL")-2, CCL-3, CCL-4, and chemokine (C-X-C motif) ligand ("CXCL")-10 in the first and second samples after stimulation;
calculating a first ratio of the amount of the mRNA in the first sample to the amount of the mRNA in the second sample;
stimulating leukocytes in vitro in a third sample comprising leukocytes from the human obtained after the administration of therapy;
stimulating leukocytes in vitro in a fourth sample comprising leukocytes from the human obtained after the administration of therapy with the control stimulus;
measuring the level of the mRNA in the third and fourth samples after stimulation;
calculating a second ratio of the amount of the mRNA in the third sample to the amount of the mRNA in the fourth sample; and
comparing the first and second ratios, wherein a significant difference in the ratios is indicative of an effective therapy,
wherein the therapy comprises inactivation of tumor necrosis factor alpha.

5. The method of claim 4, wherein stimulating leukocytes in the first and third samples comprises intermixing with the sample an agent selected from the group consisting of an anti-T cell receptor antibody, phorbol myristate acetate (PMA), phytohemagglutinin (PHA), wheat germ agglutinin (WGA), concanavalin-A (ConA), lipopolysaccharides (LPS), jacalin, fucoidan, heat-aggregated IgE, heat-aggregated IgA, heat-aggregated IgG and heat-aggregated IgM.

6. The method of claim 4 or 5, wherein the significant difference in the ratios is that the second ratio is greater than the first ratio.

7. The method of claim 4 or 5, wherein the significant difference in the ratios is that the first ratio is greater than the second ratio.

8. A method of identifying a putative agent for treating Crohn's disease, comprising:

obtaining first, second, third, and fourth samples comprising leukocytes from a human whose leukocytes demonstrate at least a 1.7-fold increase in the transcription of an mRNA selected from the group consisting of tumor necrosis factor superfamily ("TNFSF")-2, TNFSF-5, TNFSF-6, TNFSF-14, chemokine (C-C motif) ligand ("CCL")-2, CCL-3, CCL-4, and chemokine (C-X-C motif) ligand ("CXCL")-10 when exposed to an anti-T cell receptor antibody;
stimulating leukocytes in vitro in the first sample;
stimulating leukocytes in vitro in the second sample with a control stimulus;
measuring the amount of the mRNA in the first and second samples after stimulation;
calculating a first ratio of the amount of the mRNA in the first sample to the amount of the mRNA in the second sample;
exposing the third and fourth samples to the agent;
stimulating leukocytes in vitro in the third sample after the exposure;
stimulating leukocytes in vitro in the fourth sample with the control stimulus after the exposure;
measuring the level of the mRNA in the third and fourth samples after stimulation;
calculating a second ratio of the amount of the mRNA in the third sample to the amount of the mRNA in the fourth sample; and
comparing the first and second ratios, wherein a significant difference in the ratios is indicative of a putative agent.

9. The method of claim 8, wherein stimulating leukocytes in the first and third samples comprises intermixing with the sample an agent selected from the group consisting of an anti-T cell receptor antibody, phorbol myristate acetate (PMA), phytohemagglutinin (PHA), wheat germ agglutinin (WGA), concanavalin-A (ConA), lipopolysaccharides (LPS), jacalin, fucoidan, heat-aggregated IgE, heat-aggregated IgA, heat-aggregated IgG and heat-aggregated IgM.

10. The method of claim 8 or 9, wherein the significant difference in the ratios is that the first ratio is greater than the second ratio.

11. A method of evaluating the state of Crohn's disease in a human, comprising:

stimulating leukocytes in vitro in a first sample that comprises leukocytes and is obtained at a first time from the human;
after the stimulation, measuring the amount of an mRNA selected from the group consisting of tumor necrosis factor superfamily ("TNFSF")-2, TNFSF-5, TNFSF-6, TNFSF-14, chemokine (C-C motif) ligand ("CCL")-2, CCL-3, CCL-4,
and chemokine (C-X-C motif) ligand ("CXCL")-10 in the first sample;
stimulating leukocytes in vitro with a control stimulus in a second sample comprising leukocytes obtained from the human at the first time;
measuring the amount of the mRNA in the second sample after stimulation;
determining a first ratio of the amount of the mRNA in the first sample to the amount of the mRNA in the second sample;
stimulating leukocytes in vitro in a third sample that comprises leukocytes and
is obtained from the human at a second time that is subsequent to the first time;
after the stimulation, measuring the amount of the mRNA in the third sample;
stimulating leukocytes in vitro with a control stimulus in a fourth sample comprising leukocytes obtained from the human at the second time;
measuring the amount of the mRNA in the fourth sample after stimulation;
determining a second ratio of the amount of the mRNA in the third sample to the amount of the mRNA in the fourth sample; and
comparing the first and second ratios, wherein a significant difference in the first and second ratios is indicative of a change in the disease state.

12. The method of claim 11, wherein stimulating leukocytes in the first and third samples comprises intermixing with the sample an agent selected from the group consisting of an anti-T cell receptor antibody, phorbol myristate acetate (PMA), phytohemagglutinin (PHA), wheat germ agglutinin (WGA), concanavalin-A (ConA), lipopolysaccharides (LPS), jacalin, fucoidan, heat-aggregated IgE, heat-aggregated IgA, heat-aggregated IgG and heat-aggregated IgM.

13. The method of claim 11 or 12, wherein the significant difference in the ratios is that the second ratio is greater than the first ratio, and the change in disease state is a progression of the disease.

14. The method of claim 11 or 12, wherein the significant difference in the ratios is that the first ratio is greater than the second ratio, and the change in disease state is a regression of the disease.

15. The method of any one of claims 1 to 3, 4 or 5, 8 or 9, or 11 or 12, wherein the control stimulus comprises a purified control immunoglobulin.

16. The method of any one of claims 1 to 3 or 6, wherein the therapy comprises administration of infliximab.

17. The method of any one of claims 1 to 3 or 7, wherein the therapy comprises administration of an agent selected from the group consisting of cyclosporin A and tacrolimus.

18. The method of claim 4 or 5, 8 or 9, or 11 or 12, wherein at least one of the first, second, third and fourth samples comprise whole blood.

Patentansprüche

1. Verfahren zum Bestimmen, ob ein Mensch, der Morbus Crohn hat, wahrscheinlich auf eine Therapie reagiert, die auf TNF-α abzielt, umfassend:

Stimulieren von Leukozyten in vitro in einer ersten Probe, umfassend Leukozyten von dem Menschen;
nach der Stimulierung Messen der Menge einer mRNA ausgewählt aus der Gruppe bestehend aus Tumornekrosefaktor-Superfamilie ("TNFSF")-2, TNFSF-5, TNFSF-6, TNFSF-14, Chemokin (C-C-Motiv)-Ligand ("CCL")-

2, CCL-3, CCL-4 und Chemokin (C-X-C-Motiv)-Ligand ("CXCL")-10 in der ersten Probe;
Stimulieren der Leukozyten in vitro in einer zweiten Probe umfassend Leukozyten von dem Menschen mit einem Kontrollstimulus;
Messen der Menge an mRNA in der zweiten Probe nach der Stimulierung; und
Ermitteln des Verhältnisses der Menge an mRNA in der ersten Probe zu der Menge an mRNA in der zweiten Probe, wobei der Mensch wahrscheinlich auf die Therapie reagiert, wenn das Verhältnis etwa 1,7:1 oder größer ist.

2. Verfahren gemäß Anspruch 1, wobei das Stimulieren von Leukozyten in der ersten Probe das Vermischen der ersten Probe mit einem Agens umfasst, ausgewählt aus der Gruppe bestehend aus einem Anti-T-Zellrezeptor-Antikörper, Phorbolmyristatacetat (PMA), Phytohemagglutinin (PHA), Weizenkeimagglutinin (WGA), Concanavalin-A (ConA), Lipopolysacchariden (LPS), Jacalin, Fucoidan, Hitze-aggregiertem IgE, Hitze-aggregiertem IgA, Hitze-aggregiertem IgG und Hitze-aggregiertem IgM.

3. Verfahren gemäß Anspruch 1 oder 2, wobei mindestens eine der ersten und der zweiten Probe Vollblut umfasst.

4. Verfahren zum Evaluieren der Effektivität einer Morbus Crohn-Therapie in einem Menschen, umfassend:

Stimulieren von Leukozyten in vitro in einer ersten Probe umfassend Leukozyten von dem Menschen;
Stimulieren von Leukozyten in vitro in einer zweiten Probe umfassend Leukozyten von dem Menschen mit einem Kontrollstimulus;
Messen der Menge einer mRNA ausgewählt aus der Gruppe bestehend aus Tumomekrosefaktor-Superfamilie ("TNFSF")-2, TNFSF-5, TNFSF-6, TNFSF-14,
Chemokin (C-C-Motiv)-Ligand ("CCL")-2, CCL-3, CCL-4 und Chemokin (C-X-C Motiv)-Ligand ("CXCL")-10 in der ersten Probe und der zweiten Probe nach der Stimulierung;
Berechnen eines ersten Verhältnisses der Menge der mRNA in der ersten Probe zu der Menge der mRNA in der zweiten Probe;
Stimulieren von Leukozyten in vitro in einer dritten Probe umfassend Leukozyten von dem Menschen, die nach der Verabreichung der Therapie erhalten wurden;
Stimulieren von Leukozyten in vitro in einer vierten Probe umfassend Leukozyten von dem Menschen, die nach der Verabreichung der Therapie mit dem Kontrollstimulus erhalten wurden;
Messen des Gehalts an mRNA in der dritten und vierten Probe nach der Stimulierung;
Berechnen eines zweiten Verhältnisses der Menge an mRNA in der dritten Probe zu der Menge an mRNA in der vierten Probe; und
Vergleichen des ersten und zweiten Verhältnisses, wobei eine signifikante Differenz in den Verhältnissen auf eine effektive Therapie hinweist;
wobei die Therapie die Inaktivierung von Tumomekrosefaktor alpha umfasst.

5. Verfahren gemäß Anspruch 4, wobei das Stimulieren von Leukozyten in der ersten und dritten Probe das Vermischen der Probe mit einem Agens umfasst, ausgewählt aus der Gruppe bestehend aus einem Anti-T-Zellrezeptor-Antikörper, Phorbolmyristatacetat (PMA), Phytohemagglutinin (PHA), Weizenkeimagglutinin (WGA), Concanavalin-A (ConA), Lipopolysacchariden (LPS), Jacalin, Fucoidan, Hitze-aggregiertem IgE, Hitze-aggregiertem IgA, Hitze-aggregiertem IgG und Hitze-aggregiertem IgM.

6. Verfahren gemäß Anspruch 4 oder 5, wobei die signifikante Differenz in den Verhältnissen ist, dass das zweite Verhältnis größer als das erste Verhältnis ist.

7. Verfahren gemäß Anspruch 4 oder 5, wobei die signifikante Differenz in den Verhältnissen ist, dass das erste Verhältnis größer als das zweite Verhältnis ist.

8. Verfahren zum Identifizieren eines putativen Agens zur Behandlung von Morbus Crohn, umfassend:

Erhalten einer ersten, zweiten, dritten und vierten Probe umfassend Leukozyten von einem Menschen, dessen Leukozyten mindestens eine 1,7-fache Erhöhung in der Transkription einer mRNA zeigen, ausgewählt aus der Gruppe bestehend aus Tumomekrosefaktor-Superfamilie ("TNFSF")-2, TNFSF-5, TNFSF-6, TNFSF-14,
Chemokin (C-C-Motiv)-Ligand ("CCL")-2, CCL-3, CCL-4 und Chemokin (C-X-C-Motiv)-Ligand ("CXCL")-10, wenn sie einem Anti-T-Zellrezeptor-Antikörper ausgesetzt sind;
Stimulieren von Leukozyten in vitro in der ersten Probe;

Stimulieren von Leukozyten in vitro in der zweiten Probe mit einem Kontrollstimulus;
Messen der Menge an mRNA in der ersten und zweiten Probe nach der Stimulierung;
Berechnen eines ersten Verhältnisses der Menge an mRNA in der ersten Probe zu der Menge an mRNA in der zweiten Probe;
Aussetzen der dritten und vierten Probe dem Agens;
Stimulieren von Leukozyten in vitro in der dritten Probe nach der Aussetzung;
Stimulieren von Leukozyten in vitro in der vierten Probe mit dem Kontrollstimulus nach der Aussetzung;
Messen des Gehalts an mRNA in der dritten und vierten Probe nach der Stimulierung;
Berechnen eines zweiten Verhältnisses der Menge an mRNA in der dritten Probe zu der Menge an mRNA in der vierten Probe; und
Vergleichen des ersten und zweiten Verhältnisses, wobei eine signifikante Differenz in den Verhältnissen ein Hinweis auf ein putatives Agens ist.

9. Verfahren gemäß Anspruch 8, wobei das Stimulieren von Leukozyten in der ersten und dritten Probe das Vermischen der Probe mit einem Agens umfasst, ausgewählt aus der Gruppe bestehend aus einem Anti-T-Zellrezeptor-Antikörper, Phorbolmyristatacetat (PMA), Phytohemagglutinin (PHA), Weizenkeimagglutinin (WGA), Concanavalin-A (ConA), Lipopolysacchariden (LPS), Jacalin, Fucoidan, Hitze-aggregiertem IgE, Hitze-aggregiertem IgA, Hitze-aggregiertem IgG und Hitze-aggregiertem IgM.

10. Verfahren gemäß Anspruch 8 oder 9, wobei die signifikante Differenz in den Verhältnissen ist, dass das erste Verhältnis größer als das zweite Verhältnis ist.

11. Verfahren zum Evaluieren des Morbus Crohn-Zustands in einem Menschen, umfassend:

Stimulieren von Leukozyten in vitro in einer ersten Probe, die Leukozyten umfasst und die an einem ersten Zeitpunkt von dem Menschen erhalten wurde;
nach der Stimulierung Messen der Menge an mRNA ausgewählt aus der Gruppe bestehend aus Tumomekrosefaktor-Superfamilie ("TNFSF")-2, TNFSF-5, TNFSF-6, TNFSF-14, Chemokin (C-C-Motiv)-Ligand ("CCL")-2, CCL-3, CCL-4 und Chemokin (C-X-C-Motiv)-Ligand ("CXCL")-10 in der ersten Probe;
Stimulieren von Leukozyten in vitro mit einem Kontrollstimulus in einer zweiten Probe umfassend Leukozyten, die an dem ersten Zeitpunkt von dem Menschen erhalten wurden;
Messen der Menge an mRNA in der zweiten Probe nach der Stimulierung;
Bestimmen eines ersten Verhältnisses der Menge an mRNA in der ersten Probe zu der Menge an mRNA in der zweiten Probe;
Stimulieren von Leukozyten in vitro in einer dritten Probe, die Leukozyten umfasst und die von dem Menschen erhalten wurde zu einem zweiten Zeitpunkt, der nach dem ersten Zeitpunkt liegt;
nach der Stimulierung Messen der Menge an mRNA in der dritten Probe;
Stimulieren von Leukozyten in vitro mit einem Kontrollstimulus in der vierten Probe umfassend Leukozyten, die zu dem zweiten Zeitpunkt von dem Menschen erhalten wurden;
Messen der Menge an mRNA in der vierten Probe nach der Stimulierung;
Bestimmen eines zweiten Verhältnisses der Menge an mRNA in der dritten Probe zu der Menge an mRNA in der vierten Probe; und
Vergleichen des ersten und zweiten Verhältnisses, wobei eine signifikante Differenz in dem ersten und zweiten Verhältnis auf eine Änderung in dem Krankheitszustand hinweist.

12. Verfahren gemäß Anspruch 11, wobei das Stimulieren von Leukozyten in der ersten und dritten Probe das Vermischen der Probe mit einem Agens umfasst, ausgewählt aus der Gruppe bestehend aus einem Anti-T-Zellrezeptor-Antikörper, Phorbolmyristatacetat (PMA), Phytohemagglutinin (PHA), Weizenkeimagglutinin (WGA), Concanavalin-A (ConA), Lipopolysacchariden (LPS), Jacalin, Fucoidan, Hitze-aggregiertem IgE, Hitze-aggregiertem IgA, Hitze-aggregiertem IgG und Hitze-aggregiertem IgM.

13. Verfahren gemäß Anspruch 11 oder 12, wobei die signifikante Differenz in den Verhältnissen ist, dass das zweite Verhältnis größer als das erste Verhältnis ist und die Änderung des Krankheitszustands ein Fortschreiten der Krankheit ist.

14. Verfahren gemäß Anspruch 11 oder 12, wobei die signifikante Differenz in den Verhältnissen ist, dass das erste Verhältnis größer als das zweite Verhältnis ist und die Änderung des Krankheitszustands eine Regression der Krankheit ist.

15. Verfahren gemäß einem der Ansprüche 1 bis 3, 4 oder 5, 8 oder 9 oder 11 oder 12, wobei der Kontrollstimulus ein aufgereinigtes Kontrollimmunglobulin umfasst.

16. Verfahren gemäß einem der Ansprüche 1 bis 3 oder 6, wobei die Therapie die Verabreichung von Infliximab umfasst.

17. Verfahren gemäß einem der Ansprüche 1 bis 3 oder 7, wobei die Therapie die Verabreichung eines Agens ausgewählt aus der Gruppe bestehend aus Cyclosporin A und Tacrolimus umfasst.

18. Verfahren gemäß Anspruch 4 oder 5, 8 oder 9 oder 11 oder 12, wobei mindestens eine der ersten, zweiten, dritten und vierten Probe Vollblut umfasst.

Revendications

1. Procédé pour déterminer si un humain atteint de la maladie de Crohn est susceptible de répondre à une thérapie qui cible le TNF-α, consistant à :

   stimuler des leucocytes in vitro dans un premier échantillon qui comprend des leucocytes provenant de l'humain ;
   après la stimulation, mesurer la quantité d'un ARNm choisi parmi le groupe consistant en superfamille des facteurs de nécrose tumorale ("TNFSF")-2, TNFSF-5, TNFSF-6, TNFSF-14, ligand ("CCL")-2 de chimiokine (motif C-C), CCL-3, CCL-4, et ligand ("CXCL")-10 de chimiokine (motif C-X-C) dans le premier échantillon ;
   stimuler des leucocytes in vitro dans un deuxième échantillon comprenant des leucocytes provenant de l'humain avec un stimulus témoin ;
   mesurer la quantité de l'ARNm dans le deuxième échantillon après la stimulation ; et
   déterminer un rapport de la quantité de l'ARNm dans le premier échantillon par rapport à la quantité de l'ARNm dans le deuxième échantillon, où l'humain est susceptible de répondre à la thérapie si le rapport est d'environ 1,7 : 1 ou plus.

2. Procédé selon la revendication 1, où la stimulation des leucocytes dans le premier échantillon consiste à mélanger avec le premier échantillon un agent choisi parmi le groupe consistant en un anticorps du récepteur anti-lymphocyte T, phorbol myristate acétate (PMA), phytohémagglutinine (PHA), agglutinine de germe de blé (WGA), concanavaline A (ConA), lipopolysaccharides (LPS), jacaline, fucoïdane, IgE thermoagrégée, IgA thermoagrégée, IgG thermoagrégée et IgM thermoagrégée.

3. Procédé selon la revendication 1 ou 2, où au moins l'un des premier et deuxième échantillons comprend du sang total.

4. Procédé d'évaluation de l'efficacité d'une thérapie envers la maladie de Crohn chez un humain, consistant à :

   stimuler des leucocytes in vitro dans un premier échantillon comprenant des leucocytes provenant de l'humain ;
   stimuler des leucocytes in vitro dans un deuxième échantillon comprenant des leucocytes provenant de l'humain avec un stimulus témoin ;
   mesurer la quantité d'un ARNm choisi parmi le groupe consistant en superfamille des facteurs de nécrose tumorale ("TNFSF")-2, TNFSF-5, TNFSF-6, TNFSF-14, ligand ("CCL")-2 de chimiokine (motif C-C), CCL-3, CCL-4, et ligand ("CXCL")-10 de chimiokine (motif C-X-C) dans les premier et deuxième échantillons après la stimulation ;
   calculer un premier rapport de la quantité de l'ARNm dans le premier échantillon par rapport à la quantité de l'ARNm dans le deuxième échantillon ;
   stimuler des leucocytes in vitro dans un troisième échantillon comprenant des leucocytes provenant de l'humain obtenus après l'administration de la thérapie ;
   stimuler des leucocytes in vitro dans un quatrième échantillon comprenant des leucocytes provenant de l'humain obtenus après l'administration de la thérapie avec le stimulus témoin ;
   mesurer le niveau de l'ARNm dans les troisième et quatrième échantillons après la stimulation ;
   calculer un deuxième rapport de la quantité de l'ARNm dans le troisième échantillon par rapport à la quantité de l'ARNm dans le quatrième échantillon ; et
   comparer les premier et deuxième rapports, où une différence significative des rapports est indicatrice d'une thérapie efficace,
   où la thérapie comprend l'inactivation du facteur de nécrose tumorale alpha.

5. Procédé selon la revendication 4, où la stimulation des leucocytes dans les premier et troisième échantillons consiste à mélanger avec l'échantillon un agent choisi parmi le groupe consistant en un anticorps du récepteur anti-lymphocyte T, phorbol myristate acétate (PMA), phytohémagglutinine (PHA), agglutinine de germe de blé (WGA), concanavaline A (ConA), lipopolysaccharides (LPS), jacaline, fucoïdane, IgE thermoagrégée, IgA thermoagrégée, IgG thermoagrégée et IgM thermoagrégée.

6. Procédé selon la revendication 4 ou 5, où la différence significative des rapports est telle que le deuxième rapport est supérieur au premier rapport.

7. Procédé selon la revendication 4 ou 5, où la différence significative des rapports est telle que le premier rapport est supérieur au deuxième rapport.

8. Procédé d'identification d'un agent putatif destiné au traitement de la maladie de Crohn, consistant à :

obtenir des premier, deuxième, troisième, et quatrième échantillons comprenant des leucocytes provenant d'un humain dont les leucocytes démontrent au moins une augmentation d'un facteur 1,7 de la transcription d'un ARNm choisi parmi le groupe consistant en superfamille des facteurs de nécrose tumorale ("TNFSF")-2, TNFSF-5, TNFSF-6, TNFSF-14, ligand ("CCL")-2 de chimiokine (motif C-C), CCL-3, CCL-4, et ligand ("CXCL")-10 de chimiokine (motif C-X-C) lorsqu'ils sont exposés à un anticorps du récepteur anti-lymphocyte T ;
stimuler des leucocytes in vitro dans le premier échantillon;
stimuler des leucocytes in vitro dans le deuxième échantillon avec un stimulus témoin ;
mesurer la quantité de l'ARNm dans les premier et deuxième échantillons après la stimulation ;
calculer un premier rapport de la quantité de l'ARNm dans le premier échantillon par rapport à la quantité de l'ARNm dans le deuxième échantillon ;
exposer les troisième et quatrième échantillons à l'agent ;
stimuler des leucocytes in vitro dans le troisième échantillon après l'exposition;
stimuler des leucocytes in vitro dans le quatrième échantillon avec le stimulus témoin après l'exposition ;
mesurer le niveau de l'ARNm dans les troisième et quatrième échantillons après la stimulation ;
calculer un deuxième rapport de la quantité de l'ARNm dans le troisième échantillon par rapport à la quantité de l'ARNm dans le quatrième échantillon ; et
comparer les premier et deuxième rapports, où une différence significative des rapports est indicatrice d'un agent putatif.

9. Procédé selon la revendication 8, où la stimulation des leucocytes dans les premier et troisième échantillons consiste à mélanger avec l'échantillon un agent choisi parmi le groupe consistant en un anticorps du récepteur anti-lymphocyte T, phorbol myristate acétate (PMA), phytohémagglutinine (PHA), agglutinine de germe de blé (WGA), concanavaline A (ConA), lipopolysaccharides (LPS), jacaline, fucoïdane, IgE thermoagrégée, IgA thermoagrégée, IgG thermoagrégée et IgM thermoagrégée.

10. Procédé selon la revendication 8 ou 9, où la différence significative des rapports est telle que le premier rapport est supérieur au deuxième rapport.

11. Procédé d'évaluation de l'état de la maladie de Crohn chez un humain, consistant à:

stimuler les leucocytes in vitro dans un premier échantillon qui comprend des leucocytes et qui est obtenu à un premier moment à partir de l'humain ;
après la stimulation, mesurer la quantité d'un ARNm choisi parmi le groupe consistant en superfamille des facteurs de nécrose tumorale ("TNFSF")-2, TNFSF-5, TNFSF-6, TNFSF-14, ligand ("CCL")-2 de chimiokine (motif C-C), CCL-3, CCL-4, et ligand ("CXCL")-10 de chimiokine (motif C-X-C) dans le premier échantillon ;
stimuler des leucocytes in vitro avec un stimulus témoin dans un deuxième échantillon comprenant des leucocytes obtenus à partir de l'humain au premier moment ;
mesurer la quantité de l'ARNm dans le deuxième échantillon après la stimulation ;
déterminer un premier rapport de la quantité de l'ARNm dans le premier échantillon par rapport à la quantité de l'ARNm dans le deuxième échantillon ;
stimuler des leucocytes in vitro dans un troisième échantillon qui comprend des leucocytes et qui est obtenu à partir de l'humain à un deuxième moment qui est ultérieur au premier moment ;
après la stimulation, mesurer la quantité de l'ARNm dans le troisième échantillon ;
stimuler des leucocytes in vitro avec un stimulus témoin dans un quatrième échantillon comprenant des leuco-

cytes obtenus à partir de l'humain au deuxième moment ;
mesurer la quantité de l'ARNm dans le quatrième échantillon après la stimulation ;
déterminer un deuxième rapport de la quantité de l'ARNm dans le troisième échantillon par rapport à la quantité de l'ARNm dans le quatrième échantillon ; et
comparer les premier et deuxième rapports, où une différence significative des premier et deuxième rapports est indicatrice d'un changement de l'état de la maladie.

12. Procédé selon la revendication 11, où la stimulation des leucocytes dans les premier et troisième échantillons consiste à mélanger avec l'échantillon un agent choisi parmi le groupe consistant en un anticorps du récepteur anti-lymphocyte T, phorbol myristate acétate (PMA), phytohémagglutinine (PHA), agglutinine de germe de blé (WGA), concanavaline A (ConA), lipopolysaccharides (LPS), jacaline, fucoïdane, IgE thermoagrégée, IgA thermoagrégée, IgG thermoagrégée et IgM thermoagrégée.

13. Procédé selon la revendication 11 ou 12, où la différence significative des rapports est telle que le deuxième rapport est supérieur au premier rapport, et le changement de l'état de la maladie est une progression de la maladie.

14. Procédé selon la revendication 11 ou 12, où la différence significative des rapports est telle que le premier rapport est supérieur au deuxième rapport, et le changement de l'état de la maladie est une régression de la maladie.

15. Procédé selon l'une quelconque des revendications 1 à 3, 4 ou 5, 8 ou 9, ou 11 ou 12, où le stimulus témoin comprend une immunoglobuline témoin purifiée.

16. Procédé selon l'une quelconque des revendications 1 à 3 ou 6, où la thérapie comprend l'administration d'infliximab.

17. Procédé selon l'une quelconque des revendications 1 à 3 ou 7, où la thérapie comprend l'administration d'un agent choisi parmi le groupe consistant en cyclosporine A et tacrolimus.

18. Procédé selon la revendication 4 ou 5, 8 ou 9, ou 11 ou 12, où au moins l'un des premier, deuxième, troisième et quatrième échantillons comprend du sang total.

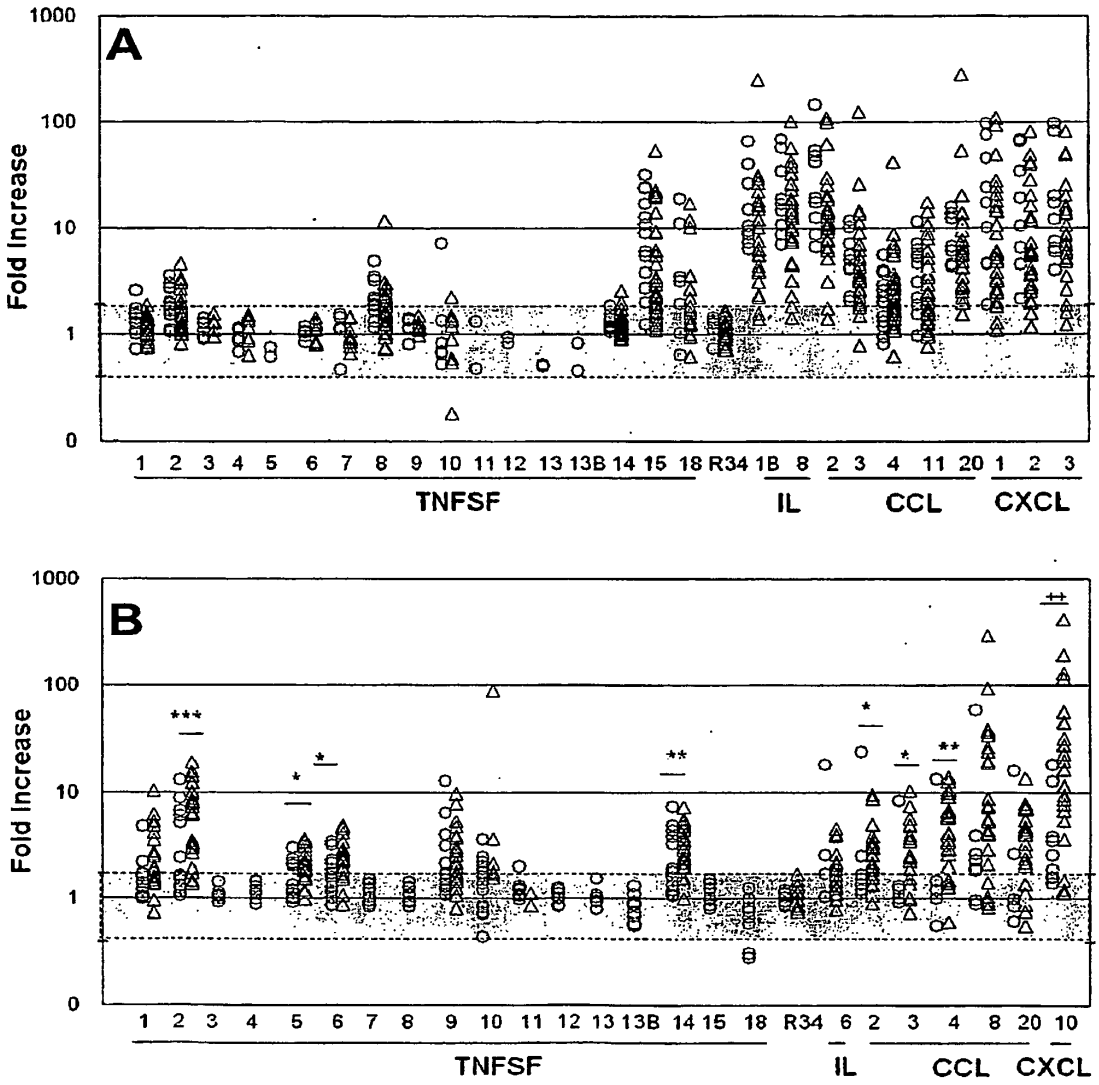
A
Fold Increase
1000
100
10
1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 13B 14 15 18 R34 1B 8 2 3 4 11 20 1 2 3
TNFSF
IL
CCL
CXCL
B
Fold Increase
1000
100
10
1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 13B 14 15 18 R34 6 2 3 4 8 20 10
TNFSF
IL
CCL
CXCL

FIG. 1

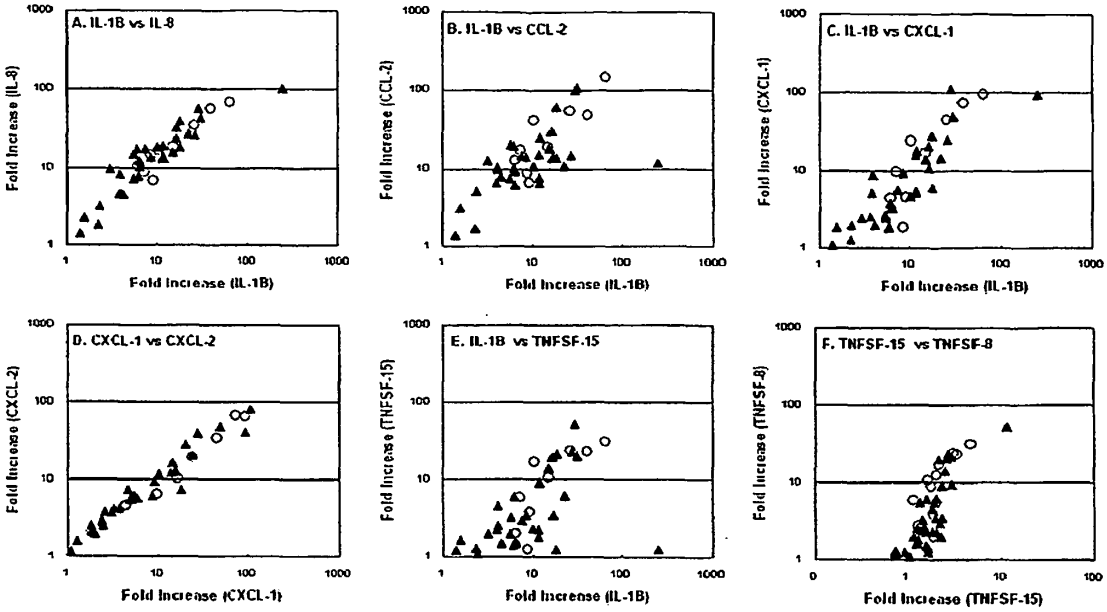
A. IL-1B vs IL-8
Fold Increase (IL-8)
Fold Increase (IL-1B)
B. IL-1B vs CCL-2
Fold Increase (CCL-2)
Fold Increase (IL-1B)
C. IL-1B vs CXCL-1
Fold Increase (CXCL-1)
Fold Increase (IL-1B)
D. CXCL-1 vs CXCL-2
Fold Increase (CXCL-2)
Fold Increase (CXCL-1)
E. IL-1B vs TNFSF-15
Fold Increase (TNFSF-15)
Fold Increase (IL-1B)
F. TNFSF-15 vs TNFSF-8
Fold Increase (TNFSF-8)
Fold Increase (TNFSF-15)

FIG. 2

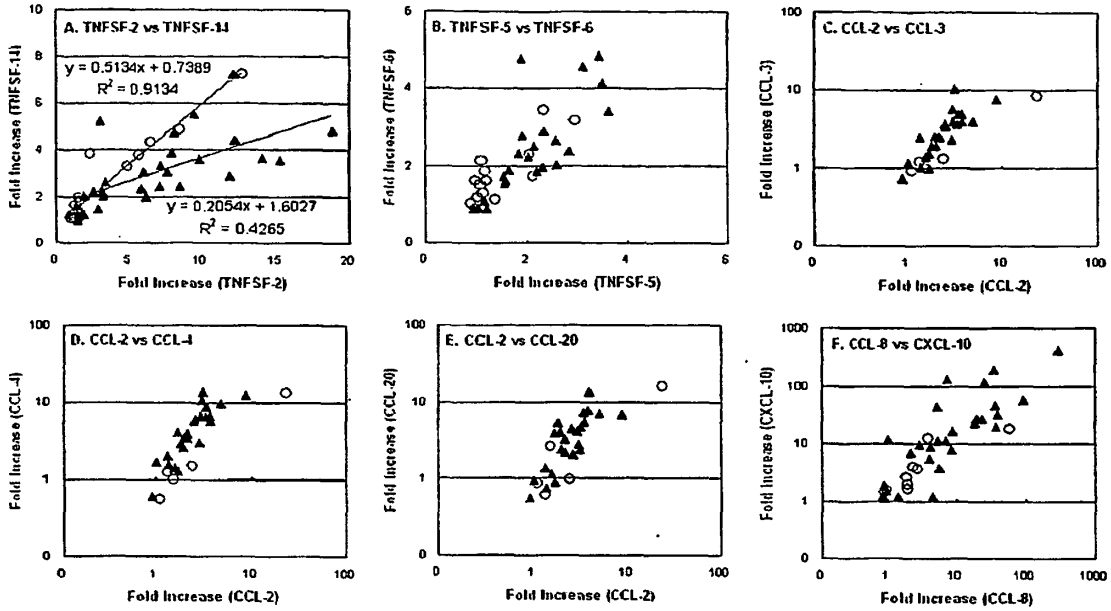
A. TNFSF-2 vs TNFSF-11
y = 0.5134x + 0.7389
R² = 0.9134
y = 0.2054x + 1.6027
R² = 0.4265
Fold Increase (TNFSF-11)
Fold Increase (TNFSF-2)
B. TNFSF-5 vs TNFSF-6
Fold Increase (TNFSF-6)
Fold Increase (TNFSF-5)
C. CCL-2 vs CCL-3
Fold Increase (CCL-3)
Fold Increase (CCL-2)
D. CCL-2 vs CCL-4
Fold Increase (CCL-4)
Fold Increase (CCL-2)
E. CCL-2 vs CCL-20
Fold Increase (CCL-20)
Fold Increase (CCL-2)
F. CCL-8 vs CXCL-10
Fold Increase (CXCL-10)
Fold Increase (CCL-8)

FIG. 3

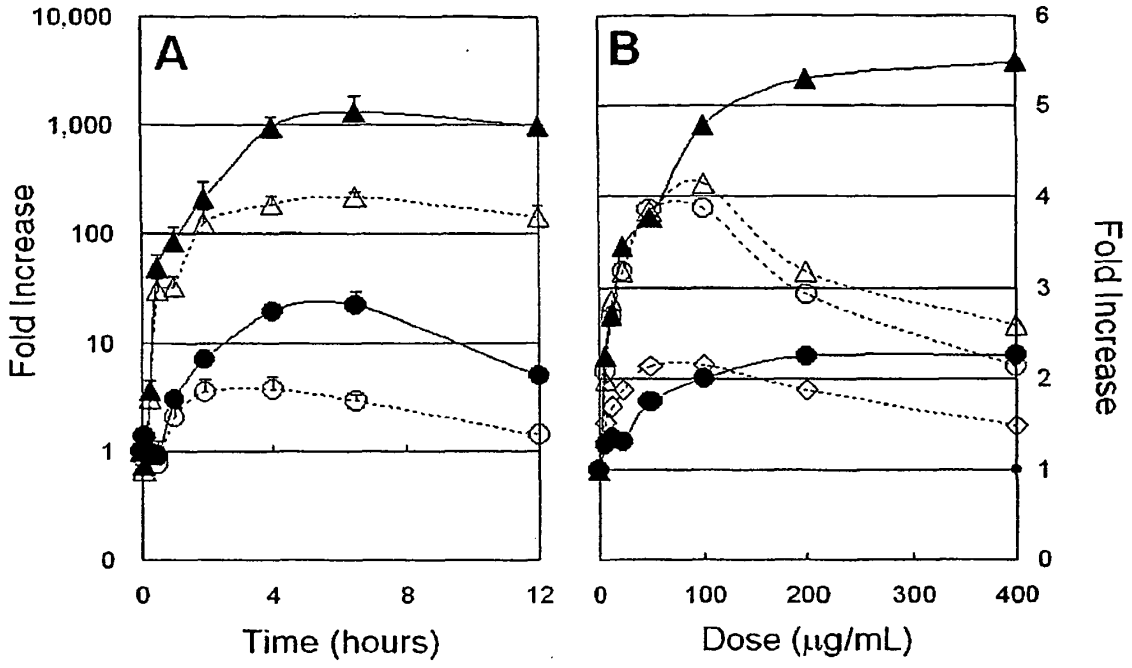
A
B
10,000
1,000
100
10
1
0
Fold Increase
0
4
8
12
Time (hours)
6
5
4
3
2
1
0
Fold Increase
0
100
200
300
400
Dose (μg/mL)

FIG. 4

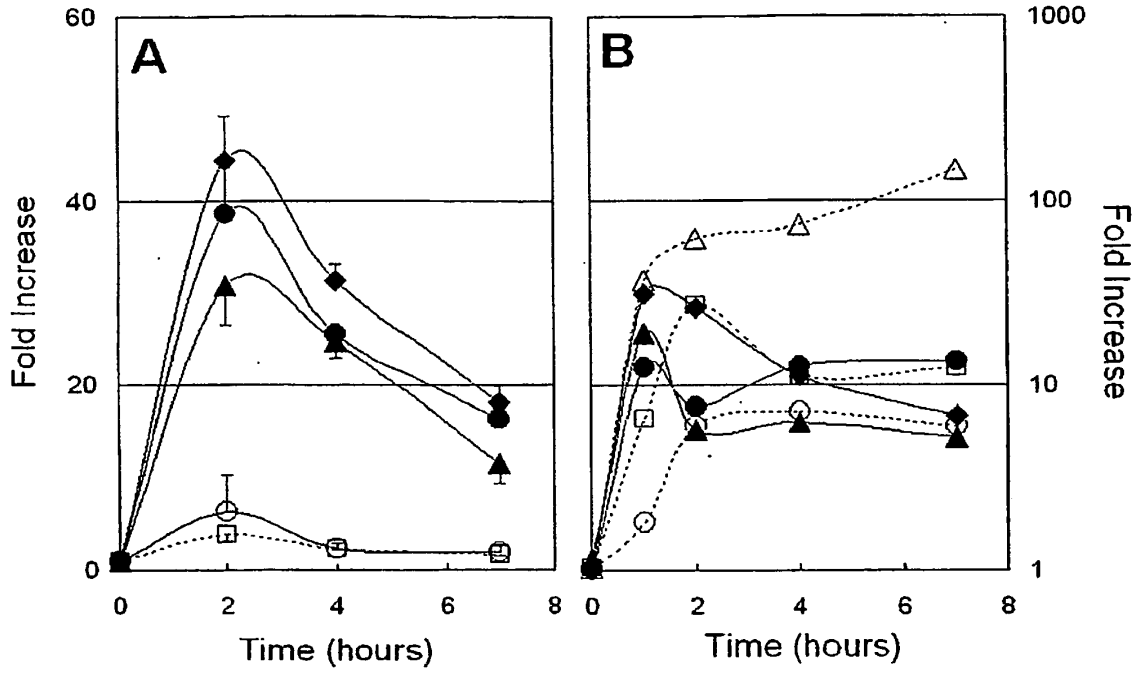

FIG. 5

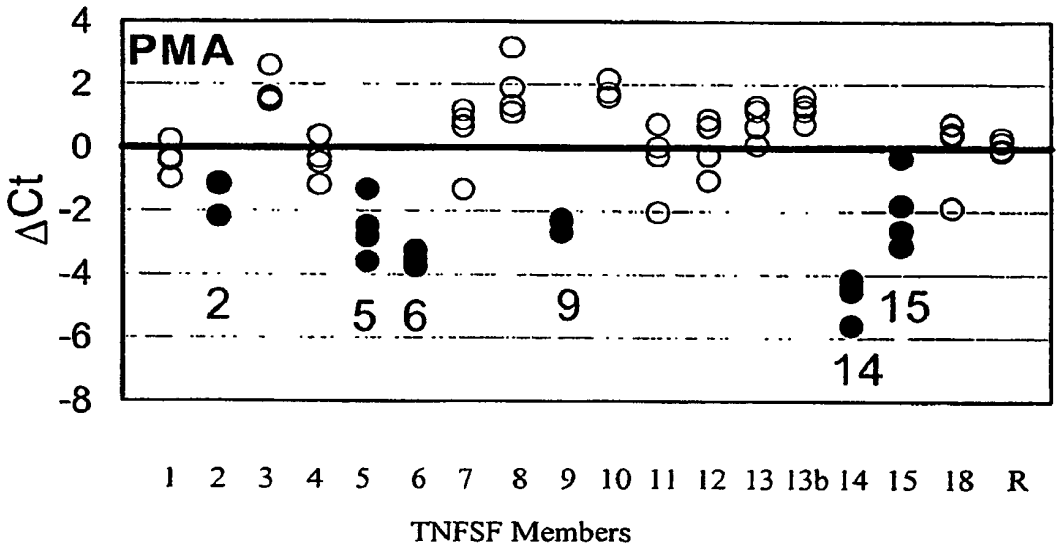
PMA
ΔCt
4
2
0
-2
-4
-6
-8
2
5 6
9
15
14
1 2 3 4 5 6 7 8 9 10 11 12 13 13b 14 15 18 R
TNFSF Members

FIG. 6

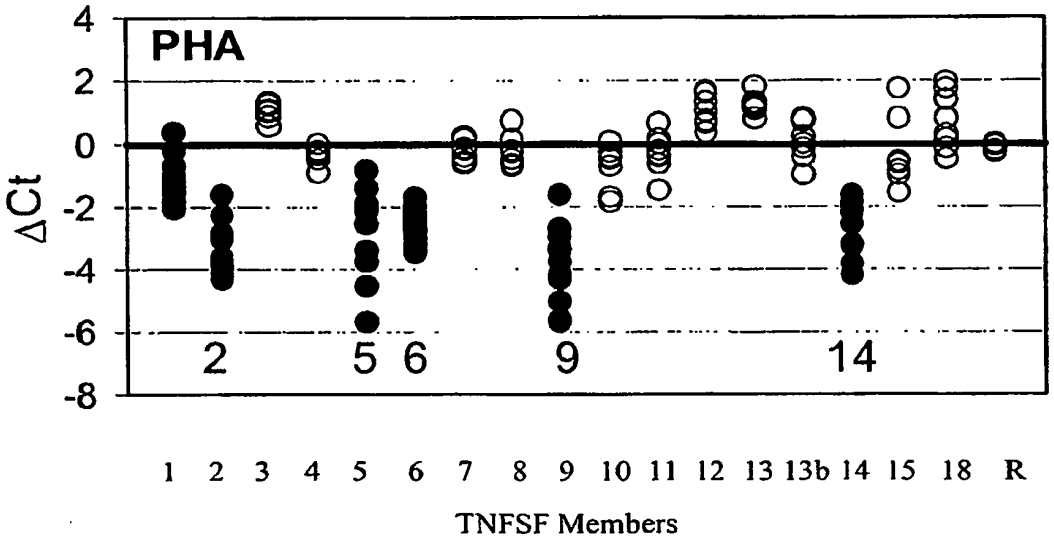
PHA
ΔCt
4
2
0
-2
-4
-6
-8
2
5
6
9
14
1 2 3 4 5 6 7 8 9 10 11 12 13 13b 14 15 18 R
TNFSF Members

FIG. 7

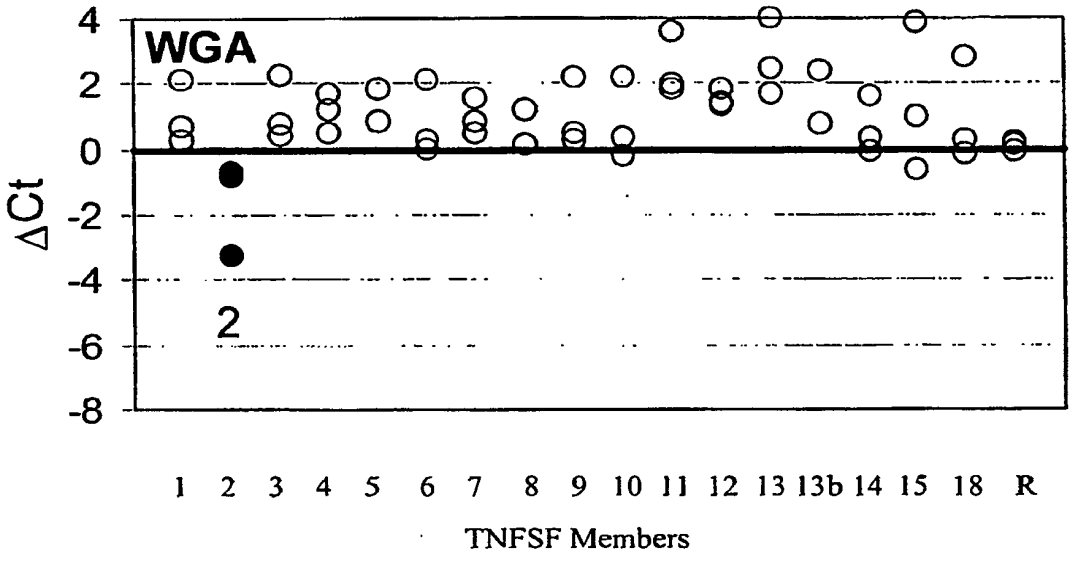
WGA
4
2
0
-2
-4
-6
-8
ΔCt
2
1 2 3 4 5 6 7 8 9 10 11 12 13 13b 14 15 18 R
TNFSF Members

FIG. 8

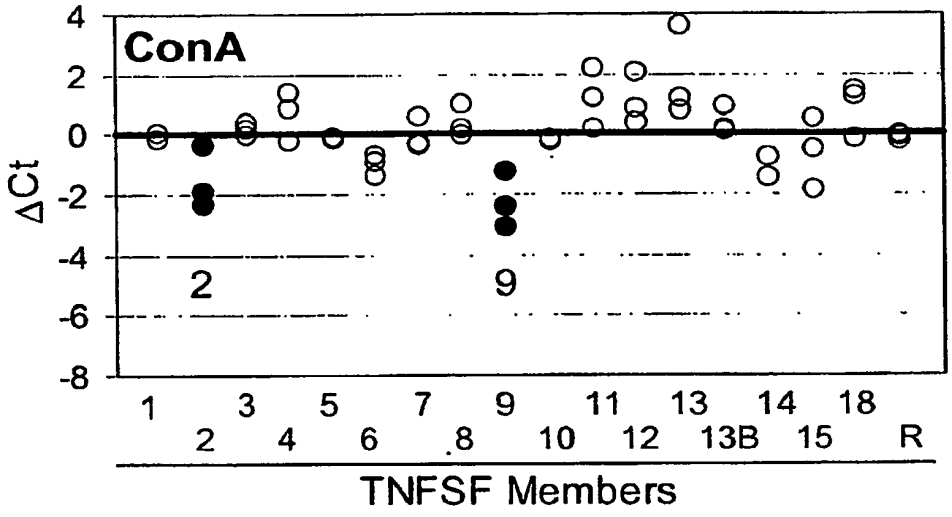
ConA
ΔCt
4
2
0
-2
-4
-6
-8
2
9
1
2
3
4
5
6
7
8
9
10
11
12
13
13B
14
15
18
R
TNFSF Members

FIG. 9

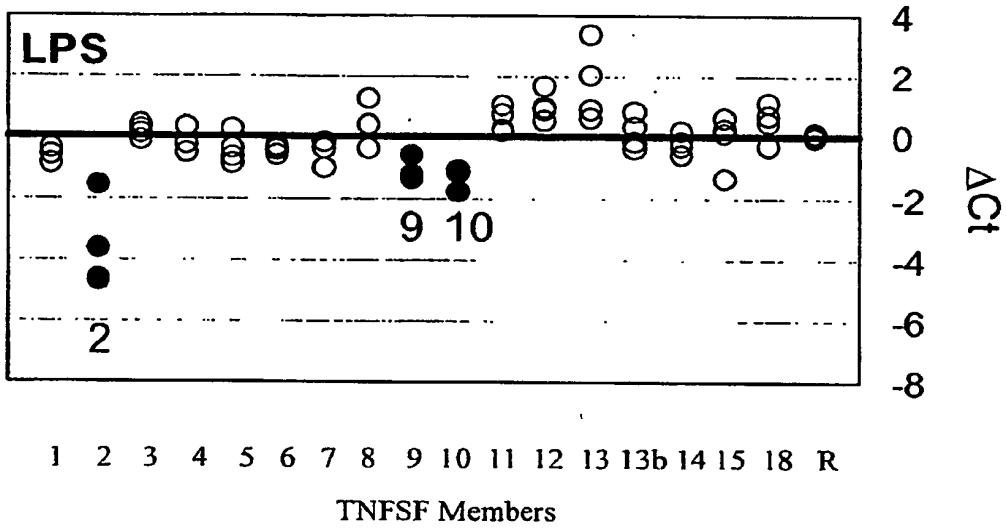
LPS
4
2
0
-2
-4
-6
-8
ΔCt
9 10
2
1 2 3 4 5 6 7 8 9 10 11 12 13 13b 14 15 18 R
TNFSF Members

FIG. 10

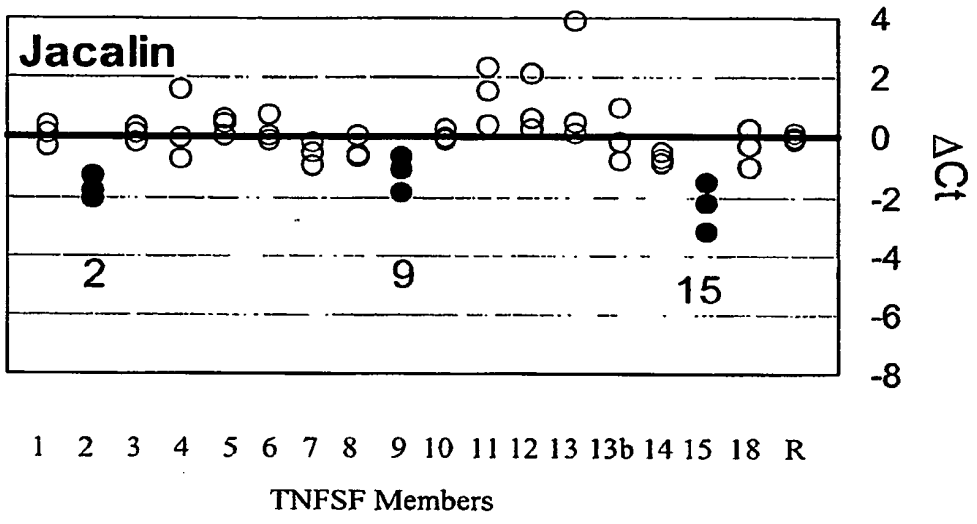
Jacalin
4
2
0
-2
-4
-6
-8
ΔCt
2
9
15
1 2 3 4 5 6 7 8 9 10 11 12 13 13b 14 15 18 R
TNFSF Members

FIG. 11

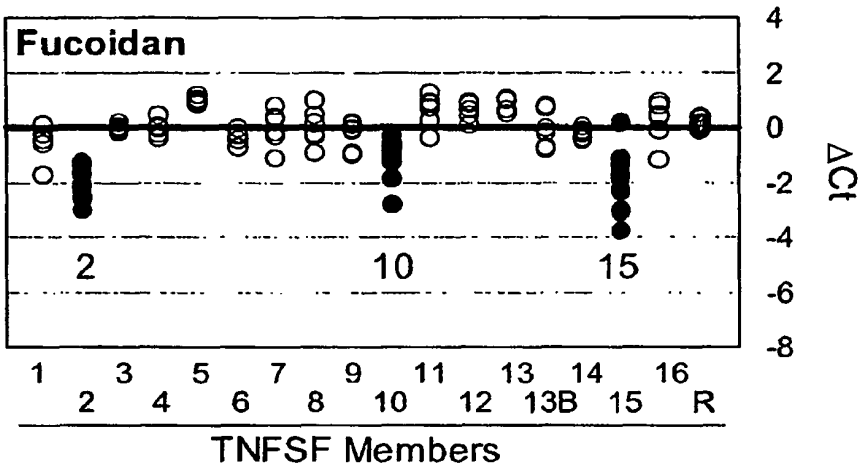
Fucoidan
4
2
0
-2
-4
-6
-8
ΔCt
2
10
15
1
2
3
4
5
6
7
8
9
10
11
12
13
13B
14
15
16
R
TNFSF Members

FIG. 12

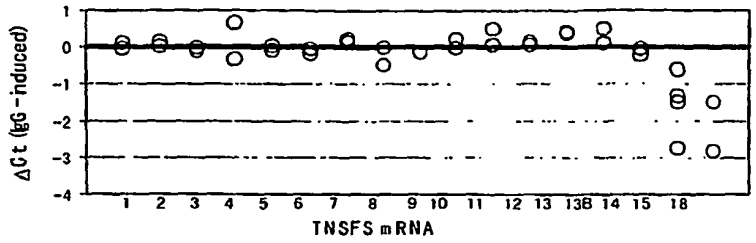

FIG. 13A

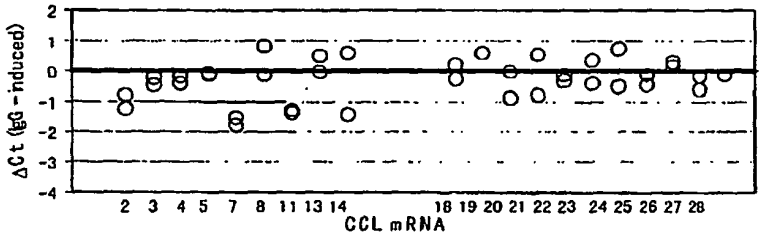

FIG. 13B

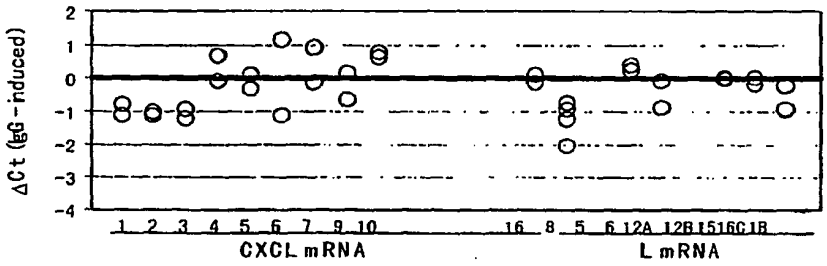

FIG. 13C

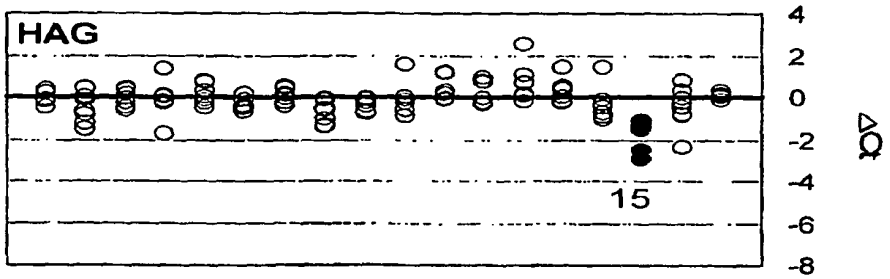

FIG. 13D

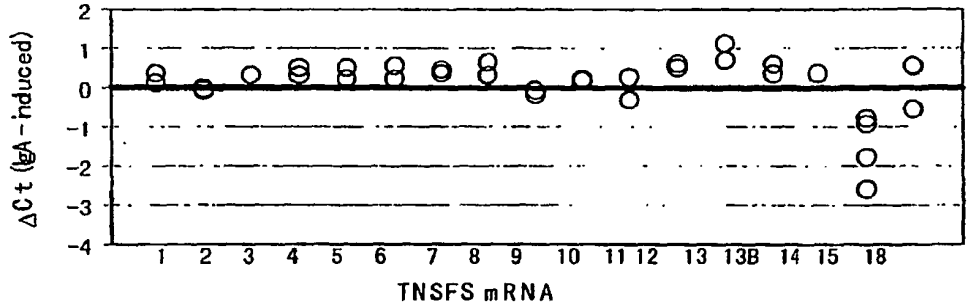

FIG. 14A

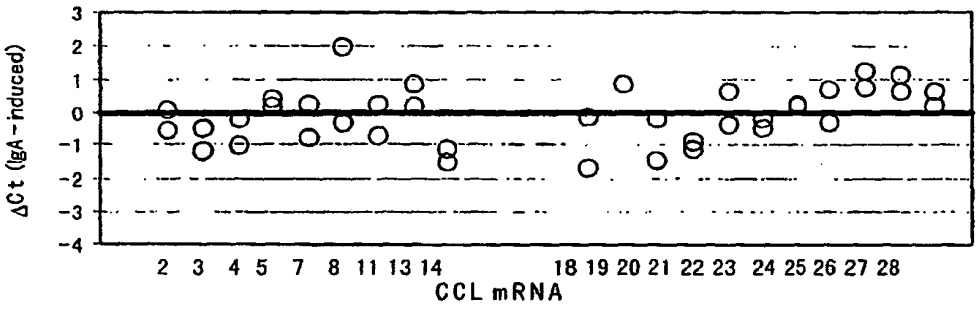

FIG. 14B

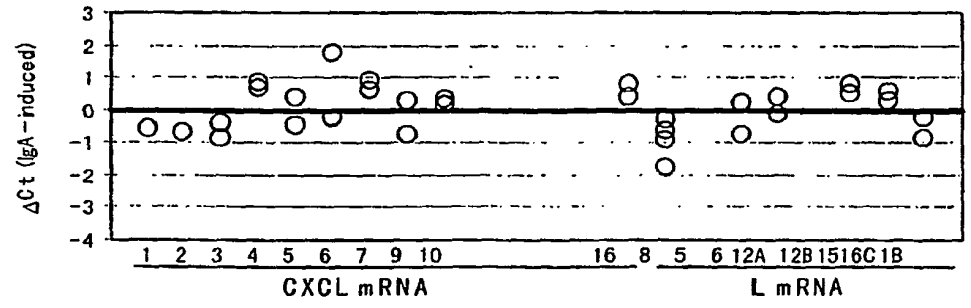

FIG. 14C

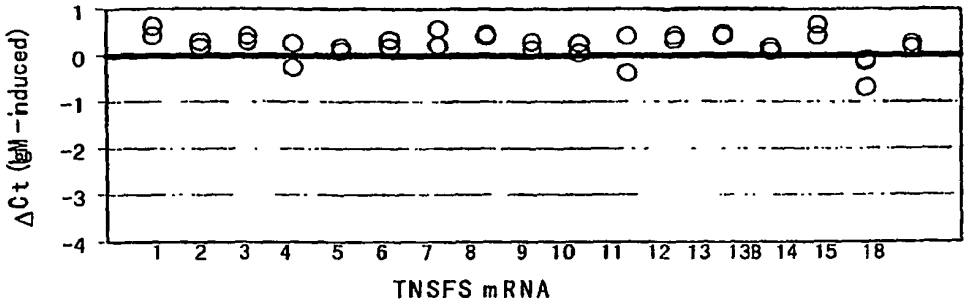

FIG. 15A

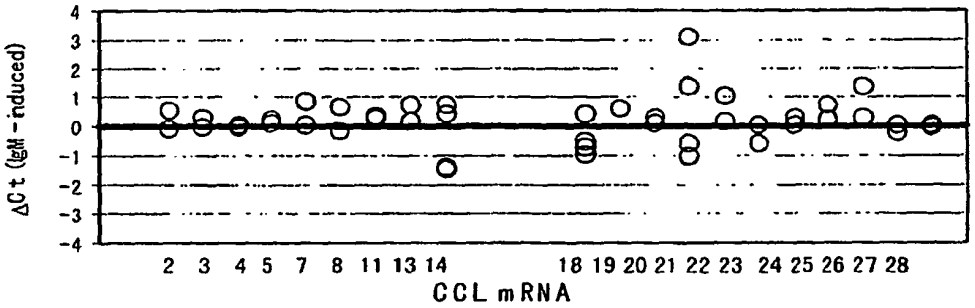

FIG. 15B

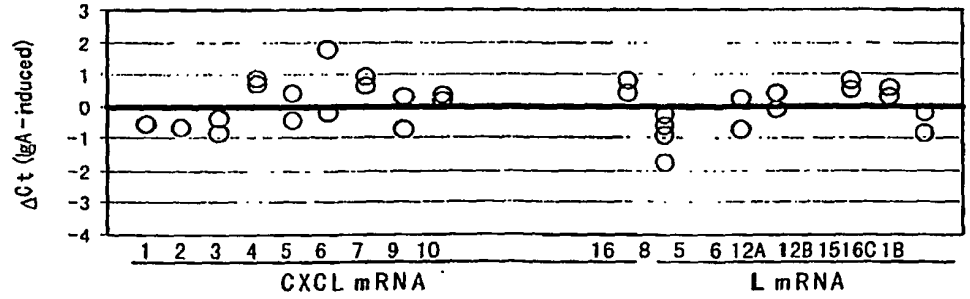

FIG. 15C

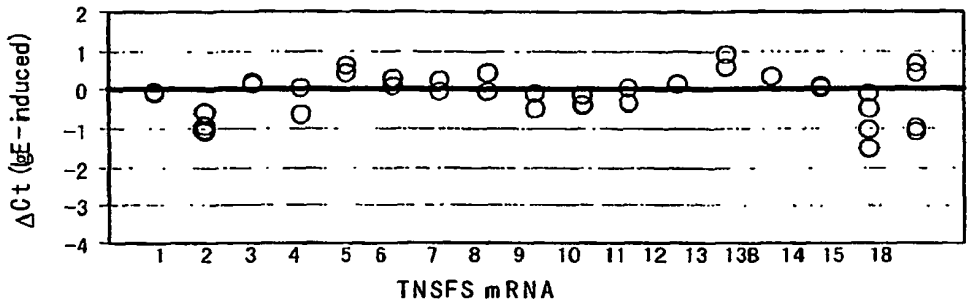

FIG. 16A

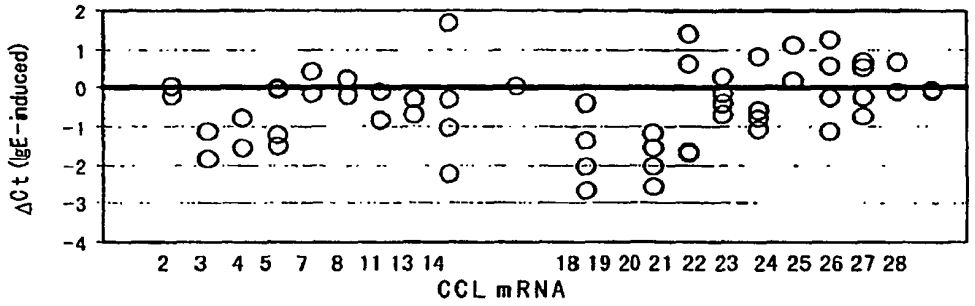

FIG. 16B

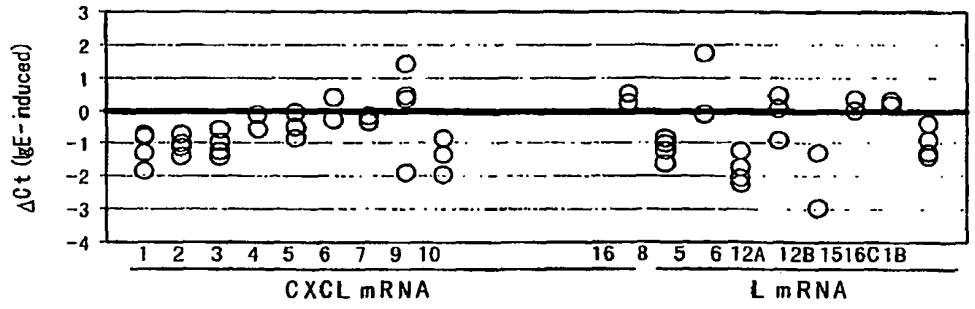

FIG. 16C

REFERENCES CITED IN THE DESCRIPTION

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专利名称(译)	克罗恩病患者外周血白细胞增强的t细胞受体介导的肿瘤坏死因子超家族和趋化因子mRNA的表达		
公开(公告)号	EP2005175A4	公开(公告)日	2009-06-03
申请号	EP2007755012	申请日	2007-04-05
[标]申请(专利权)人(译)	日立化成工业株式会社 日立化成研究中心公司 雪松-西奈医学中心		
申请(专利权)人(译)	日立化成工业有限公司. 日立化成研究中心，INC. 雪松 - 西奈医疗中心		
当前申请(专利权)人(译)	日立化成工业有限公司. 日立化成研究中心，INC. 雪松 - 西奈医疗中心		
[标]发明人	MITSUHASHI MASATO TARGAN STEPHAN R		
发明人	MITSUHASHI, MASATO TARGAN, STEPHAN, R.		
IPC分类号	G01N33/53 C12Q1/68		
CPC分类号	A61P1/04 A61P37/00 C12Q1/6883 C12Q2600/106 C12Q2600/136 C12Q2600/158		
代理机构(译)	法思博事务所		
优先权	60/790354 2006-04-07 US		
其他公开文献	EP2005175B1 EP2005175A2		
外部链接	Espacenet		

摘要(译)

公开了一种方法，用于通过测量响应于刺激的某些mRNA的水平来确定患有克罗恩病的人是否可能对靶向TNFSF成员或细胞因子的疗法起反应。还公开了一种评估克罗恩病治疗在人体中的有效性的方法。此外，公开了筛选用于治疗克罗恩病的化合物的方法。还公开了一种在克罗恩病患者中随时间监测疾病状态的方法。

Table 1: Primer sequences

Target mRNA	Forward	Reverse
TNFSF-1	CAGCTATCCACCCACACAGATG (SEQ ID NO: 1)	CGAAGGCTCCAAAGAAGACAGT (SEQ ID NO: 2)
TNFSF-2	TCAATCGGCCCGACTATCTC (SEQ ID NO: 3)	CAGGGCAATGATCCCAAAGT (SEQ ID NO: 4)
TNFSF-3	AGGGTGTACGTCAACATCAGTCA (SEQ ID NO: 5)	CACGGCCCCAAAGAAGGT (SEQ ID NO: 6)
TNFSF-4	GCCCCTCTTCCAACTGAAGAA (SEQ ID NO: 7)	GGTATTGTCAGTGGTCACATTCAAG (SEQ ID NO: 8)
TNFSF-5	CCACAGTTCCGCCAAACCT (SEQ ID NO: 9)	CACCTGGTTGCAATTCAAATACTC (SEQ ID NO: 10)
TNFSF-6	TGGCAGCATCTTCACTTCTAAATG (SEQ ID NO: 11)	GAAATGAGTCCCCAAAACATCTCT (SEQ ID NO: 12)
TNFSF-7	CACACTCTGCACCAACCTCACT (SEQ ID NO: 13)	TGCACTCCAAAGAAGGTCTCATC (SEQ ID NO: 14)
TNFSF-8	ACCACCATATCAGTCAATGTGGAT (SEQ ID NO: 15)	GAAGATGGACAACACATTCTCAAGA (SEQ ID NO: 16)
TNFSF-9	AGCTACAAAGAGGACACGAAGGA	CGCAGCTCTAGTTGAAAGAAGACA