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DESCRIPTION

FIELD OF THE INVENTION

[0001] The present invention relates to gene expression profiles in inflammatory bowel disease pathogenesis. This discovery finds use in the detection and diagnosis of inflammatory bowel disease.

BACKGROUND OF THE INVENTION

[0002] Inflammatory bowel disease (IBD), a chronic inflammatory disorder of the gastrointestinal tract suffered by approximately one million patients in the United States, is made up of two major disease groups: ulcerative colitis (UC) and Crohn's Disease (CD). In both forms of IBD, intestinal microbes may initiate the disease in genetically susceptible individuals. UC is often restricted to the colon, while CD typically occurs in the ileum of the small intestine and in the colon. (Podolsky, D.K., N. Engl. J. Med. 347:417-429 (2002). Gene expression profiling of tissue from IBD patients has provided some insight into possible targets for therapy and/or diagnosis (see, for example, Dieckgraefe, B.K. et al., Physiol. Genomics 4:1-11 (2000); Lawrance I.C. et al., Hum Mol Genet. 10:445-456 (2001); Dooley T.P. et al., Inflamm. Bowel Dis. 10:1-14 (2004); and Uthoff S.M., Int J Oncol. 19:803-810 (2001)). Further investigations of gene dysregulation in patients experiencing inflammatory bowel disease include, or example, Lawrance, I.C. et al., who disclosed distinctive gene expression profiles for several genes in UC and CD (Lawrance, I.C. et al., Human Mol. Genetics 10(5):445-456 (2001)). Uthoff, S.M.S. et al. disclosed the identification of candidate genes for UC and CD using micro array analysis (Uthoff, S.M.S. et al., Int'l. J. Oncology 19:803-810 (2001). Dooley, T.P. et al. disclosed correlation of gene expression in IBD with drug treatment for the disorder (Dooley, T.P. et al., Inflamm. Bowel Dis. 10(1):1-14 (2004).

[0003] US 5,368,854 describes the use of IL-10 to treat inflammatory bowel disease. WO 00/77026 and WO 00/61629 describe various human secreted proteins.

[0004] There is a need for the identification of additional biological markers of inflammatory bowel disease for use in diagnosis of this chronic disease. The present disclosure fills that need.

SUMMARY OF THE INVENTION

[0005] Disclosed herein is the unique finding that members of the LY6 superfamily of genes are upregulated on the surface of intestinal epithelial cells (IEC) in models of murine colitis and in intestinal tissue of human patients experiencing IBD, which genes are not expressed on healthy IEC. The majority of LY6 family members are GPI-anchored cell surface glycoproteins with broad distribution on cells of hematopoietic origin, and more limited expression on non-hematopoietic cells. Though widely used as markers of differentiation of immune cells (Sunderkotter, C. et al., J. Immunol. 172:4410-4417 (2004)), the functions that the LY6 family possesses have been difficult to elucidate (Shevach, E.M. and P.E. Korty, Immunol. Today 10:195-200 (1989)). Reports have shown that LY6 molecules are involved in a diverse array of functions including T cell activation (Zhang, Z.X. et al., Eur. J. Immunol. 32:1584-1592 (2002) and Henderson, S.C. et al., J. Immunol. 168:118-126 (2002), olfaction (Chou, J.H. et al., Genetics 157:211-224 (2001) and cellular adhesion (Jaakkola, I. et al., J. Immunol. 170:1283-1290 (2003)).

[0006] In the broadest sense, disclosed is a method of detecting increased expression of genes of the human LY6 gene family in intestinal tissue in intestinal tissue from a first mammal experiencing an intestinal disorder relative to a control mammal. In a more directed sense, the method is expected to be applicable to the diagnosis of disorders related to intestinal disorders associated with human LY6H, LYPD1, LYPD3, and LYPD5 expression, which disorders include without limitation inflammatory bowel disease (IBD), such as ulcerative colitis (UC) and Crohn's Disease (CD). In one embodiment, the method of the invention is useful to detect responders and nonrespnders of IBD therapeutic treatment. In one embodiment, the IBD is ulcerative colitis (UC). In one embodiment, the IBD is Crohn's Disease (CD). In one embodiment, the intestinal tissue is colon tissue. In one embodiment, the colon tissue is sigmoid colon. In one embodiment, LY6H, LYPD1, LYPD3 and/or LYPD5 gene expression is increased in intestinal tissue (such as colon tissue) in an IBD, UC or CD mammal relative to normal intestinal (such as normal colon tissue) of a mammal not experiencing IBD, CD or UC. In an embodiment, the LY6H gene comprises the nucleic acid of SEQ ID NO:1 and encodes the LY6H polypeptide comprising SEQ ID NO:2. In an embodiment, the LYPD1 gene comprises the nucleic acid of SEQ ID NOS:3 or 4 and encodes the LYPD1 polypeptide comprising SEQ ID NO:5. In an embodiment, the LYPD3 gene comprises the nucleic acid of SEQ ID NO:6 and encodes the LYPD3 polypeptide comprising SEQ ID NO:7. In an embodiment, the LYPD5 gene comprises the

nucleic acid of SEQ ID NOS:8 or 9 and encodes the LYPD5 polypeptide comprising SEQ ID NO: 10.

[0007] In one embodiment, the method of the invention comprises obtaining a tissue sample from a test mammal suspected of experiencing an intestinal disorder, contacting the tissue with a detectable agent that interacts with LY6H, LYPD1, LYPD3 and/or LYPD5 protein or with nucleic acid encoding LY6H, LYPD1, LYPD3 and/or LYPD5 and determining the level of LY6H, LYPD1, LYPD3 and/or LYPD5 expression relative to a control tissue. In one embodiment increased expression of LY6H, LYPD1, LYPD3 and/or LYPD5 relative to control is indicative of IBD in the test mammal. In one embodiment, increased expression of LY6H, LYPD1, LYPD3 and/or LYPD5 in test intestinal tissue relative to control intestinal tissue is indicative of UC in the test mammal. In one embodiment, increased expression of LY6H, LYPD1, LYPD3 and/or LYPD5 in test intestinal tissue relative to control intestinal tissue is indicative of CD in the test mammal. In one embodiment the tissue or cells from the test and control mammal are from the colon.

[0008] In one embodiment, LY6H, LYPD1, LYPD3 and/or LYPD5 expression is determine by detection of gene expression, such as by detection of mRNA encoding LY6H, LYPD1, LYPD3 and/or LYPD5 in a tissue sample or cells. In an embodiment, a control sample is a sample of tissue or cells of the same tissue or cell type obtained from a mammal known not to be experiencing a gastrointestinal disorder, such as IBD, UC or CD. In an emobiment, a control sample is a universal standard comprising RNA from several normal tissues or from multiple cell lines. In microarray analysis, such universal standards are useful for monitoring and controlling intra- and inter-experimental variation. In one embodiment, a universal standard (or Universal Reference RNA (URR) is prepared as provided in Novoradovskaya, N. et al., (2004) BMC Genomics 5:20. In one embodiment, for use as a control in microarray analysis of mouse RNA, the URR is a Universal Mouse Reference RNA from Stratagene® (catalog #740100, Stratagene®, La Jolla, CA). In one embodiment, for use as a control in microarray analysis of human RNA, the URR is a Universal Human Reference RNA from Stratagene® (catalog #740000). In one embodiment, for use as a control in microarray analysis of rat RNA, the URR is a Universal Rat Reference RNA from Stratagene® (catalog #740200). In one embodiment, where the RNA is mouse RNA, the cell lines from which total RNA is extracted comprise cell lines derived from embryo, embryo fibroblast, kidney, liver hepatocyte, lung alveolar macrophage, B-lymphocyte, T-lymphocyte (thymus), mammary gland, muscle myoblast, skin, and testis. In one embodiment, where the RNA is human RNA, the cell lines from which total RNA is extracted comprise cell lines derived from mammary gland adenocarcinoma, liver hepatoblastoma, cervix adenocarcinoma, embryonal carcinoma or testis, brain glioblastoma, melanoma, liposarcoma, histiocytic lymphoma (macrophage, histocyte), T lymphoblast lymphoblastic leukemia, B lymphocyte plasmacytoma melanoma. In one embodiment where the RNA is rat RNA, the cell lines from which total RNA is extracted comprise cell lines derived from blood basophilic leukemia, blood T-lymphocyte lymphoma, blood B-lymphoblast hybridoma, brain glioma, embryo yolk sac carcinoma, embryo normal fibroblast, normal kidney, liver hepatoma, lung normal alveolar macrophage, lung normal alveolar type II, mammary gland adenocarcinoma, muscle myoblast, normal skin, and testis leydig cell tumor.

[0009] In one aspect, disclosed is an article of manufacture comprising a container and a composition of matter contained within the container, wherein the composition of matter comprises a nucleic acid encoding LY6H, LYPD1, LYPD3 and/or LYPD5 or their complements, and/or an anti- LY6H, LYPD1, LYPD3 and/or LYPD5 antibody or antibodies, or anti- LY6H-, LYPD1-, LYPD3- and/or LYPD5-binding fragment thereof, wherein the nucleic acids and/or antiobodies are detectable. In one embodiment, the composition of matter comprises detecting agents for detecting nucleic acid binding, such as without limitation LY6H-, LYPD1-, LYPD3- and/or LYPD5-encoding nucleic acids or their complements, to LY6H, LYPD1, LYPD3 and/or LYPD5 nucleic acid in a tissue sample of a test mammal suspected of experiencing an intestinal disorder. In one embodiment, the compositions of matter comprises detecting agents for detecting antibody binding to, for example, LY6H, LYPD 1, LYPD3 and/or LYPD5 in a tissue sample of a test mammal suspected of experiencing an intestinal disorder. In one embodiment, the antibody of the composition is detectably labeled. In one embodiment, the antibody of the composition is detectable by a second antibody, which second antibody is detectable or detectably labeled. The article may further optionally comprise a label affixed to the container, or a package insert included with the container, that refers to the use the LY6H, LYPD1, LYPD3 and/or LYPD5 nucleic acid or its complement and/or the anti-LY6H, anti-LYPD1, anti-LYPD3 and/or anti-LYPD5 antibody or LY6H, LYPD1, LYPD3 and/or LYPD5 binding fragment thereof in the detection of increased expression of LY6H, LYPD1, LYPD3 and/or LYPD5 in intestinal tissue, including without limitation, colon tissue. In an embodiment, the intestinal disorder is IBD. In an embodiment the intestinal disorder is UC or CD. In an embodiment the LYPD1 polypeptide and the anti-LYPD1 antibody is an antibody as disclosed in US7,157,558 and US7,144,990, respectively.

[0010] In one aspect, disclosed is a method of diagnosing the presence of an intestinal disorder in a mammal, comprising detecting the level of expression of a gene encoding LY6H, LYPD1, LYPD3 and/or LYPD5 polypeptide (a) in a test sample of tissue or cells obtained from said mammal, and (b) in a control sample of known normal cells from a mammal not experiencing an intestinal disorder of the same tissue origin or type, wherein a higher level of expression of the LY6H, LYPD1, LYPD3 and/or LYPD5 polypeptide in the test sample, as compared to the control sample, is indicative of the presence of an intestinal disorder in the mammal from which the test sample was obtained. In an embodiment, the intestinal disorder in IBD. In an emobiment, the IBD

is UC. In an embodiment, the IBD is CD. In an embodiment, the detecting is by contacting an antibody to LY6H, LYPD1, LYPD3 and/or LYPD5 polypeptide, or binding fragment of the antibody, with the test and control samples and determining the relative amount of antibody-polypeptide complex formation. A higher level of antibody-polypeptide complex formation in the test sample relative of the control sample is indicative of intestinal disorder, such as IBD, UC or CD, in the test mammal. The antibody of the invention is detectably labeled or, alternatively, the antibody is detected by subsequent binding of a second antibody which is detectable.

[0011] In yet a further embodiment, disclosed is a method of diagnosing the presence of an intestinal disorder in a mammal, comprising (a) contacting a test sample comprising tissue or cells obtained from the test mammal with an oligonucleotide that hybridizes at high stringency to LY6H, LYPD1, LYPD3 and/or LYPD5 nucleic acid (or its complement) or an antibody that binds specifically to LY6H, LYPD1, LYPD3 and/or LYPD5 polypeptide and (b) detecting the formation of a complex between the oligonucleotide or antibody and the LY6H, LYPD1, LYPD3 and/or LYPD5 nucleic acid (or its completment) or LY6H, LYPD1, LYPD3 and/or LYPD5 polypeptide, respectively, in the test sample, wherein the formation of more of such complex in the test sample relative to a control sample is indicative of the presence of an intestinal disorder (such as IBD, UC or CD) in the test mammal. In one embodiment, the intestinal disorder is IBD. In one embodiment, the disorder is UC. In one embodiment the disorder is CD. In one embodiment the tissue of the test and control mammals is colon tissue. Optionally, the LY6H, LYPD1, LYPD3 and/or LYPD5 polypeptide binding antibody or LY6H, LYPD1, LYPD3 and/or LYPD5 gene hybridizing oligonucleotide employed by the method of the invention is detecable, detectably labeled, attached to a solid support, or the like, and/or the test sample of tissue or cells is obtained from an individual suspected of experiencing an intestinal disorder, wherein the disorder is IBD, such as without limitation, UC or CD.

[0012] In yet a further embodiment, disclosed is the use of (a) a LY6H, LYPD1, LYPD3 and/or LYPD5 polypeptide, (b) a nucleic acid encoding a LY6H, LYPD1, LYPD3 and/or LYPD5 polypeptide or a vector or host cell comprising the nucleic acid of (a), (c) an anti-LY6H, LYPD1, LYPD3 and/or LYPD5 polypeptide antibody, or (d) a LY6H, LYPD1, LYPD3 and/or LYPD5-binding oligopeptide, in the preparation of a medicament useful for the diagnostic detection of an intestinal disorder, including without limitation, IBD CD or UC, in an intestinal tissue of a mammal, including without limitation colon tissue.

[0013] In one aspect, the invention comprises a method of detecting a therapeutic drug response in a mammal treated with an IBD therapeutic agent, wherein the method comprises determining LY6H, LYPD1, LYPD3 and/or LYPD5 expression in gastrointestinal tissue of a test mammal relative to a control gastrointestinal tissue of a control mammal, where a higher level of expression of LY6H, LYPD1, LYPD3 and/or LYPD5 in a test tissue relative to a control tissue indicates a disease state or continuation of the disease state. A difference in LY6H, LYPD1, LYPD3 and/or LYPD5 expression in the test tissue that is not significantly higher than normal control expression levels or are within a range of normal expression levels for LY6H, LYPD1, LYPD3 and/or LYPD5 in a population of mammals indicates improvement or resolution of the intestinal disorder, which improvement or resolution may be attributed to the therapeutic agent. In one embodiment, a therapeutic response is determined when the levels of expression of LY6H, LYPD1, LYPD3 and/or LYPD5 in gastrointestinal or colon tissues or cells of the mammal treated with a therapeutic agent are different (expression is more similar to normal control, i.e., LY6H, LYPD1, LYPD3 and/or LYPD5 levels are lower than LY6H, LYPD1, LYPD3 and/or LYPD5 expression levels were in the mammal prior to treatment.

[0014] Yet further embodiments of the present invention will be evident to the skilled artisan upon a reading of the present specification.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015]

Figures 1A and 1B depict the nucleic acid sequence (SEQ ID NO:l) encoding human LY6H polypeptide and the amino acid sequence of human LY6H polypeptide (SEQ ID NO:2).

Figures 2A and 2B depict nucleic acid sequences (SEQ ID NOS:3 and 4) encoding the human LYPD1 polypeptide and the amino acid sequence of human LYPD1 polypeptide shown in **Figure 2C** (SEQ ID NO:5).

Figures 3A and 3B depict the nucleic acid sequence (SEQ ID NO:6) encoding human LYPD3 polypeptide and the amino acid sequence of human LYPD3 polypeptide (SEQ ID **NO:7).**

Figures 4A and 4B depict nucleic acid sequences (SEQ ID NOS:8 and 9) encoding human LYPD5 polypeptide and the amino acid sequence of human LYPD5 polypeptide shown in **Figure 4C** (SEQ ID NO: 10).

Figures 5A and 5B depict the nucleic acid sequence (SEQ ID NO: 11) encoding human LY6D polypeptide and the amino acid sequence of human LY6D polypeptide (SEQ ID NO: 12).

Figures 6A and 6B depict the nucleic acid sequence (SEQ ID NO:13) encoding human LY6E polypeptide and the amino acid sequence of human LY6E polypeptide (SEQ ID NO:14).

Figures 7A and 7B depict the nucleic acid sequence (SEQ ID NO: 15) encoding human LYPD2 polypeptide and the amino acid sequence of human LYPD2 polypeptide (SEQ ID NO:16).

Figures 8A-8H depict sequences of GLG-1 (ESL-1) molecules: (A-B) Accession No. U64791, nucleic acid sequence (SEQ ID NO:17) encoding human GLG-1 (ESL-1) polypeptide (SEQ ID NO:18); (C-D) Accession No. NM_012201, nucleic acid sequence (SEQ ID NO:19) encoding human GLG-1 (ESL-1) polypeptide (SEQ ID NO:20); (E-F) Accession No. AK172806, nucleic acid sequence (SEQ ID NO:21) encoding human GLG-1 (ESL-1) polypeptide (SEQ ID NO:22); and Accession No. AK131501, nucleic acid sequence (SEQ ID NO:23) encoding human GLG-1 (ESL-1) polypeptide (SEQ ID NO:24).

Figures 9A and 9B depict the nucleic acid sequence (SEQ ID NO:25) encoding murine LY6A polypeptide and the amino acid sequence of murine LY6A polypeptide (SEQ ID NO:26).

Figures 10A and 10B depict the nucleic acid sequence (SEQ ID NO:27) encoding murine LY6C polypeptide and the amino acid sequence of murine LY6C polypeptide (SEQ ID NO:28).

Figures 11A and 11B depict the nucleic acid sequence (SEQ ID NO:29) encoding murine LY6D polypeptide and the amino acid sequence of murine LY6D polypeptide (SEQ ID NO:30).

Figures 12A and 12B depict the nucleic acid sequence (SEQ ID NO:31) encoding murine LY6E polypeptide and the amino acid sequence of murine LY6E polypeptide (SEQ ID NO:32).

Figures 13A and 13B depict the nucleic acid sequence (SEQ ID NO:33) encoding murine LY6F polypeptide and the amino acid sequence of murine LY6F polypeptide (SEQ ID NO:34).

Figures 14A and 14B depict the nucleic acid sequence (SEQ ID NO:35) encoding murine LY6I polypeptide and the amino acid sequence of murine LY6I polypeptide (SEQ ID NO:36).

Figures 15A and 15B depict the nucleic acid sequence (SEQ ID NO:37) encoding murine LY6K polypeptide and the amino acid sequence of murine LY6K polypeptide (SEQ ID NO:38).

Figures 16A and 16B depict the nucleic acid sequence (SEQ ID NO:45) encoding murine LYPD3 polypeptide and the amino acid sequence of murine LYPD3 polypeptide (SEQ ID NO:46).

Figures 17A and 17B depict the nucleic acid sequence (SEQ ID NO:47) encoding murine LY6H polypeptide and the amino acid sequence of murine LY6H polypeptide (SEQ ID NO:48).

Figures 18A and 18B depict the nucleic acid sequence (SEQ ID NO:49) encoding murine LYPD1 polypeptide and the amino acid sequence of murine LYPD1 polypeptide (SEQ ID NO:50).

Figures 19A and 19B depict the nucleic acid sequence (SEQ ID NO:51) encoding murine LYPD2 polypeptide and the amino acid sequence of murine LYPD2 polypeptide (SEQ ID NO:52).

Figures 20A and 20B depict the nucleic acid sequence (SEQ ID NO:53) encoding murine LY6g5c polypeptide and the amino acid sequence of murine LY6g5c polypeptide (SEQ ID NO:54).

Figures 21A and 22B depict the nucleic acid sequence (SEQ ID NO:55) encoding murine LY6g6c polypeptide and the amino acid sequence of murine LY6g6c polypeptide (SEQ ID NO:56).

Figures 22A and 22B depict the nucleic acid sequence (SEQ ID NO:57) encoding murine SLURP2/LYNX1 polypeptide and the amino acid sequence of murine SLURP2/LYNX1 polypeptide (SEQ ID NO:58).

Figure 23 shows that LY6 family members are upregulated in IEC in murine models of colitis. IEC in both the $IL10^{-/-}$ (Figure 23A) and $CD45RB^{Hi}$ transfer colitis model (Figure 23B) were isolated by LCM and RNA was purified. Microarray analysis was performed and analyzed as described in the Examples. Numbers represent the mean of the fold change compared to a universal standard RNA of colitic mice over healthy mice. Numbers below the heatmap indicate the inflammation score of the individual mouse.

Figures 24A-24D show that surface expression of LY6 molecules is upregulated on IEC in the IL10-/- model of colitis. Wild type (Figure 24A) or IL10 -/- mice (Figure 24B) were stained for surface expression of LY6A (green, with DAPI counterstain). Similarly, wild type (Figure 24C) or IL10 -/- mice (Figure 24D) were stained for surface expression of LY6C.

Figures 25A-25I show that surface expression of LY6A and LY6C are upregulated in response to inflammatory cytokines, particularly IFNγ. YAMC cells were treated with the indicated cytokine for 15 hours and stained for surface expression of LY6C (Figure 25A) and LY6A (Figure 25B). YAMC cells were cultured for 15 hours in the presence of increasing doses of IFNγ and analyzed by flow cytometry for expression of LY6C (Figure 25C) and LY6A (Figure 25D). IFNγ stimulated YAMC cells were collected at various time points, as indicated, and analyzed by flow cytometry for expression of LY6C (Figure 25E) and LY6A (Figure 25F). IL-22 upregulated expression of both LY6C (Figure 25G) and LY6A (Figure 25H). Levels of both LY6A and LY6C were upregulated in the murine IEC line, CMT93 in response to treatment with IFNγ (Figure 25I).

Figures 26A-26E Lipid raft depletion results in an inhibition of LY6C-mediated chemokine production. Cholesterol depleted (dark bars) or non-depleted (open bars) YAMC cells were incubated with plate-bound nati-KLH or anti-LY6C as indicated for 15 hours. RNA was collected and expression levels of CXCL2, CXCL5, and CCL7 were determined (Figures 26A-26C). Surface levels of LY6A (Figure 26D) and LY6C (Figure 26E) where decreased in response to cholesterol depletion.

Figures 27A-27D show that crosslinking of LY6C, but not LY6A, induces upregulation of surface expression of LY6A and LY6C. YAMC cells were incubated for 24 hours on plates coated with anti-KLH control, anti-LY6A or anti-LY6C and analyzed by flow cytometry for expression of LY6C (Figure 27A) or LY6A (Figure 27B). Cells were pretreated for 12 hours with 100 U/ml of IFNγ and similarly plated on antibody coated plates and analyzed for expression of LY6C (Figure 27C) or LY6A (Figure 27D).

Figures 28A-28C show that crosslinking LY6C, but not LY6A, induces secretion of chemokines. Figure 28A: YAMC cells were preincubated or not, as indicated, with 100 U/ml of IFNγ for 15 hours and cultured on plates coated with 10 µg/ml of anti-LY6A (black bars) or anti-LY6C (hatched bars) or anti-KLH control (open bars). RNA was isolated at 24 (left), 48 (center) and 72 (right) hours and analyzed for expression of CXCL5 or CCL7 (A). Data indicates mean ± SD of the fold change (as determined by $2^{-\Delta\Delta Ct}$ method) compared to untreated, isotype crosslinked cells. Figure 28B: Supernatants were collected at 48 hours in cells crosslinked, as above, with 1, 5 or 10 µg/ml (as indicated) of antibody and CXCL5 secretion into the supernatant was deterimined by ELISA. *<0.05. Figure 28C: Levels of both CXCL5 and CXCL2 in response to LY6C crosslinking were diminished when LY6C levels were knocked down with siRNA.

Figures 29A-29B show that IEC in colitis possess a similar chemokine gene expression pattern. IEC in both the $IL10^{-/-}$ (Figure 29A) and $CD45RB^{Hi}$ transfer colitis model (Figure 29B) were isolated by LCM and RNA was purified. Microarray analysis was performed and analyzed as described in the Examples. Numbers represent the mean of the fold change compared to the universal standard RNA of colitic mice over healthy mice. Numbers below the heatmap indicate the inflammation score of the individual mouse.

Figures 30A-30C show that expression of human LY6 family genes is upregulated in colon cells treated with cytokines. Human Colo-205 cells were treated with the indicated cytokines, or combinations of cytokines, for 18 or 24 hours. The fold increase in expression of human LY6H (Figure 30A), human LYPD3 (Figure 30B), and human LYPD5 (Figure 30C) are shown relative to human β-actin control.

Figures 31A-31B show that patients with Crohn's Disease have elevated levels of LYPD1 (Figure 31 A) and LYPD5 (Figure 31B) in the colon. Tissue samples from human IBD patients were obtained and LYPD1 and LYPD5 gene expression was determined. Statistically significant increases in expression of LYPD1 and LYPD5 were observed in inflamed tissue of CD patients. A statistically significant increase in expression of LYPD5 was also observed in inflamed tissue of UC patients. Y-axis values reflect gene expression relative to a universal RNA standard.

Figures 32A and 32B shows (A) untransfected COS cells, and (B) COS cells transfected with GLG-1 (ESL-1) polypeptide and stained with LYPD5-Fc protein.

Figure 33A depicts the structure of GLG-1 or ESL-1 and various fragments suitable for characterizing the binding of LYPD5 and **Figure 33B** shows the results of a co-immunoprecipitation study characterizing the binding of LYPD5 and an LYPD5 ligand.

Figure 34A depicts the structure of GLG-1 or ESL-1 and various fragments suitable for characterizing the binding of LYPD5 and **Figure 34B** shows the results of a co-immunoprecipitation study characterizing the binding of LYPD5 and an LYPD5 ligand.

Figure 35A depicts the structure of GLG-1 or ESL-1 and various fragments suitable for characterizing the binding of LYPD5 and **Figure 35B** shows the results of a co-immunoprecipitation study characterizing the binding of LYPD5 and an LYPD5 ligand.

Figures 36A and 36B depict the nucleic acid sequence (SEQ ID NO: 68) encoding human integrin, beta 7, and the amino acid

sequence of human integrin, beta 7 polypeptide (SEQ ID NO: 69).

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[0016] "Inflammatory Bowel Disease" or "IBD" is used interchangeably herein to refer to diseases of the bowel that cause inflammation and/or ulceration and includes without limitation Crohn's disease and ulcerative colitis.

[0017] "Crohn's disease (CD)" or "ulcerative colitis (UC)" are chronic inflammatory bowel diseases of unknown etiology. Crohn's disease, unlike ulcerative colitis, can affect any part of the bowel. The most prominent feature Crohn's disease is the granular, reddish-purple edmatous thickening of the bowel wall. With the development of inflammation, these granulomas often lose their circumscribed borders and integrate with the surrounding tissue. Diarrhea and obstruction of the bowel are the predominant clinical features. As with ulcerative colitis, the course of Crohn's disease may be continuous or relapsing, mild or severe, but unlike ulcerative colitis, Crohn's disease is not curable by resection of the involved segment of bowel. Most patients with Crohn's disease require surgery at some point, but subsequent relapse is common and continuous medical treatment is usual.

[0018] Crohn's disease may involve any part of the alimentary tract from the mouth to the anus, although typically it appears in the ileocolic, small-intestinal or colonic-anorectal regions. Histopathologically, the disease manifests by discontinuous granulomatomas, crypt abscesses, fissures and aphthous ulcers. The inflammatory infiltrate is mixed, consisting of lymphocytes (both T and B cells), plasma cells, macrophages, and neutrophils. There is a disproportionate increase in IgM- and IgG-secreting plasma cells, macrophages and neutrophils.

[0019] Anti-inflammatory drugs sulfasalazine and 5-aminosalisylic acid (5-ASA) are useful for treating mildly active colonic Crohn's disease and is commonly perscribed to maintain remission of the disease. Metroidazole and ciprofloxacin are similar in efficacy to sulfasalazine and appear to be particularly useful for treating perianal disease. In more severe cases, corticosteroids are effective in treating active exacerbations and can even maintain remission. Azathioprine and 6-mercaptopurine have also shown success in patients who require chronic administration of cortico steroids. It is also possible that these drugs may play a role in the long-term prophylaxis. Unfortunately, there can be a very long delay (up to six months) before onset of action in some patients.

[0020] Antidiarrheal drugs can also provide symptomatic relief in some patients. Nutritional therapy or elemental diet can improve the nutritional status of patients and induce symtomatic improvement of acute disease, but it does not induce sustained clinical remissions. Antibiotics are used in treating secondary small bowel bacterial overgrowth and in treatment of pyogenic complications.

[0021] "Ulcerative colitis (UC)" afflicts the large intestine. The course of the disease may be continuous or relapsing, mild or severe. The earliest lesion is an inflammatory infiltration with abscess formation at the base of the crypts of Lieberkühn. Coalescence of these distended and ruptured crypts tends to separate the overlying mucosa from its blood supply, leading to ulceration. Symptoms of the disease include cramping, lower abdominal pain, rectal bleeding, and frequent, loose discharges consisting mainly of blood, pus and mucus with scanty fecal particles. A total colectomy may be required for acute, severe or chronic, unremitting ulcerative colitis.

[0022] The clinical features of UC are highly variable, and the onset may be insidious or abrupt, and may include diarrhea, tenesmus and relapsing rectal bleeding. With fulminant involvement of the entire colon, toxic megacolon, a life-threatening emergency, may occur. Extraintestinal manifestations include arthritis, pyoderma gangrenoum, uveitis, and erythema nodosum.

[0023] Treatment for UC includes sulfasalazine and related salicylate-containing drugs for mild cases and corticosteroid drugs in severe cases. Topical adminstration of either salicylates or corticosteroids is sometimes effective, particularly when the disease is limited to the distal bowel, and is associated with decreased side effects compared with systemic use. Supportive measures such as administration of iron and antidiarrheal agents are sometimes indicated. Azathioprine, 6-mercaptopurine and methotrexate are sometimes also prescribed for use in refractory corticosteroid-dependent cases.

[0024] As used herein, "LY6 gene family member" or "LY6 gene superfamily member" is used interchangeably herein to refer to a gene having homology to members of the LY6 gene family, the majority of which gene family members are GPI-anchored cell surface glycoproteins with broad distribution on cells of hematopoietic origin and more limited expression on non-hematopoietic cells. Members of this gene family are used as markers of differentiation of immune cells (Sunderkotter, C. et al., J. Immunol. 172:4410-4417 (2004)). Genes of the LY6 family have been examined (Shevach, E.M. and P.E. Korty, Immunol. Today 10:195-200 (1989)) and functions include T cell activation (Zhang, Z.X. et al., Eur. J. Immunol. 32:1584-1592 (2002) and Henderson, S.C. et al., J. Immunol. 168:118-126 (2002), olfaction (Chou, J.H. et al., Genetics 157:211-224 (2001) and cellular adhesion (Jaakkola, I. et al., J. Immunol. 170:1283-1290 (2003)). Members of the LY6 gene family include without limitation members of the mammalian LY6 gene family, such as the LY6 family genes of mouse or human. As use here, "LY6 gene" refers to a LY6 gene family member and "LY6 polypeptide" refers to the polypeptide encoded by a LY6 gene. Murine LY6 gene family members include, without limitation, LY6A (M_010738, nucleic acid SEQ ID NO:25 which encodes polypeptide SEQ ID NO:26), LY6C (NM_010741, nucleic acid SEQ ID NO:27 which encodes polypeptide SEQ ID NO:28), LY6D (NM_003695, nucleic acid SEQ ID NO:29 which encodes polypeptide SEQ ID NO:30), LY6E (NM_002346, nucleic acid SEQ ID NO:31 which encodes polypeptide SEQ ID NO:32), LY6F (NM_008530, nucleic acid SEQ ID NO:33 which encodes polypeptide SEQ ID NO:34), LY6I (NM_020498, nucleic acid SEQ ID NO:35 which encodes polypeptide SEQ ID NO:36), and LY6K (NM_017527, nucleic acid SEQ ID NO:37 which encodes polypeptide SEQ ID NO:38). Human LY6 gene family members include, without limitation, LY6H (NM_002347, nucleic acid SEQ ID NO:1 which encodes polypeptide SEQ ID NO:2), LYPD1 (NM_144586, nucleic acid SEQ ID NOS:3 or 4 which encodes polypeptide SEQ ID NO:5), LYPD3 (NM_014400, nucleic acid SEQ ID NO:6 which encodes polypeptide SEQ ID NO:7), LYPD5 (NM_182573, nucleic acid SEQ ID NOS:8 or 9 which encodes polypeptide SEQ ID NO:10), LY6D (NM_003695, nucleic acid SEQ ID NO:11 which encodes polypeptide SEQ ID NO:12), LY6E (NMNM_002346, nucleic acid SEQ ID NO:13 which encodes polypeptide SEQ ID NO:14), LYPD2 (NM_205545, nucleic acid SEQ ID NO: 15 which encodes polypeptide SEQ ID NO: 16). In embodiments, the polynucleotide of each LY6 gene family member disclosed herein comprises at least 15, at least 25, at least, at least 50, at least 100, at least 250, at least 500, at least 750, at least 1000, at least 1250, at least 1500, at least 1750, at least 2000, or at least 2040 contiguous nucleotides of SEQ ID NOs:1, 3, 4, 6, 8, 9, 11, 13, 15, 25, 27, 29, 31, 33, 35, 37, 45, 47, 49, 51, 53, 55, or 57, or the LY6 gene family member polynucleotide comprises SEQ ID NOS:1, 3, 4, 6, 8, 9, 11, 13, 15, 25, 27, 29, 31, 33, 35, 37, 45, 47, 49, 51, 53, 55, or 57. In one embodiment, a polynucleotide that binds a LY6 gene family member polynucleotide (SEQ ID NOs:1, 3, 4, 6, 8, 9, 11, 13, 15, 25, 27, 29, 31, 33, 35, 37, 45, 47, 49, 51, 53, 55, or 57), or fragment thereof, has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99% or 100% sequence identity with the LY6 polypeptide or fragment thereof. In one embodiment, the LY6 gene family member polypeptide comprises at least 10, at least 25, at least 50, at least 75, at least 100, at least 125, at least 150, at least 175, at least 200, at least 225, at least 250, at least 275, at least 300, or at least 325, at least contiguous amino acids of SEQ ID NOs:2, 5, 7, 10, 12, 14, 26, 28, 30, 32, 34, 36, 38, 46, 48, 50, 52, 54, 56, or 58, or the LY6 gene family polypeptide comprises SEQ ID NOs:2, 5, 7, 10, 12, 14, 26, 28, 30, 32, 34, 36, 38, 46, 48, 50, 52, 54, 56, or 58).

[0025] A "native sequence polypeptide" of any of the LY6 gene family members comprises a polypeptide having the same amino acid sequence as the corresponding LY6 gene family member polypeptide derived from nature. Such native sequence LY6 polypeptides can be isolated from nature or can be produced by recombinant or synthetic means. The term "native sequence LY6 polypeptide" specifically encompasses naturally-occurring truncated or secreted forms of the specific LY6 polypeptide (*e.g.*, an extracellular domain sequence), naturally-occurring variant forms (*e.g.*, alternatively spliced forms) and naturally-occurring allelic variants of the polypeptide. In one specific aspect, the native sequence LY6 polypeptides disclosed herein are mature or full-length native sequence polypeptides corresponding to the sequences in Figures 1-7 and SEQ ID NOs:2, 5, 7, 10, 12, 14, 26, 28, 30, 32, 34, 36, 38, 46, 48, 50, 52, 54, 56, or 58.

[0026] As used herein, a "LY6 polypeptide variant" means a LY6 polypeptide, preferably biologically active forms thereof, as defined herein, having at least about 80% amino acid sequence identity with a full-length native sequence LY6 polypeptide sequence, as disclosed herein, and variant forms thereof lacking the signal peptide, an extracellular domain, or any other fragment of a full length native sequence LY6 polypeptide such as those referenced herein. Such variant polypeptides include, for instance, polypeptides wherein one or more amino acid residues are added, or deleted, at the N- or C-terminus of the full-length native amino acid sequence. In a specific aspect, such variant polypeptides will have at least about 80% amino acid sequence identity, alternatively at least about 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity, to a full-length native sequence LY6 polypeptide sequence polypeptide, as disclosed herein, and variant forms thereof lacking a signal peptide, an extracellular domain, or any other fragment of a full length native sequence LY6 polypeptide such as those disclosed herein.

[0027] "Percent (%) amino acid sequence identity" with respect to a LY6 polypeptide sequence identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific LY6 polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of

determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2, wherein the complete source code for the ALIGN-2 program is provided in Table 1 below. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc. and the source code shown in Table 1 below has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available through Genentech, Inc., South San Francisco, California or may be compiled from the source code provided in Table 1 below. The ALIGN-2 program should be compiled for use on a UNIX operating system, preferably digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

[0028] As used herein "LY6 variant polynucleotide" or "LY6 variant nucleic acid sequence," or "LY6 gene" refers to a nucleic acid molecule which encodes a LY6 gene family member polypeptide, preferably biologically active forms thereof, as defined herein, and which have at least about 80% nucleic acid sequence identity with a nucleotide acid sequence encoding a full-length native sequence LY6 polypeptide sequence identified herein, or any other fragment of the respective full-length LY6 polypeptide sequence as identified herein (such as those encoded by a nucleic acid that represents only a portion of the complete coding sequence for a full-length LY6 polypeptide). Ordinarily, such variant polynucleotides will have at least about 80% nucleic acid sequence identity, alternatively at least about 81 %, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% nucleic acid sequence identity with a nucleic acid sequence encoding the respective full-length native sequence LY6 polypeptide sequence or any other fragment of the respective full-length LY6 polypeptide sequence identified herein. Such variant polynucleotides do not encompass the native nucleotide sequence.

[0029] Ordinarily, such variant polynucleotides vary at least about 50 nucleotides in length from the native sequence polypeptide, alternatively the variance can be at least about 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, or 1000 nucleotides in length, wherein in this context the term "about" means the referenced nucleotide sequence length plus or minus 10% of that referenced length.

[0030] "Percent (%) nucleic acid sequence identity" with respect to a LY6 gene polypeptide-encoding nucleic acid sequences identified herein is defined as the percentage of nucleotides in a candidate sequence that are identical with the nucleotides in the LY6 gene nucleic acid sequence of interest, respectively, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. For purposes herein, however, % nucleic acid sequence identity values are generated using the sequence comparison computer program ALIGN-2, wherein the complete source code for the ALIGN-2 program is provided in Table 1 below. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc. and the source code shown in Table 1 below has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available through Genentech, Inc., South San Francisco, California or may be compiled from the source code provided in Table 1 below. The ALIGN-2 program should be compiled for use on a UNIX operating system, preferably digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

[0031] In situations where ALIGN-2 is employed for nucleic acid sequence comparisons, the % nucleic acid sequence identity of a given nucleic acid sequence C to, with, or against a given nucleic acid sequence D (which can alternatively be phrased as a given nucleic acid sequence C that has or comprises a certain % nucleic acid sequence identity to, with, or against a given nucleic acid sequence D) is calculated as follows:

100 times the fraction W/Z

where W is the number of nucleotides scored as identical matches by the sequence alignment program ALIGN-2 in that program=s alignment of C and D, and where Z is the total number of nucleotides in D. It will be appreciated that where the length of nucleic acid sequence C is not equal to the length of nucleic acid sequence D, the % nucleic acid sequence identity of C to D will not equal the % nucleic acid sequence identity of D to C. As examples of % nucleic acid sequence identity calculations, Tables 4 and 5, demonstrate how to calculate the % nucleic acid sequence identity of the nucleic acid sequence designated "Comparison

DNA" to the nucleic acid sequence designated "REF-DNA", wherein "REF-DNA" represents a hypothetical LY6 gene-encoding nucleic acid sequence of interest, "Comparison DNA" represents the nucleotide sequence of a nucleic acid molecule against which the "REF-DNA" nucleic acid molecule of interest is being compared, and "N", "L" and "V" each represent different hypothetical nucleotides. Unless specifically stated otherwise, all % nucleic acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

[0032] In other embodiments, LY6 gene variant polynucleotides are nucleic acid molecules that encode LY6 polypeptide, respectively, and which are capable of hybridizing, preferably under stringent hybridization and wash conditions, to nucleotide sequences encoding a full-length LY6 polypeptide, respectively, as disclosed herein. Such variant polypeptides may be those that are encoded by such variant polynucleotides.

[0033] "Isolated", when used to describe the various LY6 polypeptides disclosed herein, means polypeptide that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, such polypeptides will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Such isolated polypeptides includes the corresponding polypeptides *in situ* within recombinant cells, since at least one component of the LY6 polypeptide from its natural environment will not be present. Ordinarily, however, such isolated polypeptides will be prepared by at least one purification step.

[0034] An "isolated" LY6 polypeptide-encoding nucleic acid is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the polypeptide-encoding nucleic acid. Any of the above such isolated nucleic acid molecule is other than in the form or setting in which it is found in nature. Any such nucleic acid molecules therefore are distinguished from the specific polypeptide-encoding nucleic acid molecule as it exists in natural cells.

[0035] The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

[0036] Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

[0037] As used herein "expression" as applied to gene expression, refers to transcription of a gene encoding a protein to produce mRNA as well as translation of the mRNA to produce the protein encoded by the gene. Thus, increased or decreased expression refers to increased or decreased transcription of a gene and/or increased or decreased translation of mRNA resulting from transcription.

[0038] "Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing temperature, and salt concentration. In general, longer probes require higher temperatures for proper annealing, while shorter probes need lower temperatures. Hybridization generally depends on the ability of denatured DNA to reanneal when complementary strands are present in an environment below their melting temperature. The higher the degree of desired homology between the probe and hybridizable sequence, the higher the relative temperature which can be used. As a result, it follows that higher relative temperatures would tend to make the reaction conditions more stringent, while lower temperatures less so. For additional details and explanation of stringency of hybridization reactions, see Ausubel et al., Current Protocols in Molecular Biology, Wiley Interscience Publishers, (1995).

[0039] "Stringent conditions" or "high stringency conditions", as defined herein, may be identified by those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50EC; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v)

formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50mM sodium phosphate buffer at pH 6.5 with 750 mM sodium chloride, 75 mM sodium citrate at 42EC; or (3) overnight hybridization in a solution that employs 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt=s solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42EC, with a 10 minute wash at 42EC in 0.2 x SSC (sodium chloride/sodium citrate) followed by a 10 minute high-stringency wash consisting of 0.1 x SSC containing EDTA at 55EC.

[0040] "Moderately stringent conditions" may be identified as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, New York: Cold Spring Harbor Press, 1989, and include the use of washing solution and hybridization conditions (e.g., temperature, ionic strength and %SDS) less stringent that those described above. An example of moderately stringent conditions is overnight incubation at 37EC in a solution comprising: 20% formamide, 5 x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5 x Denhardt=s solution, 10% dextran sulfate, and 20 mg/ml denatured sheared salmon sperm DNA, followed by washing the filters in 1 x SSC at about 37-50EC. The ordinarily skilled artisan will recognize how to adjust the temperature, ionic strength, *etc.* as necessary to accommodate factors such as probe length and the like.

[0041] The term "epitope tagged" when used herein refers to a chimeric polypeptide comprising an LY6 polypeptide, or LY6 polypeptide binding agent fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, yet is short enough such that it does not interfere with the activity of the polypeptide to which it is fused. The tag polypeptide preferably also is sufficiently unique so that such antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least six amino acid residues and usually between about 8 and 50 amino acid residues (preferably, between about 10 and 20 amino acid residues).

[0042] "Active" or "activity" for the purposes herein refers to form(s) of polypeptides which retain a biological and/or an immunological activity of native or naturally-occurring polypeptide, wherein "biological" activity refers to a biological function (either inhibitory or stimulatory) caused by a native or naturally-occurring polypeptide other than the ability to induce the production of an antibody against an antigenic epitope possessed by a native or naturally-occurring 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 polypeptide. An active polypeptide, as used herein, is an antigen that is differentially expressed, either from a qualitative or quantitative perspective, in IBD tissue, relative to its expression on similar tissue that is not afflicted with IBD.

[0043] The term "antagonist" is used in the broadest sense, and includes any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity of a native polypeptide disclosed herein. Suitable antagonist molecules specifically include antagonist antibodies or antibody fragments, fragments or amino acid sequence variants of native polypeptides, peptides, antisense oligonucleotides, small organic molecules, *etc.* Methods for identifying antagonists may comprise contacting such a polypeptide, including a cell expressing it, with a candidate agonist or antagonist molecule and measuring a detectable change in one or more biological activities normally associated with such polypeptide.

[0044] "Treating" or "treatment" or "alleviation" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) the progression of a disease. Treatment may also refer the modification of the progression of an IBD.

[0045] "Diagnosing" refers to the process of identifying or determining the distinguishing characteristics of a disease including without limitation IBD, UC and/or Crohn's Disease. The process of diagnosing is sometimes also expressed as staging or disease classification based on severity or disease progression as well as on location (such as, for example, location within or along the gastrointestinal tract at which inflammation and/or altered gene expression is found).

[0046] Subjects in need of diagnosis include those already experiencing with aberrant LY6 expression as well as those prone to having or those in whom aberrant LY6 expression is to be prevented. Accordingly, an aspect of the invention is the detection of a therapeutic drug response in a mammal treated with a therapeutic agent for the treatment of IBD, wherein the method comprises determining lh LY6 expression in gastrointestinal tissue of a test mammal relative to a control and determining that the LY6 expression levels are within not significantly different from normal control expression levels. In one embodiment, a therapeutic response is determined when the levels of expression of LY6 of the mammal treated with a therapeutic agent are different (expression is more similar to normal control, i.e., LY6 expression levels are lower than LY6 expression levels were in the mammal prior to treatment).

[0047] The above parameters for assessing successful treatment and improvement in the disease are readily measurable by

routine procedures familiar to a physician. For IBD therapy, efficacy can be measured, for example, by assessing the time to disease progression (TTP) and/or determining the response rate (RR). Biopsies may be taken to assess gene expression and observe histopathology of gastrointestinal tissue from the patient. The invention described herein relating to the process of prognosing and/or diagnosing involves the determination and evaluation of LY6 gene expression upregulation.

[0048] "Mammal" or "mammalian subject" for purposes of the treatment of, alleviating the symptoms of or diagnosis of a IBD refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, cats, cattle, horses, sheep, pigs, goats, rabbits, ferrets, etc. Preferably, the mammal is human.

[0049] Administration "in combination with" one or more further therapeutic agents includes simultaneous (concurrent) and consecutive administration in any order.

[0050] "Carriers" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers which are nontoxic 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 dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN7, polyethylene glycol (PEG), and PLURONICS7.

[0051] By "solid phase" or "solid support" is meant a non-aqueous matrix to which a polypeptide, nucleic acid, antibody or LY6 binding agent can adhere or attach. 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 phase 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. Patent No. 4,275,149.

[0052] A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

[0053] A "small molecule" or "small organic molecule" is defined herein to have a molecular weight below about 500 Daltons.

[0054] An "effective amount" of an antagonist agent is an amount sufficient to bring about a physiological effect, such as without limitation to inhibit, partially or entirely, function of gene or its encoded protein. An "effective amount" may be determined empirically and in a routine manner, in relation to this purpose.

[0055] The term "therapeutically effective amount" refers to an antagonist or other drug effective to "treat" a disease or disorder in a subject or mammal. In the case of IBD, the therapeutically effective amount of the drug will restore aberrant LY6 expression to normal physiological levels; reduce gastrointestinal inflammation; reduce the number of gastrointestinal lesions; and/or relieve to some extent one or more of the symptoms associated with IBD, UC and/or CD. See the definition herein of "treating".

[0056] A "growth inhibitory amount" of an antagonist is an amount capable of inhibiting the growth of a cell, especially tumor, *e.g.*, cancer cell, either *in vitro* or *in vivo*. For purposes of inhibiting neoplastic cell growth, such an amount may be determined empirically and in a routine manner.

[0057] A "cytotoxic amount" of an antagonist is an amount capable of causing the destruction of a cell, especially a proliferating cell, *e.g.,* cancer cell, either *in vitro* or *in vivo*. For purposes of inhibiting neoplastic cell growth may be determined empirically and in a routine manner.

[0058] The term "antibody" is used in the broadest sense and specifically covers, for example, anti- LY6 monoclonal antibodies (including antagonist and neutralizing antibodies), anti- LY6 antibody compositions with polyepitopic specificity, polyclonal antibodies, single chain anti- LY6 antibodies, multispecific antibodies (*e.g.*, bispecific) and antigen binding fragments (see below) of all of the above enumerated antibodies as long as they exhibit the desired biological or immunological activity. The term "immunoglobulin" (Ig) is used interchangeably with antibody herein.

[0059] An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural

environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody *in situ* within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

[0060] The basic 4-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains (an IgM antibody consists of 5 of the basic heterotetramer unit along with an additional polypeptide called J chain, and therefore contain 10 antigen binding sites, while secreted IgA antibodies can polymerize to form polyvalent assemblages comprising 2-5 of the basic 4-chain units along with J chain). In the case of IgGs, the 4-chain unit is generally about 150,000 daltons. Each L chain is linked to an H chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. Each H and L chain also has regularly spaced intrachain disulfide bridges. Each H chain has at the N-terminus, a variable domain (V_H) followed by three constant domains (C_H) for each of the α and γ chains and four C_H domains for μ and ε isotypes. Each L chain has at the N-terminus, a variable domain (V_L) followed by a constant domain (C_L) at its other end. The V_L is aligned with the V_H and the C_L is aligned with the first constant dmain of the heavy chain (C_H1). Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains. The pairing of a V_H and V_L together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, see, *e.g.*, Basic and Clinical Immunology, 8th edition, Daniel P. Stites, Abba I. Terr and Tristram G. Parslow (eds.), Appleton & Lange, Norwalk, CT, 1994, page 71 and Chapter 6.

[0061] The L chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains (C_H), immunoglobulins can be assigned to different classes or isotypes. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, having heavy chains designated α, δ, ε, γ, and μ, respectively. The γ and α classes are further divided into subclasses on the basis of relatively minor differences in C_H sequence and function, e.g., humans express the following subclasses: IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2.

[0062] The term "variable" refers to the fact that certain segments of the variable domains differ extensively in sequence among antibodies. The V domain mediates antigen binding and define specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the approximately 110-amino acid span of the variable domains. Instead, the V regions consist of relatively invariant stretches called framework regions (FRs) of 15-30 amino acids separated by shorter regions of extreme variability called "hypervariable regions" that are each 9-12 amino acids long. The variable domains of native heavy and light chains each comprise four FRs, largely adopting a β-sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the β-sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC).

[0063] The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region generally comprises amino acid residues from a "complementarity determining region" or "CDR" (*e.g.* around about Kabat residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the V_L, and around about Kabat residues 31-35B (H1), 50-65 (H2) and 95-102 (H3) in the V_H (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a "hypervariable loop" (*e.g.* around about Chothia residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the V_L, and 26-32 (H1), 52A-55 (H2) and 96-101 (H3) in the V_H (Chothia and Lesk J. Mol. Biol. 196:901-917 (1987)).

[0064] The term "monoclonal antibody" as used herein refers to an antibody from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope(s), except for possible variants that may arise during production of the monoclonal antibody, such variants generally being present in minor amounts. Such monoclonal antibody typically includes an antibody comprising a polypeptide sequence that binds a target, wherein the target-binding polypeptide sequence was obtained by a process that includes the selection of a single target binding

polypeptide sequence from a plurality of polypeptide sequences. For example, the selection process can be the selection of a unique clone from a plurality of clones, such as a pool of hybridoma clones, phage clones or recombinant DNA clones. It should be understood that the selected target binding sequence can be further altered, for example, to improve affinity for the target, to humanize the target binding sequence, to improve its production in cell culture, to reduce its immunogenicity *in vivo*, to create a multispecific antibody, *etc.*, and that an antibody comprising the altered target binding sequence is also a monoclonal antibody of this invention. In contrast to polyclonal antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. In addition to their specificity, the monoclonal antibody preparations are advantageous in that they are typically uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including, for example, the hybridoma method (*e.g.*, Kohler et al., Nature, 256:495 (1975); Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas 563-681, (Elsevier, N.Y., 1981)), recombinant DNA methods (see, *e.g.*, U.S. Patent No. 4,816,567), phage display technologies (see, *e.g.*, Clackson et al., Nature, 352:624-628 (1991); Marks et al., J. Mol. Biol., 222:581-597 (1991); Sidhu et al., J. Mol. Biol. 338(2):299-310 (2004); Lee et al., J.Mol.Biol.340(5):1073-1093 (2004); Fellouse, Proc. Nat. Acad. Sci. USA 101(34): 12467-12472 (2004); and Lee et al. J. Immunol. Methods 284(1-2):119-132 (2004), and technologies for producing human or human-like antibodies in animals that have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences (see, e.g., WO 1998/24893; WO 1996/34096; WO 1996/33735; WO 1991/10741; Jakobovits et al., Proc. Natl. Acad. Sci. USA, 90:2551 (1993); Jakobovits et al., Nature, 362:255-258 (1993); Bruggemann et al., Year in Immuno., 7:33 (1993); U.S. Patent Nos. 5,545,806; 5,569,825; 5,591,669 (all of GenPharm); 5,545,807; WO 1997/17852; U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016; Marks et al., Bio/Technology, 10: 779-783 (1992); Lonberg et al., Nature, 368: 856-859 (1994); Morrison, Nature, 368: 812-813 (1994); Fishwild et al., Nature Biotechnology, 14: 845-851 (1996); Neuberger, Nature Biotechnology, 14: 826 (1996); and Lonberg and Huszar, Intern. Rev. Immunol., 13: 65-93 (1995).

[0065] "Chimeric" antibodies (immunoglobulins) have a portion of the heavy and/or light chain identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; and Morrison et al., Proc. Natl. Acad. Sci. USA 81:6851-6855 (1984)). Humanized antibody as used herein is a subset of chimeric antibodies.

[0066] "Humanized" forms of non-human (*e.g.*, murine) antibodies are chimeric antibodies which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient or acceptor antibody) in which hypervariable region residues of the recipient are replaced by hypervariable region residues from a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance such as binding affinity. Generally, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence although the FR regions may include one or more amino acid substitutions that improve binding affinity. The number of these amino acid substitutions in the FR are typically no more than 6 in the H chain, and in the L chain, no more than 3. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., Nature 321:522-525 (1986); Reichmann et al., Nature 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol. 2:593-596 (1992).

[0067] "Antibody fragments" comprise a portion of an intact antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies (see U.S. Patent No. 5,641,870, Example 2; Zapata et al., Protein Eng. 8(10): 1057-1062 [1995]); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments.

[0068] Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. The Fab fragment consists of an entire L chain along with the variable region domain of the H chain (VH), and the first constant domain of one heavy chain (C_H1). Each Fab fragment is monovalent with respect to antigen binding, i.e., it has a single antigen-binding site. Pepsin treatment of an antibody yields a

single large $F(ab')_2$ fragment which roughly corresponds to two disulfide linked Fab fragments having divalent antigen-binding activity and is still capable of cross-linking antigen. Fab= fragments differ from Fab fragments by having additional few residues at the carboxy terminus of the C_H1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ 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.

[0069] The Fc fragment comprises the carboxy-terminal portions of both H chains held together by disulfides. The effector functions of antibodies are determined by sequences in the Fc region, which region is also the part recognized by Fc receptors (FcR) found on certain types of cells.

[0070] "Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0071] "Single-chain Fv" also abbreviated as "sFv" or "scFv" are antibody fragments that comprise the V_H and V_L antibody domains connected into a single polypeptide chain. Preferably, the sFv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the sFv to form the desired structure for antigen binding. For a review of sFv, see Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994); Borrebaeck 1995, *infra*.

[0072] As used herein "LY6 binding polypeptide" is an oligopeptide that binds, preferably specifically, to a LY6 polypeptide, ligand or signaling component, respectively, or a LY6 binding portion or fragment thereof. Such oligopeptides may be chemically synthesized using known oligopeptide synthesis methodology or may be prepared and purified using recombinant technology. Such oligopeptides are usually at least about 5 amino acids in length, alternatively at least about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 amino acids in length or more. Such oligopeptides may be identified without undue experimentation using well known techniques. In this regard, it is noted that techniques for screening oligopeptide libraries for oligopeptides that are capable of specifically binding to a polypeptide target are well known in the art (see, *e.g.*, U.S. Patent Nos. 5,556,762, 5,750,373, 4,708,871, 4,833,092, 5,223,409, 5,403,484, 5,571,689, 5,663,143; PCT Publication Nos. WO 84/03506 and WO84/03564; Geysen et al., Proc. Natl. Acad. Sci. U.S.A., 81:3998-4002 (1984); Geysen et al., Proc. Natl. Acad. Sci. U.S.A., 82:178-182 (1985); Geysen et al., in Synthetic Peptides as Antigens, 130-149 (1986); Geysen et al., J. Immunol. Meth., 102:259-274 (1987); Schoofs et al., J. Immunol., 140:611-616 (1988), Cwirla, S. E. et al. Proc. Natl. Acad. Sci. USA, 87:6378 (1990); Lowman, H.B. et al. Biochemistry, 30:10832 (1991); Clackson, T. et al. Nature, 352: 624 (1991); Marks, J. D. et al., J. Mol. Biol., 222:581 (1991); Kang, A.S. et al. Proc. Natl. Acad. Sci. USA, 88:8363 (1991), and Smith, G. P., Current Opin. Biotechnol., 2:668 (1991).

[0073] An LY6 antagonist (*e.g.*, antibody, polypeptide, oligopeptide or small molecule) "which binds" a target antigen of interest, *e.g.* LY6 is one that binds the target with sufficient affinity so as to be a useful diagnostic, prognostic and/or therapeutic agen,t in targeting a cell or tissue expressing the antigen, and does not significantly cross-react with other proteins. The extent of binding to a non-desired marker polypeptide will be less than about 10% of the binding to the particular desired target, as determinable by common techniques such as fluorescence activated cell sorting (FACS) analysis or radioimmunoprecipitation (RIA).

[0074] Moreover, the term "specific binding" or "specifically binds to" or is "specific for" a particular LY6 polypeptide or an epitope on a particular LY6 polypeptide target means binding that is measurably different from a non-specific interaction. Specific binding can be measured, for example, by determining binding of a molecule compared to binding of a control molecule, which generally is a molecule of similar structure that does not have binding activity. For example, specific binding can be determined by competition with a control molecule that is similar to the target, for example, an excess of non-labeled target. In this case, specific binding is indicated if the binding of the labeled target to a probe is competitively inhibited by excess unlabeled target. In one embodiment, such terms refer to binding where a molecule binds to a particular polypeptide or epitope on a particular polypeptide without substantially binding to any other polypeptide or polypeptide epitope. Alternatively, such terms can be described by a molecule having a Kd for the target of at least about 10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, 10^{-12} M, or greater.

[0075] A gastrointestinal cell or tissue that "overexpresses" LY6 if that cell or tissue is shown to have increased nucleic acid encoding LY6 in a cells or if that cell or tissue over produces and secretes LY6 protein, compared to a normal gastrointestinal cell or tissue of the same tissue type. Such overexpression may result from gene amplification or by increased transcription or translation. Various diagnostic or prognostic assays are known that measure altered expression levels resulting in increased or decreased levels at the cell surface or increased or decreased levels of secreted protein and include without limitation immunohistochemistry assay using anti- LY6 antibodies, FACS analysis, *etc.* Alternatively, the levels of LY6 encoding nucleic acid or mRNA can be measured in the cell, *e.g.*, via fluorescent *in situ* hybridization using a nucleic acid based probe corresponding to a LY6-encoding nucleic acid or the complement thereof; (FISH; see WO98/45479 published October, 1998), Southern blotting, Northern blotting, or polymerase chain reaction (PCR) techniques, such as real time quantitative PCR (RT-PCR). Alternatively, LY6 polypeptide overexpression is determinable by measuring shed antigen in a biological fluid such as serum, *e.g*, using antibody-based assays (see also, *e.g.*, U.S. Patent No. 4,933,294 issued June 12, 1990; WO91/05264 published April 18, 1991; U.S. Patent 5,401,638 issued March 28, 1995; and Sias et al., J. Immunol. Methods 132:73-80 (1990)). In addition to the above assays, various *in* vivo assays are available to the skilled practitioner. For example, one may expose cells within the body of the patient to an antibody which is optionally labeled with a detectable label, *e.g.*, a radioactive isotope, and binding of the antibody to cells in the patient can be evaluated, *e.g.*, by external scanning for radioactivity or by analyzing a biopsy taken from a patient previously exposed to the therapeutic agent.

[0076] As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the binding specificity of a heterologous protein (an "adhesin") with the effector functions of immunoglobulin constant domains. Structurally, the immunoadhesins comprise a fusion of an amino acid sequence with the desired binding specificity which is other than the antigen recognition and binding site of an antibody (*i.e.*, is "heterologous"), and an immunoglobulin constant domain sequence. The adhesin part of an immunoadhesin molecule typically is a contiguous amino acid sequence comprising at least the binding site of a receptor or a ligand. The immunoglobulin constant domain sequence in the immunoadhesin may be obtained from any immunoglobulin, such as IgG-1, IgG-2, IgG-3, or IgG-4 subtypes, IgA (including IgA-1 and IgA-2), IgE, IgD or IgM.

[0077] The word "label" when used herein refers to a detectable compound or composition which is conjugated directly or indirectly to the antibody, oligopeptide or other organic molecule so as to generate a "labeled" antibody, oligopeptide or other organic molecule. The label may be detectable by itself (*e.g.* radioisotope labels or fluorescent labels) or, in the case of an enzymatic label, may catalyze chemical alteration of a substrate compound or composition which is detectable.

[0078] The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (*e.g.*, At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32} and radioactive isotopes of Lu), chemotherapeutic agents, enzymes and fragments thereof such as nucleolytic enzymes, antibiotics, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof, and the various antitumor or anticancer agents disclosed below. Other cytotoxic agents are described below. A tumoricidal agent causes destruction of tumor cells.

[0079] A "chemotherapeutic agent" or "therapeutic agent" is a chemical compound useful in the treatment of a disorder or disease. Examples of chemotherapeutic or therapeutic agents for the treatment of IBD include without limitation anti-inflammatory drugs sulfasalazine and 5-aminosalisylic acid (5-ASA); metroidazole and ciprofloxacin are similar in efficacy to sulfasalazine and appear to be particularly useful for treating perianal disease; in more severe cases, corticosteroids are effective in treating active exacerbations and can even maintain remission; azathioprine, 6-mercaptopurine, and methotrexate have also shown success in patients who require chronic administration of cortico steroids; antidiarrheal drugs can also provide symptomatic relief in some patients; nutritional therapy or elemental diet can improve the nutritional status of patients and induce symtomatic improvement of acute disease; antibiotics are used in treating secondary small bowel bacterial overgrowth and in treatment of pyogenic complications. IBD chemotherapeutic agents further include biologicals and other agents as follows: anti-beta7 antibodies (see, for example, WO2006026759), anti-alpha4 antibodies (such as ANTEGEN®), anti-TNF antibody (REMICADE®)) or non-protein compounds including without limitation 5-ASA compounds ASACOL®, PENTASA™, ROWASA™, COLAZAL™, and other compounds such as Purinethol and steroids such as prednisone. Examples of chemotherapeutic agents for the treatment of cancer include hydroxyureataxanes (such as paclitaxel and doxetaxel) and/or anthracycline antibiotics; alkylating agents such as thiotepa and CYTOXAN7 cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethiylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL7); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN7), CPT-11 (irinotecan, CAMPTOSAR7), acetylcamptothecin, scopolectin, and 9-aminocamptothecin); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin

and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (e. g., calicheamicin, especially calicheamicin gamma1I and calicheamicin omegaI1 (see, *e.g.*, Agnew, Chem Intl. Ed. Engl., 33: 183-186 (1994)); dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN7 doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti- adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK7 polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE7, FILDESIN7); dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); thiotepa; taxoids, *e.g.*, TAXOL7 paclitaxel (Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANETM Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumberg, Illinois), and TAXOTERE7 doxetaxel (Rhône-Poulenc Rorer, Antony, France); chloranbucil; gemcitabine (GEMZAR7); 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine (VELBAN7); platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine (ONCOVIN7); oxaliplatin; leucovovin; vinorelbine (NAVELBINE7); novantrone; edatrexate; daunomycin; aminopterin; ibandronate; topoisomerase inhibitor RFS 2000; difluorometlhylornithine (DMFO); retinoids such as retinoic acid; capecitabine (XELODA7); pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone, and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATINTM) combined with 5-FU and leucovovin.

[0080] The term "cytokine" is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor-α and -β; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF-β; platelet-growth factor; transforming growth factors (TGFs) such as TGF-α and TGC-β; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon -α, -β, and -γ; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as It-1, IL- 1a, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12; a tumor necrosis factor such as TNF-α or TNF-β; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

[0081] The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products.

[0082] "Epithelia," "epithelial" and "epithelium" refer to the cellular covering of internal and external body surfaces (cutaneous, mucous and serous), including the glands and other structures derived therefrom, *e.g.*, corneal, esophageal, epidermal, and hair follicle epithelial cells. Other exemplary epithelial tissue includes: olfactory epithelium - the pseudostratified epithelium lining the olfactory region of the nasal cavity, and containing the receptors for the sense of smell; glandular epithelium - the epithelium composed of secreting cells squamous epithelium; squamous epithelium - the epithelium comprising one or more cell layers, the

most superficial of which is comosed of flat, scalelike or platelike cells. Epithelium can also refer to transitional epithelium, like that which is characteristically found lining hollow organs that are subject to great mechanical change due to contraction and distention, *e.g.*, tissue which represents a transition between stratified squamous and columnar epithelium.

[0083] The "growth state" of a cell refers to the rate of proliferation of the cell and/or the state of differentiation of the cell. An "altered growth state" is a growth state characterized by an abnormal rate of proliferation, *e.g.*, a cell exhibiting an increased or decreased rate of proliferation relative to a normal cell.

[0084] The term "LY6" or "LY6 polypeptide" is used herein to refer generically to any of the mammalian homologs of the mammalian LY6 gene family. The term "LY6" may be used to describe protein or nucleic acid.

[0085] The term "overexpression" as used herein, refers to cellular gene expression levels of a tissue that is higher than the normal expression levels for that tissue. The term "underexpression" as used herein, refers to cellular gene expression levels of a tissue that is lower than the normal expression levels for that tissue. In either case, the higher or lower expression is significantly different from normal expression under controlled conditions of the study.

[0086] A "control" includes a sample obtained for use in determining base-line or normal expression or activity in a mammal that is not experiencing IBD. Accordingly, a control sample may be obtained by a number of means including from tissue or cells not affected by inflammation and/or IBD, UC or CD (as determined by standard techniques); non-IBD cells or tissue e.g., from cells of a subject not experiencing IBD; from subjects not having an IBD, Crohn's disease, or ulcerative colitis disorder; from subjects not suspected of being at risk for an IBD, CD or UC; or from cells or cell lines derived from such subjects. A control also includes a previously established standard. For assays, such as mRNA assays, including microarray assays, a control may be a universal control. Such universal control refers to RNA expression information of a particular LY6 gene obtained from RNA isolated from a mixture of healthy tissues or from a mixture of cell lines derived from various tissues such as, without limitation, universal reference RNAs disclosed herein. Accordingly, any test or assay conducted according to the invention may be compared with the established standard and it may not be necessary to obtain a control sample for comparison each time.

Table 1

```
/*
 *
 * C-C increased from 12 to 15
 * Z is average of EQ
 * B is average of ND
 * match with stop is _M; stop-stop = 0; J (joker) match = 0
 */
#define _M      -8          /* value of a match with a stop */

int        _day[26][26] = {
/*      A  B  C  D  E  F  G  H  I  J  K  L  M  N  O  P  Q  R  S  T  U  V  W  X  Y  Z */
/* A */    { 2, 0,-2, 0, 0,-4, 1,-1,-1, 0,-1,-2,-1, 0,_M, 1, 0,-2, 1, 1, 0, 0,-6, 0,-3, 0},
/* B */    { 0, 3,-4, 3, 2,-5, 0, 1,-2, 0, 0,-3,-2, 2,_M,-1, 1, 0, 0, 0, 0,-2,-5, 0,-3, 1},
/* C */    {-2,-4,15,-5,-5,-4,-3,-3,-2, 0,-5,-6,-5,-4,_M,-3,-5,-4, 0,-2, 0,-2,-8, 0, 0,-5},
/* D */    { 0, 3,-5, 4, 3,-6, 1, 1,-2, 0, 0,-4,-3, 2,_M,-1, 2,-1, 0, 0, 0,-2,-7, 0,-4, 2},
/* E */    { 0, 2,-5, 3, 4,-5, 0, 1,-2, 0, 0,-3,-2, 1,_M,-1, 2,-1, 0, 0, 0,-2,-7, 0,-4, 3},
/* F */    {-4,-5,-4,-6,-5, 9,-5,-2, 1, 0,-5, 2, 0,-4,_M,-5,-5,-4,-3,-3, 0,-1, 0, 0, 7,-5},
/* G */    { 1, 0,-3, 1, 0,-5, 5,-2,-3, 0,-2,-4,-3, 0,_M,-1,-1,-3, 1, 0, 0,-1,-7, 0,-5, 0},
/* H */    {-1, 1,-3, 1, 1,-2,-2, 6,-2, 0, 0,-2,-2, 2,_M, 0, 3, 2,-1,-1, 0,-2,-3, 0, 0, 2},
/* I */    {-1,-2,-2,-2,-2, 1,-3,-2, 5, 0,-2, 2, 2,-2,_M,-2,-2,-2,-1, 0, 0, 4,-5, 0,-1,-2},
/* J */    { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,_M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* K */    {-1, 0,-5, 0, 0,-5,-2, 0,-2, 0, 5,-3, 0, 1,_M,-1, 1, 3, 0, 0, 0,-2,-3, 0,-4, 0},
/* L */    {-2,-3,-6,-4,-3, 2,-4,-2, 2, 0,-3, 6, 4,-3,_M,-3,-2,-3,-3,-1, 0, 2,-2, 0,-1,-2},
/* M */    {-1,-2,-5,-3,-2, 0,-3,-2, 2, 0, 0, 4, 6,-2,_M,-2,-1, 0,-2,-1, 0, 2,-4, 0,-2,-1},
/* N */    { 0, 2,-4, 2, 1,-4, 0, 2,-2, 0, 1,-3,-2, 2,_M,-1, 1, 0, 1, 0, 0,-2,-4, 0,-2, 1},
/* O */    {_M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M, _M,
0,_M, _M,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M},
/* P */    { 1,-1,-3,-1,-1,-5,-1, 0,-2, 0,-1,-3,-2,-1,_M, 6, 0, 0, 1, 0, 0,-1,-6, 0,-5, 0},
/* Q */    { 0, 1,-5, 2, 2,-5,-1, 3,-2, 0, 1,-2,-1, 1,_M, 0, 4, 1,-1,-1, 0,-2,-5, 0,-4, 3},
/* R */    {-2, 0,-4,-1,-1,-4,-3, 2,-2, 0, 3,-3, 0, 0,_M, 0, 1, 6, 0,-1, 0,-2, 2, 0,-4, 0},
/* S */    { 1, 0, 0, 0, 0,-3, 1,-1,-1, 0, 0,-3,-2, 1,_M, 1,-1, 0, 2, 1, 0,-1,-2, 0,-3, 0},
/* T */    { 1, 0,-2, 0, 0,-3, 0,-1, 0, 0, 0,-1,-1, 0,_M, 0,-1,-1, 1, 3, 0, 0,-5, 0,-3, 0},
/* U */    { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,_M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* V */    { 0,-2,-2,-2,-2,-1,-1,-2, 4, 0,-2, 2, 2,-2,_M,-1,-2,-2,-1, 0, 0, 4,-6, 0,-2,-2},
/* W */    {-6,-5,-8,-7,-7, 0,-7,-3,-5, 0,-3,-2,-4,-4,_M,-6,-5, 2,-2,-5, 0,-6,17, 0, 0,-6},
/* X */    { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,_M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
/* Y */    {-3,-3, 0,-4,-4, 7,-5, 0,-1, 0,-4,-1,-2,-2,_M,-5,-4,-4,-3,-3, 0,-2, 0, 0,10,-4},
/* Z */    { 0, 1,-5, 2, 3,-5, 0, 2,-2, 0, 0,-2,-1, 1,_M, 0, 3, 0, 0, 0, 0,-2,-6, 0,-4, 4}
};
```

Table 1 (cont. 1)

```
/*
 */
#include <stdio.h>
#include <ctype.h>

#define MAXJMP      16      /* max jumps in a diag */
#define MAXGAP      24      /* don't continue to penalize gaps larger than this */
#define JMPS        1024    /* max jmps in an path */
#define MX          4       /* save if there's at least MX-1 bases since last jmp */

#define DMAT        3       /* value of matching bases */
#define DMIS        0       /* penalty for mismatched bases */
#define DINS0       8       /* penalty for a gap */
#define DINS1       1       /* penalty per base */
#define PINS0       8       /* penalty for a gap */
#define PINS1       4       /* penalty per residue */

struct jmp {
        short           n[MAXJMP];      /* size of jmp (neg for dely) */
        unsigned short  x[MAXJMP];      /* base no. of jmp in seq x */
};                                      /* limits seq to 2^16 -1 */

struct diag {
        int             score;          /* score at last jmp */
        long            offset;         /* offset of prev block */
        short           ijmp;           /* current jmp index */
        struct jmp      jp;             /* list of jmps */
};

struct path {
        int     spc;            /* number of leading spaces */
        short   n[JMPS];        /* size of jmp (gap) */
        int     x[JMPS];        /* loc of jmp (last elem before gap) */
};

char            *ofile;                 /* output file name */
char            *namex[2];              /* seq names: getseqs( ) */
char            *prog;                  /* prog name for err msgs */
char            *seqx[2];                       /* seqs: getseqs( ) */
int             dmax;                   /* best diag: nw( ) */
int             dmax0;                  /* final diag */
int             dna;                    /* set if dna: main( ) */
int             endgaps;                /* set if penalizing end gaps */
int             gapx, gapy;             /* total gaps in seqs */
int             len0, len1;             /* seq lens */
int             ngapx, ngapy;           /* total size of gaps */
int             smax;                   /* max score: nw( ) */
int             *xbm;                   /* bitmap for matching */
long            offset;                 /* current offset in jmp file */
struct  diag    *dx;                    /* holds diagonals */
struct  path    pp[2];                  /* holds path for seqs */

char            *calloc( ), *malloc( ), *index( ), *strcpy( );
char            *getseq( ), *g_calloc( );
```

Table 1 (cont. 2)

```
/* Needleman-Wunsch alignment program
 *
 * usage: progs file1 file2
 *   where file1 and file2 are two dna or two protein sequences.
 *   The sequences can be in upper- or lower-case an may contain ambiguity
 *   Any lines beginning with ';', '>' or '<' are ignored
 *   Max file length is 65535 (limited by unsigned short x in the jmp struct)
 *   A sequence with 1/3 or more of its elements ACGTU is assumed to be DNA
 *   Output is in the file "align.out"
 *
 * The program may create a tmp file in /tmp to hold info about traceback.
 * Original version developed under BSD 4.3 on a vax 8650
 */
#include "nw.h"
#include "day.h"

static    _dbval[26] = {
        1,14,2,13,0,0,4,11,0,0,12,0,3,15,0,0,0,5,6,8,8,7,9,0,10,0
};

static    _pbval[26] = {
        1, 2|(1<<('D'-'A'))|(1<<('N'-'A')), 4, 8, 16, 32, 64,
        128, 256, 0xFFFFFFF, 1<<10, 1<<11, 1<<12, 1<<13, 1<<14,
        1<<15, 1<<16, 1<<17, 1<<18, 1<<19, 1<<20, 1<<21, 1<<22,
        1<<23, 1<<24, 1<<25|(1<<('E'-'A'))|(1<<('Q'-'A'))
};

main(ac, av)                                                              main
        int     ac;
        char    *av[];
{
        prog = av[0];
        if (ac != 3) {
                fprintf(stderr,"usage: %s file1 file2\n", prog);
                fprintf(stderr,"where file1 and file2 are two dna or two protein sequences.\n");
                fprintf(stderr,"The sequences can be in upper- or lower-case\n");
                fprintf(stderr,"Any lines beginning with ';' or '<' are ignored\n");
                fprintf(stderr,"Output is in the file \"align.out\"\n");
                exit(1);
        }
        namex[0] = av[1];
        namex[1] = av[2];
        seqx[0] = getseq(namex[0], &len0);
        seqx[1] = getseq(namex[1], &len1);
        xbm = (dna)? _dbval : _pbval;

        endgaps = 0;                    /* 1 to penalize endgaps */
        ofile = "align.out";            /* output file */

        nw( );          /* fill in the matrix, get the possible jmps */
        readjmps( );    /* get the actual jmps */
        print( );       /* print stats, alignment */

        cleanup(0);     /* unlink any tmp files */
}
```

Table 1 (cont. 3)

```
/* do the alignment, return best score: main( )
 * dna: values in Fitch and Smith, PNAS, 80, 1382-1386, 1983
 * pro: PAM 250 values
 * When scores are equal, we prefer mismatches to any gap, prefer
 * a new gap to extending an ongoing gap, and prefer a gap in seqx
 * to a gap in seq y.
 */
nw( )                                                                    nw
{
        char            *px, *py;               /* seqs and ptrs */
        int             *ndely, *dely;  /* keep track of dely */
        int             ndelx, delx;    /* keep track of delx */
        int             *tmp;           /* for swapping row0, row1 */
        int             mis;            /* score for each type */
        int             ins0, ins1;     /* insertion penalties */
        register        id;             /* diagonal index */
        register        ij;             /* jmp index */
        register        *col0, *col1;   /* score for curr, last row */
        register        xx, yy;         /* index into seqs */

        dx = (struct diag *)g_calloc("to get diags", len0+len1+1, sizeof(struct diag));

        ndely = (int *)g_calloc("to get ndely", len1+1, sizeof(int));
        dely = (int *)g_calloc("to get dely", len1+1, sizeof(int));
        col0 = (int *)g_calloc("to get col0", len1+1, sizeof(int));
        col1 = (int *)g_calloc("to get col1", len1+1, sizeof(int));
        ins0 = (dna)? DINS0 : PINS0;
        ins1 = (dna)? DINS1 : PINS1;

        smax = -10000;
        if (endgaps) {
                for (col0[0] = dely[0] = -ins0, yy = 1; yy <= len1; yy++) {
                        col0[yy] = dely[yy] = col0[yy-1] - ins1;
                        ndely[yy] = yy;
                }
                col0[0] = 0;            /* Waterman Bull Math Biol 84 */
        }
        else
                for (yy = 1; yy <= len1; yy++)
                        dely[yy] = -ins0;

        /* fill in match matrix
           */
        for (px = seqx[0], xx = 1; xx <= len0; px++, xx++) {
                /* initialize first entry in col
                   */
                if (endgaps) {
                        if (xx == 1)
                                col1[0] = delx = -(ins0+ins1);
                        else
                                col1[0] = delx = col0[0] - ins1;
                        ndelx = xx;
                }
                else {
                        col1[0] = 0;
                        delx = -ins0;
                        ndelx = 0;
                }
```

Table 1 (cont. 4)

...nw

```
for (py = seqx[1], yy = 1; yy <= len1; py++, yy++) {
        mis = col0[yy-1];
        if (dna)
                mis += (xbm[*px-'A']&xbm[*py-'A'])? DMAT : DMIS;
        else
                mis += _day[*px-'A'][*py-'A'];

        /* update penalty for del in x seq;
         * favor new del over ongong del
         * ignore MAXGAP if weighting endgaps
         */
        if (endgaps || ndely[yy] < MAXGAP) {
                if (col0[yy] - ins0 >= dely[yy]) {
                        dely[yy] = col0[yy] - (ins0+ins1);
                        ndely[yy] = 1;
                } else {
                        dely[yy] -= ins1;
                        ndely[yy]++;
                }
        } else {
                if (col0[yy] - (ins0+ins1) >= dely[yy]) {
                        dely[yy] = col0[yy] - (ins0+ins1);
                        ndely[yy] = 1;
                } else
                        ndely[yy]++;
        }

        /* update penalty for del in y seq;
         * favor new del over ongong del
         */
        if (endgaps || ndelx < MAXGAP) {
                if (col1[yy-1] - ins0 >= delx) {
                        delx = col1[yy-1] - (ins0+ins1);
                        ndelx = 1;
                } else {
                        delx -= ins1;
                        ndelx++;
                }
        } else {
                if (col1[yy-1] - (ins0+ins1) >= delx) {
                        delx = col1[yy-1] - (ins0+ins1);
                        ndelx = 1;
                } else
                        ndelx++;
        }

        /* pick the maximum score; we're favoring
         * mis over any del and delx over dely
         */
```

Table 1 (cont. 5)

```
                id = xx - yy + len1 - 1;                                        ...nw
                if (mis >= delx && mis >= dely[yy])
                        col1[yy] = mis;
                else if (delx >= dely[yy]) {
                        col1[yy] = delx;
                        ij = dx[id].ijmp;
                        if (dx[id].jp.n[0] && (!dna  || (ndelx >= MAXJMP
                        && xx > dx[id].jp.x[ij]+MX) || mis > dx[id].score+DINS0)) {
                                dx[id].ijmp++;
                                if (++ij >= MAXJMP) {
                                        writejmps(id);
                                        ij = dx[id].ijmp = 0;
                                        dx[id].offset = offset;
                                        offset += sizeof(struct jmp) + sizeof(offset);
                                }
                        }
                        dx[id].jp.n[ij] = ndelx;
                        dx[id].jp.x[ij] = xx;
                        dx[id].score = delx;
                }
                else {
                        col1[yy] = dely[yy];
                        ij = dx[id].ijmp;
        if (dx[id].jp.n[0] && (!dna || (ndely[yy] >= MAXJMP
                        && xx > dx[id].jp.x[ij]+MX) || mis > dx[id].score+DINS0)) {
                                dx[id].ijmp++;
                                if (++ij >= MAXJMP) {
                                        writejmps(id);
                                        ij = dx[id].ijmp = 0;
                                        dx[id].offset = offset;
                                        offset += sizeof(struct jmp) + sizeof(offset);
                                }
                        }
                        dx[id].jp.n[ij] = -ndely[yy];
                        dx[id].jp.x[ij] = xx;
                        dx[id].score = dely[yy];
                }
                if (xx == len0 && yy < len1) {
                        /* last col
                         */
                        if (endgaps)
                                col1[yy] -= ins0+ins1*(len1-yy);
                        if (col1[yy] > smax) {
                                smax = col1[yy];
                                dmax = id;
                        }
                }
        }
        if (endgaps && xx < len0)
                col1[yy-1] -= ins0+ins1*(len0-xx);
        if (col1[yy-1] > smax) {
                smax = col1[yy-1];
                dmax = id;
        }
        tmp = col0; col0 = col1; col1 = tmp;
}
(void) free((char *)ndely);
(void) free((char *)dely);
(void) free((char *)col0);
(void) free((char *)col1);
}
```

Table 1 (cont. 6)

```
/*
 *
 * print( ) -- only routine visible outside this module
 *
 * static:
 * getmat( ) -- trace back best path, count matches: print( )
 * pr_align( ) -- print alignment of described in array p[]: print( )
 * dumpblock( ) -- dump a block of lines with numbers, stars: pr_align( )
 * nums( ) -- put out a number line: dumpblock( )
 * putline( ) -- put out a line (name, [num], seq, [num]): dumpblock( )
 * stars( ) - -put a line of stars: dumpblock( )
 * stripname( ) -- strip any path and prefix from a seqname
 */

#include "nw.h"

#define SPC     3
#define P_LINE  256     /* maximum output line */
#define P_SPC   3       /* space between name or num and seq */

extern  _day[26][26];
int     olen;           /* set output line length */
FILE    *fx;            /* output file */

print( )                                                                    print
{
        int     lx, ly, firstgap, lastgap;      /* overlap */

        if ((fx = fopen(ofile, "w")) == 0) {
                fprintf(stderr,"%s: can't write %s\n", prog, ofile);
                cleanup(1);
        }
        fprintf(fx, "<first sequence: %s (length = %d)\n", namex[0], len0);
        fprintf(fx, "<second sequence: %s (length = %d)\n", namex[1], len1);
        olen = 60;
        lx = len0;
        ly = len1;
        firstgap = lastgap = 0;
        if (dmax < len1 - 1) {          /* leading gap in x */
                pp[0].spc = firstgap = len1 - dmax - 1;
                ly -= pp[0].spc;
        }
        else if (dmax > len1 - 1) {  /* leading gap in y */
                pp[1].spc = firstgap = dmax - (len1 - 1);
                lx -= pp[1].spc;
        }
        if (dmax0 < len0 - 1) {         /* trailing gap in x */
                lastgap = len0 - dmax0 -1;
                lx -= lastgap;
        }
        else if (dmax0 > len0 - 1) {            /* trailing gap in y */
                lastgap = dmax0 - (len0 - 1);
                ly -= lastgap;
        }
        getmat(lx, ly, firstgap, lastgap);
        pr_align( );
}
```

Table 1 (cont. 7)

```
/*
 * trace back the best path, count matches
 */
static
getmat(lx, ly, firstgap, lastgap)                                    getmat
        int     lx, ly;                 /* "core" (minus endgaps) */
        int     firstgap, lastgap;      /* leading trailing overlap */
{
        int             nm, i0, i1, siz0, siz1;
        char            outx[32];
        double          pct;
        register        n0, n1;
        register char   *p0, *p1;

        /* get total matches, score
         */
        i0 = i1 = siz0 = siz1 = 0;
        p0 = seqx[0] + pp[1].spc;
        p1 = seqx[1] + pp[0].spc;
        n0 = pp[1].spc + 1;
        n1 = pp[0].spc + 1;

        nm = 0;
        while ( *p0 && *p1 ) {
                if (siz0) {
                        p1++;
                        n1++;
                        siz0--;
                }
                else if (siz1) {
                        p0++;
                        n0++;
                        siz1--;
                }
                else {
                        if (xbm[*p0-'A']&xbm[*p1-'A'])
                                nm++;
                        if (n0++ == pp[0].x[i0])
                                siz0 = pp[0].n[i0++];
                        if (n1++ == pp[1].x[i1])
                                siz1 = pp[1].n[i1++];
                        p0++;
                        p1++;
                }
        }

        /* pct homology:
         * if penalizing endgaps, base is the shorter seq
         * else, knock off overhangs and take shorter core
         */
        if (endgaps)
                lx = (len0 < len1)? len0 : len1;
        else
                lx = (lx < ly)? lx : ly;
        pct = 100.*(double)nm/(double)lx;
        fprintf(fx, "\n");
        fprintf(fx, "<%d match%s in an overlap of %d: %.2f percent similarity\n",
                nm, (nm == 1)? "" : "es", lx, pct);
```

Table 1 (cont. 8)

```
        fprintf(fx, "<gaps in first sequence: %d", gapx);                                   ...getmat
        if (gapx) {
                (void) sprintf(outx, " (%d %s%s)",
                        ngapx, (dna)? "base":"residue", (ngapx == 1)? "":"s");
                fprintf(fx,"%s", outx);

        fprintf(fx, ", gaps in second sequence: %d", gapy);
        if (gapy) {
                (void) sprintf(outx, " (%d %s%s)",
                        ngapy, (dna)? "base":"residue", (ngapy == 1)? "":"s");
                fprintf(fx,"%s", outx);
        }
        if (dna)
                fprintf(fx,
                "\n<score: %d (match = %d, mismatch = %d, gap penalty = %d + %d per base)\n",
                smax, DMAT, DMIS, DINS0, DINS1);
        else
                fprintf(fx,
                "\n<score: %d (Dayhoff PAM 250 matrix, gap penalty = %d + %d per residue)\n",
                smax, PINS0, PINS1);
        if (endgaps)
                fprintf(fx,
                "<endgaps penalized. left endgap: %d %s%s, right endgap: %d %s%s\n",
                firstgap, (dna)? "base" : "residue", (firstgap == 1)? "" : "s",
                lastgap, (dna)? "base" : "residue", (lastgap == 1)? "" : "s");
        else
                fprintf(fx, "<endgaps not penalized\n");
}

static          nm;             /* matches in core -- for checking */
static          lmax;           /* lengths of stripped file names */
static          ij[2];          /* jmp index for a path */
static          nc[2];          /* number at start of current line */
static          ni[2];          /* current elem number -- for gapping */
static          siz[2];
static char     *ps[2];         /* ptr to current element */
static char     *po[2];         /* ptr to next output char slot */
static char     out[2][P_LINE]; /* output line */
static char     star[P_LINE];   /* set by stars( ) */
/*
 * print alignment of described in struct path pp[]
 */
static
pr_align( )                                                                         pr_align
{
        int             nn;     /* char count */
        int             more;
        register        i;

        for (i =  0, lmax = 0; i < 2; i++) {
                nn = stripname(namex[i]);
                if (nn > lmax)
                        lmax = nn;

                nc[i] = 1;
                ni[i] = 1;
                siz[i] = ij[i] = 0;
                ps[i] = seqx[i];
                po[i] = out[i];
        }
```

Table 1 (cont. 9)

```
        for (nn = nm = 0, more = 1; more; ) {                                   ...pr_align
                for (i = more = 0; i < 2; i++) {
                        /*
                         * do we have more of this sequence?
                         */
                        if (!*ps[i])
                                continue;

                        more++;

                        if (pp[i].spc) {        /* leading space */
                                *po[i]++ = ' ';
                                pp[i].spc--;
                        }
                        else if (siz[i]) {      /* in a gap */
                                *po[i]++ = '-';
                                siz[i]--;
                        }
                        else {                  /* we're putting a seq element
                                                 */
                                *po[i] = *ps[i];
                                if (islower(*ps[i]))
                                        *ps[i] = toupper(*ps[i]);
                                po[i]++;
                                ps[i]++;

                                /*
                                 * are we at next gap for this seq?
                                 */
                                if (ni[i] == pp[i].x[ij[i]]) {
                                        /*
                                         * we need to merge all gaps
                                         * at this location
                                         */
                                        siz[i] = pp[i].n[ij[i]++];
                                        while (ni[i] == pp[i].x[ij[i]])
                                                siz[i] += pp[i].n[ij[i]++];
                                }
                                ni[i]++;
                        }
                }
                if (++nn == olen || !more && nn) {
                        dumpblock( );
                        for (i = 0; i < 2; i++)
                                po[i] = out[i];
                        nn = 0;
                }
        }
}

/*
 * dump a block of lines, including numbers, stars: pr_align( )
 */
static
dumpblock( )                                                                    dumpblock
{
        register i;
        for (i = 0; i < 2; i++)
                *po[i]-- = '\0';
```

Table 1 (cont. 10)

...dumpblock

```
        (void) putc('\n', fx);
        for (i = 0; i < 2; i++) {
                if (*out[i] && (*out[i] != ' ' || *(po[i]) != ' ')) {
                        if (i == 0)
                                nums(i);
                        if (i == 0 && *out[1])
                                stars( );
                        putline(i);
                        if (i == 0 && *out[1])
                                fprintf(fx, star);
                        if (i == 1)
                                nums(i);
                }
        }
}

/*
 * put out a number line: dumpblock( )
 */
static
nums(ix)                                                                nums
        int     ix;     /* index in out[] holding seq line */
{
        char            nline[P_LINE];
        register        i, j;
        register char   *pn, *px, *py;

        for (pn = nline, i = 0; i < lmax+P_SPC; i++, pn++)
                *pn = ' ';
        for (i = nc[ix], py = out[ix]; *py; py++, pn++) {
                if (*py == ' ' || *py == '-')
                        *pn = ' ';
                else {
                        if (i%10 == 0 || (i == 1 && nc[ix] != 1)) {
                                j = (i < 0)? -i : i;
                                for (px = pn; j; j /= 10, px--)
                                        *px = j%10 + '0';
                                if (i < 0)
                                        *px = '-';
                        }
                        else
                                *pn = ' ';
                        i++;
                }
        }
        *pn = '\0';
        nc[ix] = i;
        for (pn = nline; *pn; pn++)
                (void) putc(*pn, fx);
        (void) putc('\n', fx);
}

/*
 * put out a line (name, [num], seq, [num]): dumpblock( )
 */
static
putline(ix)                                                             putline
        int                             ix;
{
```

Table 1 (cont. 11)

...putline

```
        int             i;
        register char   *px;

        for (px = namex[ix], i = 0; *px && *px != ':'; px++, i++)
                (void) putc(*px, fx);
        for (; i < lmax+P_SPC; i++)
                (void) putc(' ', fx);

        /* these count from 1:
         * ni[] is current element (from 1)
         * nc[] is number at start of current line
         */
        for (px = out[ix]; *px; px++)
                (void) putc(*px&0x7F, fx);
        (void) putc('\n', fx);
}


/*
 * put a line of stars (seqs always in out[0], out[1]): dumpblock( )
 */
static
stars( )                                                        stars
{
        int             i;
        register char   *p0, *p1, cx, *px;

        if (!*out[0] || (*out[0] == ' ' && *(po[0]) == ' ') ||
            !*out[1] || (*out[1] == ' ' && *(po[1]) == ' '))
                return;
        px = star;
        for (i = lmax+P_SPC; i; i--)
                *px++ = ' ';

        for (p0 = out[0], p1 = out[1]; *p0 && *p1; p0++, p1++) {
                if (isalpha(*p0) && isalpha(*p1)) {

                        if (xbm[*p0-'A']&xbm[*p1-'A']) {
                                cx = '*';
                                nm++;
                        }
                        else if (!dna && _day[*p0-'A'][*p1-'A'] > 0)
                                cx = '.';
                        else
                                cx = ' ';
                }
                else
                        cx = ' ';
                *px++ = cx;
        }
        *px++ = '\n';
        *px = '\0';
}
```

Table 1 (cont. 12)

```
/*
 * strip path or prefix from pn, return len: pr_align( )
 */
static
stripname(pn)                                                   stripname
        char    *pn;    /* file name (may be path) */
{
        register char   *px, *py;

        py = 0;
        for (px = pn; *px; px++)
                if (*px == '/')
                        py = px + 1;
        if (py)
                (void) strcpy(pn, py);
        return(strlen(pn));

}
```

Table 1 (cont. 13)

```
/*
 * cleanup( ) -- cleanup any tmp file
 * getseq( ) -- read in seq, set dna, len, maxlen
 * g_calloc( ) -- calloc( ) with error checkin
 * readjmps( ) -- get the good jmps, from tmp file if necessary
 * writejmps( ) -- write a filled array of jmps to a tmp file: nw( )
 */
#include "nw.h"
#include <sys/file.h>

char    *jname = "/tmp/homgXXXXXX";             /* tmp file for jmps */
FILE    *fj;

int     cleanup( );                             /* cleanup tmp file */
long    lseek( );

/*
 * remove any tmp file if we blow
 */
cleanup(i)                                                              cleanup
        int     i;
{
        if (fj)
                (void) unlink(jname);
        exit(i);
}

/*
 * read, return ptr to seq, set dna, len, maxlen
 * skip lines starting with ';', '<', or '>'
 * seq in upper or lower case
 */
char    *
getseq(file, len)                                                       getseq
        char    *file;  /* file name */
        int     *len;   /* seq len */
{
        char            line[1024], *pseq;
        register char   *px, *py;
        int             natgc, tlen;
        FILE            *fp;

        if ((fp = fopen(file,"r")) == 0) {
                fprintf(stderr,"%s: can't read %s\n", prog, file);
                exit(1);
        }
        tlen = natgc = 0;
        while (fgets(line, 1024, fp)) {
                if (*line == ';' || *line == '<' || *line == '>')
                        continue;
                for (px = line; *px != '\n'; px++)
                        if (isupper(*px) || islower(*px))
                                tlen++;
        }
        if ((pseq = malloc((unsigned)(tlen+6))) == 0) {
                fprintf(stderr,"%s: malloc( ) failed to get %d bytes for %s\n", prog, tlen+6, file);
                exit(1);
        }
        pseq[0] = pseq[1] = pseq[2] = pseq[3] = '\0';
```

Table 1 (cont. 14)

...getseq

```
        py = pseq + 4;
        *len = tlen;
        rewind(fp);

        while (fgets(line, 1024, fp)) {
                if (*line == ';' || *line == '<' || *line == '>')
                        continue;
                for (px = line; *px != '\n'; px++) {
                        if (isupper(*px))
                                *py++ = *px;
                        else if (islower(*px))
                                *py++ = toupper(*px);
                        if (index("ATGCU",*(py-1)))
                                natgc++;
                }
        }
        *py++ = '\0';
        *py = '\0';
        (void) fclose(fp);
        dna = natgc > (tlen/3);
        return(pseq+4);
}

char    *
g_calloc(msg, nx, sz)                                                   g_calloc
        char    *msg;           /* program, calling routine */
        int     nx, sz;         /* number and size of elements */
{
        char            *px, *calloc( );

        if ((px = calloc((unsigned)nx, (unsigned)sz)) == 0) {
                if (*msg) {
                        fprintf(stderr, "%s: g_calloc( ) failed %s (n=%d, sz=%d)\n", prog, msg, nx, sz);
                        exit(1);
                }
        }
        return(px);
}

/*
 * get final jmps from dx[] or tmp file, set pp[], reset dmax: main( )
 */
readjmps( )                                                             readjmps
{
        int             fd = -1;
        int             siz, i0, i1;
        register i, j, xx;

        if (fj) {
                (void) fclose(fj);
                if ((fd = open(jname, O_RDONLY, 0)) < 0) {
                        fprintf(stderr, "%s: can't open( ) %s\n", prog, jname);
                        cleanup(1);
                }
        }
        for (i = i0 = i1 = 0, dmax0 = dmax, xx = len0; ; i++) {
                while (1) {
                        for (j = dx[dmax].ijmp; j >= 0 && dx[dmax].jp.x[j] >= xx; j--)
                                ;
```

Table 1 (cont. 15)

...readjmps

```
                if (j < 0 && dx[dmax].offset && fj) {
                        (void) lseek(fd, dx[dmax].offset, 0);
                        (void) read(fd, (char *)&dx[dmax].jp, sizeof(struct jmp));
                        (void) read(fd, (char *)&dx[dmax].offset, sizeof(dx[dmax].offset));
                        dx[dmax].ijmp = MAXJMP-1;
                }
                else
                        break;
        }
        if (i >= JMPS) {
                fprintf(stderr, "%s: too many gaps in alignment\n", prog);
                cleanup(1);
        }
        if (j >= 0) {
                siz = dx[dmax].jp.n[j];
                xx = dx[dmax].jp.x[j];
                dmax += siz;
                if (siz < 0) {                  /* gap in second seq */
                        pp[1].n[i1] = -siz;
                        xx += siz;
                        /* id = xx - yy + len1 - 1
                         */
                        pp[1].x[i1] = xx - dmax + len1 - 1;
                        gapy++;
                        ngapy -= siz;
/* ignore MAXGAP when doing endgaps */
                        siz = (-siz < MAXGAP || endgaps)? -siz : MAXGAP;
                        i1++;
                }
                else if (siz > 0) {  /* gap in first seq */
                        pp[0].n[i0] = siz;
                        pp[0].x[i0] = xx;
                        gapx++;
                        ngapx += siz;
/* ignore MAXGAP when doing endgaps */
                        siz = (siz < MAXGAP || endgaps)? siz : MAXGAP;
                        i0++;
                }
        }
        else
                break;
    }

    /* reverse the order of jmps
     */
    for (j = 0, i0--; j < i0; j++, i0--) {
        i = pp[0].n[j]; pp[0].n[j] = pp[0].n[i0]; pp[0].n[i0] = i;
        i = pp[0].x[j]; pp[0].x[j] = pp[0].x[i0]; pp[0].x[i0] = i;
    }
    for (j = 0, i1--; j < i1; j++, i1--) {
        i = pp[1].n[j]; pp[1].n[j] = pp[1].n[i1]; pp[1].n[i1] = i;
        i = pp[1].x[j]; pp[1].x[j] = pp[1].x[i1]; pp[1].x[i1] = i;
    }
    if (fd >= 0)
        (void) close(fd);
    if (fj) {
        (void) unlink(jname);
        fj = 0;
        offset = 0;
    }
```

Table 1 (cont. 16)

```
/*
 * write a filled jmp struct offset of the prev one (if any): nw( )
 */
writejmps(ix)                                                          writejmps
    int     ix;
{
    char    *mktemp( );

    if (!fj) {
        if (mktemp(jname) < 0) {
            fprintf(stderr, "%s: can't mktemp( ) %s\n", prog, jname);
            cleanup(1);
        }
        if ((fj = fopen(jname, "w")) == 0) {
            fprintf(stderr, "%s: can't write %s\n", prog, jname);
            exit(1);
        }
    }
    (void) fwrite((char *)&dx[ix].jp, sizeof(struct jmp), 1, fj);
    (void) fwrite((char *)&dx[ix].offset, sizeof(dx[ix].offset), 1, fj);
}
```

Table 2

Reference	XXXXXXXXXXXXXX	(Length = 15 amino acids)
Comparison Protein	XXXXXYYYYYY	(Length = 12 amino acids)
% amino acid sequence identity = (the number of identically matching amino acid residues between the two polypeptide sequences as determined by ALIGN-2) divided by (the total number of amino acid residues of the reference polypeptide) = 5 divided by 15 = 33.3%		

Table 3

Reference	XXXXXXXXXX	(Length = 10 amino acids)
Comparison Protein	XXXXXYYYYYYZZYZ	(Length = 15 amino acids)
% amino acid sequence identity = (the number of identically matching amino acid residues between the two polypeptide sequences as determined by ALIGN-2) divided by (the total number of amino acid residues of the reference polypeptide) = 5 divided by 10 = 50%		

Table 4

Reference-DNA	NNNNNNNNNNNNNN	(Length = 14 nucleotides)
Comparison DNA	NNNNNNLLLLLLLLL	(Length = 16 nucleotides)
% nucleic acid sequence identity = (the number of identically matching nucleotides between the two nucleic acid sequences as determined by ALIGN-2) divided by (the total number of nucleotides of the reference-DNA nucleic acid sequence) = 6 divided by 14 = 42.9%		

Table 5

Reference-DNA	NNNNNNNNNNN	(Length = 12 nucleotides)
Comparison DNA	NNNNLLLW	(Length = 9 nucleotides)
% nucleic acid sequence identity = (the number of identically matching nucleotides between the two nucleic acid sequences as determined by ALIGN-2) divided by (the total number of nucleotides of the reference-DNA nucleic acid sequence) = 4 divided by 12 = 33.3%		

Diagnostic Method

[0087] It is further contemplated that use of therapeutic agents for IBD may be specifically targeted to disorders where the affected tissue and/or cells exhibit increased LY6 expression relative to control. Accordingly, it is contemplated that the detection of increased LY6 expression may be used to detect IBD, such as CD or UC, in the gastrointestinal tissue of a mammal and/or to identify tissues and disorders that will particularly benefit from treatment with an IBD therapeutic agent, including a chemotherapeutic agent, useful in ameliorating IBD, UC and/or CD in a human patient.

[0088] In preferred embodiments, LY6 expression levels are detected, either by direct detection of the gene transcript or by detection of protein levels or activity. Transcripts may be detected using any of a wide range of techniques that depend primarily on hybrization or probes to the LY6 mRNA transcripts, to cDNAs synthesized therefrom, or to DNA where LY6 gene amplification is present. Well known techniques include Northern blotting, reverse-transcriptase PCR and microarray analysis of transcript levels. Methods for detecting LY6 protein levels include Western blotting, immunoprecipitation, two-dimensional polyacrylatmide gel electrophoresis (2D SDS-PAGE - preferably compared against a standard wherein the position of the LY6 proteins has been determined), and mass spectroscopy. Mass spectroscopy may be coupled with a series of purification steps to allow high-throughput indentification of many different protein levels in a particular sample. Mass spectroscopy and 2D SDS-PAGE can also be used to identify post-transcriptional modifications to proteins including proteolytic events, ubiquitination, phosphorylation, lipid modification, etc. LY6 activity may also be assessed by analyzing binding to substrate DNA or in vitro transcriptional activiaton of target promoters. Gel shift assay, DNA footprinting assays and DNA-protein crosslinking assays are all methods that may be used to assess the presence of a protein capable of binding to Gli binding sites on DNA. J Mol. Med 77(6):459-68 (1999); Cell 100(4): 423-34 (2000); Development 127(19): 4923-4301 (2000).

[0089] In certain embodiments, LY6 transcript levels are measured, and diseased or disordered tissues showing significantly elevated LY6 levels relative to control are treated with an IBD therapeutic compound. Accordingly, LY6 expression levels are a powerful diagnostic measure for determining whether a patient is experiencing IBD and whether that patient should receive an IBD therapeutic agent.

Antibody Compositions for Use in the Methods of the Invention

A. Anti-LY6 Antibodies

[0090] In one embodiment, the present invention provides the use of anti-LY6 antibodies, which may find use herein as therapeutic, diagnostic and/or prognostic agents in determining the existence, severity of and/or prognosing the disease course of an inflammatory bowel disease such as UC. Exemplary antibodies that may be used for such purposes include polyclonal, monoclonal, humanized, bispecific, and heteroconjugate antibodies. The term "antibodies" sometimes also include antigen-binding fragments. Anti-LY6 antibodies are available commercially, such as for example, from R&D Systems, Minneapolis, MN. Antiobodies that bind specifically to LY6 as antigen may be obtained commercially or prepared by standard methods known in the art of antibody and protein chemistry for use in the method of the invention. Antiobodies to LYPD1 are disclosed, for example in USUS7,144,990.

1. Polyclonal Antibodies

[0091] Polyclonal antibodies are preferably raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen (especially when synthetic peptides are used) to a protein that is immunogenic in the species to be immunized. For example, the antigen can be conjugated to keyhole limpet hemocyanin (KLH), serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor, using a bifunctional or derivatizing agent, *e.g.*, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride, $SOCl_2$, or $R^1N=C=NR$, where R and R^1 are different alkyl groups.

[0092] Animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining, *e.g.*, 100 µg or 5 µg of the protein or conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later, the animals are boosted with 1/5 to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later, the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

2. Monoclonal Antibodies

[0093] Monoclonal antibodies may be made using the hybridoma method first described by Kohler et al., Nature, 256:495 (1975), or may be made by recombinant DNA methods (U.S. Patent No. 4,816,567).

[0094] In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as described above to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized *in vitro.* After immunization, lymphocytes are isolated and then fused with a myeloma cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice, pp. 59-103 (Academic Press, 1986)).

[0095] The hybridoma cells thus prepared are seeded and grown in a suitable culture medium which medium preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells (also referred to as fusion partner). For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the selective culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

[0096] Preferred fusion partner myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a selective medium that selects against the unfused parental cells. Preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California USA, and SP-2 and derivatives *e.g.,* X63-Ag8-653 cells available from the American Type Culture Collection, Manassas, Virginia, USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, J. Immunol., 133:3001 (1984); and Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

[0097] Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA).

[0098] The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis described in Munson et al., Anal. Biochem., 107:220 (1980).

[0099] Once hybridoma cells that produce antibodies of the desired specificity, affinity, and/or activity are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, Monoclonal Antibodies: Principles and Practice, pp.59-103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown *in vivo* as ascites tumors in an animal e.g" by i.p. injection of the cells into mice.

[0100] The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional antibody purification procedures such as, for example, affinity chromatography (e.g., using protein A or protein G-Sepharose) or ion-exchange chromatography, hydroxylapatite chromatography, gel electrophoresis, dialysis, *etc.*

[0101] DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce antibody protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra et al., Curr. Opinion in Immunol., 5:256-262 (1993) and Plückthun, Immunol. Revs. 130:151-188 (1992).

[0102] In a further embodiment, monoclonal antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques described in McCafferty et al., Nature, 348:552-554 (1990). Clackson et al., Nature, 352:624-628 (1991) and Marks et al., J. Mol. Biol., 222:581-597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling *(*Marks et al., Bio/Technology, 10:779-783 (1992)), as well as combinatorial infection and *in vivo* recombination as a strategy for constructing very large phage libraries *(*Waterhouse et al., Nuc. Acids. Res. 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

[0103] The DNA that encodes the antibody may be modified to produce chimeric or fusion antibody polypeptides, for example, by substituting human heavy chain and light chain constant domain (C_H and C_L) sequences for the homologous murine sequences (U.S. Patent No. 4,816,567; and Morrison, et al., Proc. Natl Acad. Sci. USA, 81:6851 (1984)), or by fusing the immunoglobulin coding sequence with all or part of the coding sequence for a non-immunoglobulin polypeptide (heterologous polypeptide). The non-immunoglobulin polypeptide sequences can substitute for the constant domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

3. <u>Human and Humanized Antibodies</u>

[0104] The anti- LY6 antibodies useful in the practice of the invention may further comprise humanized antibodies or human antibodies. Humanized forms of non-human (*e.g.*, murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin [Jones et al.,

Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol., 2:593-596 (1992)].

[0105] Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[0106] The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity and HAMA response (human anti-mouse antibody) when the antibody is intended for human therapeutic use. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable domain sequences. The human V domain sequence which is closest to that of the rodent is identified and the human framework region (FR) within it accepted for the humanized antibody (Sims et al., J. Immunol. 151:2296 (1993); Chothia et al., J. Mol. Biol., 196:901 (1987)). Another method uses a particular framework region derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter et al., Proc. Natl. Acad. Sci. USA, 89:4285 (1992); Presta et al., J. Immunol. 151:2623 (1993)).

[0107] It is further important that antibodies be humanized with retention of high binding affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, *i.e.*, the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the hypervariable region residues are directly and most substantially involved in influencing antigen binding.

[0108] Various forms of a humanized anti-LY6 antibody antibodies are contemplated. For example, the humanized antibody may be an antibody fragment, such as a Fab, which is optionally conjugated with one or more cytotoxic agent(s) in order to generate an immunoconjugate. Alternatively, the humanized antibody may be an intact antibody, such as an intact IgG1 antibody.

[0109] As an alternative to humanization, human antibodies can be generated. For example, it is now possible to produce transgenic animals (*e.g.*, mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array into such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, *e.g.*, Jakobovits et al., Proc. Natl. Acad. Sci. USA, 90:2551 (1993); Jakobovits et al., Nature, 362:255-258 (1993); Bruggemann et al., Year in Immuno. 7:33 (1993); U.S. Patent Nos. 5,545,806, 5,569,825, 5,591,669 (all of GenPharm); 5,545,807; and WO 97/17852.

[0110] Alternatively, phage display technology (McCafferty et al., Nature 348:552-553 (1990]) can be used to produce human antibodies and antibody fragments *in vitro,* from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B-cell. Phage display can be performed in a variety of formats, reviewed in, *e.g.*, Johnson, Kevin S. and Chiswell, David J., Current Opinion in Structural Biology 3:564-571 (1993). Several sources of V-gene segments can be used for phage display. Clackson et al., Nature, 352:624-628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V

genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks et al., J. Mol. Biol. 222:581-597 (1991), or Griffith et al., EMBO J. 12:725-734 (1993). See, also, U.S. Patent Nos. 5,565,332 and 5,573,905.

[0111] As discussed above, human antibodies may also be generated by *in vitro* activated B cells (see U.S. Patents 5,567,610 and 5,229,275).

4. Antibody fragments

[0112] In certain circumstances there are advantages of using antibody fragments, rather than whole antibodies. The smaller size of the fragments allows for rapid clearance, while retaining similar antigen binding specificity of the corresponding full length molecule, and may lead to improved access to solid tumors.

[0113] Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, *e.g.*, Morimoto et al., Journal of Biochemical and Biophysical Methods 24:107-117 (1992); and Brennan et al., Science, 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. Fab, Fv and scFv antibody fragments can all be expressed in and secreted from *E. coli,* thus allowing the facile production of large amounts of these fragments. Antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form $F(ab')_2$ fragments (Carter et al., Bio/Technology 10:163-167 (1992)). According to another approach, $F(ab')_2$ fragments can be isolated directly from recombinant host cell culture. Fab and $F(ab')_2$ fragment with increased in vivo half-life comprising a salvage receptor binding epitope residues are described in U.S. Patent No. 5,869,046. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv). See WO 93/16185; U.S. Patent No. 5,571,894; and U.S. Patent No. 5,587,458. Fv and sFv are the only species with intact combining sites that are devoid of constant regions; thus, they are suitable for reduced nonspecific binding during in vivo use. sFv fusion proteins may be constructed to yield fusion of an effector protein at either the amino or the carboxy terminus of an sFv. See *Antibody Engineering,* ed. Borrebaeck, supra. The antibody fragment may also be a "linear antibody", *e.g.*, as described in U.S. Patent 5,641,870 for example. Such linear antibody fragments may be monospecific or bispecific.

5. Bispecific Antibodies

[0114] Bispecific antibodies are antibodies that have binding specificities for at least two different epitopes. Exemplary bispecific antibodies may bind separate antigens or bind to two different epitopes of a particular LY6 polypeptide described herein. Other such antibodies may combine the above LY6 binding site with a binding site for another protein. Where the bispecific antibody is useful in the diagnostic method of the invention, the second antibody arm may bind a detectable polypeptide. Bispecific antibodies can be prepared as full length antibodies or antibody fragments (*e.g.*, $F(ab')_2$ bispecific antibodies).

[0115] Methods for making bispecific antibodies are known in the art. Traditional production of full length bispecific antibodies is based on the co-expression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Millstein et al., Nature 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, and in Traunecker et al., EMBO J. 10:3655-3659 (1991).

[0116] According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. Preferably, the fusion is with an Ig heavy chain constant domain, comprising at least part of the hinge, C_H2, and C_H3 regions. It is preferred to have the first heavy-chain constant region (C_H1) containing the site necessary for light chain bonding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host cell. This provides for greater flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yield of the desired bispecific antibody. It is, however, possible to insert the coding sequences for two or all three polypeptide chains into a single expression vector when the expression of at least two polypeptide chains in equal ratios

results in high yields or when the ratios have no significant affect on the yield of the desired chain combination.

[0117] In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690. For further details of generating bispecific antibodies see, for example, Suresh et al., Methods in Enzymology 121:210 (1986).

[0118] According to another approach described in U.S. Patent No. 5,731,168, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the C_H3 domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (*e.g.*, tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g., alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

[0119] Bispecific antibodies include cross-linked or "heteroconjugate" antibodies. For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360, WO 92/200373, and EP 03089). Heteroconjugate antibodies also find use in the present method of the invention by providing multiple (either different or the same) detectable markers on each antibody for improved assay detection. Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Patent No. 4,676,980, along with a number of cross-linking techniques.

[0120] Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan et al., Science 229:81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate $F(ab')_2$ fragments. These fragments are reduced in the presence of the dithiol complexing agent, sodium arsenite, to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

[0121] Recent progress has facilitated the direct recovery of Fab'-SH fragments from *E. coli,* which can be chemically coupled to form bispecific antibodies. Shalaby et al., J. Exp. Med. 175: 217-225 (1992) describe the production of a fully humanized bispecific antibody $F(ab')_2$ molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

[0122] Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny et al., J. Immunol. 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then reoxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger et al., Proc. Natl. Acad. Sci. USA 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a V_H connected to a V_L by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber et al., J. Immunol., 152:5368 (1994).

[0123] Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt et al., J. Immunol. 147:60 (1991).

6. <u>Multivalent Antibodies</u>

[0124] A multivalent antibody may be internalized (and/or catabolized) faster than a bivalent antibody by a cell expressing an antigen to which the antibodies bind. The antibodies of the present invention can be multivalent antibodies (which are other than of the IgM class) with three or more antigen binding sites (*e.g.* tetravalent antibodies), which can be readily produced by recombinant expression of nucleic acid encoding the polypeptide chains of the antibody. The multivalent antibody can comprise a dimerization domain and three or more antigen binding sites. The preferred dimerization domain comprises (or consists of) an Fc region or a hinge region. In this scenario, the antibody will comprise an Fc region and three or more antigen binding sites amino-terminal to the Fc region. The preferred multivalent antibody herein comprises (or consists of) three to about eight, but preferably four, antigen binding sites. The multivalent antibody comprises at least one polypeptide chain (and preferably two polypeptide chains), wherein the polypeptide chain(s) comprise two or more variable domains. For instance, the polypeptide chain(s) may comprise VD1-$(X1)_n$VD2-$(X2)_n$-Fc, wherein VD1 is a first variable domain, VD2 is a second variable domain, Fc is one polypeptide chain of an Fc region, X1 and X2 represent an amino acid or polypeptide, and n is 0 or 1. For instance, the polypeptide chain(s) may comprise: VH-CH1-flexible linker-VH-CH1-Fc region chain; or VH-CH1-VH-CH1-Fc region chain. The multivalent antibody herein preferably further comprises at least two (and preferably four) light chain variable domain polypeptides. The multivalent antibody herein may, for instance, comprise from about two to about eight light chain variable domain polypeptides. The light chain variable domain polypeptides contemplated here comprise a light chain variable domain and, optionally, further comprise a CL domain.

7. Effector Function Engineering

[0125] It may be desirable to modify the antibody of the invention with respect to effector function, *e.g.*, so as to enhance antigen-dependent cell-mediated cytotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC) of the antibody. This may be achieved by introducing one or more amino acid substitutions in an Fc region of the antibody. Alternatively or additionally, cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron et al., J. Exp Med. 176:1191-1195 (1992) and Shopes, B. J. Immunol. 148:2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff et al., Cancer Research 53:2560-2565 (1993). Alternatively, an antibody can be engineered which has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson et al., Anti-Cancer Drug Design 3:219-230 (1989). To increase the serum half life of the antibody, one may incorporate a salvage receptor binding epitope into the antibody (especially an antibody fragment) as described in U.S. Patent 5,739,277, for example. As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (*e.g.*, IgG_1, IgG_2, IgG_3, or IgG_4) that is responsible for increasing the *in vivo* serum half-life of the IgG molecule.

8. Immunoconjugates

[0126] The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, a growth inhibitory agent, a toxin (*e.g.*, an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope *(i.e.,* a radioconjugate) and/or a detectable label.

a. Chemotherapeutic agents

[0127] Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa),* ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include ^{212}Bi, ^{131}I ^{131}In, ^{90}Y, and ^{186}Re. Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine),

diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science, 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026.

[0128] Conjugates of an antibody and one or more small molecule toxins, such as a calicheamicin, maytansinoids, a trichothene, and CC1065, and the derivatives of these toxins that have toxin activity, are also contemplated herein.

B. LY6 Binding Oligopeptides

[0129] LY6 binding oligopeptides of the present invention are oligopeptides that bind, preferably specifically, to a LY6 polypeptide as described herein. LY6 binding oligopeptides may be chemically synthesized using known oligopeptide synthesis methodology or may be prepared and purified using recombinant technology. LY6 binding oligopeptides are usually at least about 5 amino acids in length, alternatively at least about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 amino acids in length or more, wherein such oligopeptides that are capable of binding, preferably specifically, to a LY6 polypeptide as described herein. LY6 binding oligopeptides may be identified without undue experimentation using well known techniques. In this regard, it is noted that techniques for screening oligopeptide libraries for oligopeptides that are capable of specifically binding to a polypeptide target are well known in the art (see, *e.g.,* U.S. Patent Nos. 5,556,762, 5,750,373, 4,708,871, 4,833,092, 5,223,409, 5,403,484, 5,571,689, 5,663,143; PCT Publication Nos. WO 84/03506 and WO84/03564; Geysen et al., Proc. Natl. Acad. Sci. U.S.A., 81:3998-4002 (1984); Geysen et al., Proc. Natl. Acad. Sci. U.S.A., 82:178-182 (1985); Geysen et al., in Synthetic Peptides as Antigens, 130-149 (1986); Geysen et al., J. Immunol. Meth., 102:259-274 (1987); Schoofs et al., J. Immunol., 140:611-616 (1988), Cwirla, S. E. et al. (1990) Proc. Natl. Acad. Sci. USA, 87:6378; Lowman, H.B. et al. (1991) Biochemistry, 30:10832; Clackson, T. et al. (1991) Nature, 352: 624; Marks, J. D. et al. (1991), J. Mol. Biol., 222:581; Kang, A.S. et al. (1991) Proc. Natl. Acad. Sci. USA, 88:8363, and Smith, G. P. (1991) Current Opin. Biotechnol., 2:668).

[0130] In this regard, bacteriophage (phage) display is one well known technique which allows one to screen large oligopeptide libraries to identify member(s) of those libraries which are capable of specifically binding to a polypeptide target. Phage display is a technique by which variant polypeptides are displayed as fusion proteins to the coat protein on the surface of bacteriophage particles (Scott, J.K. and Smith, G. P. (1990) Science 249: 386). The utility of phage display lies in the fact that large libraries of selectively randomized protein variants (or randomly cloned cDNAs) can be rapidly and efficiently sorted for those sequences that bind to a target molecule with high affinity. Display of peptide (Cwirla, S. E. et al. (1990) Proc. Natl. Acad. Sci. USA, 87:6378) or protein (Lowman, H.B. et al. (1991) Biochemistry, 30:10832; Clackson, T. et al. (1991) Nature, 352: 624; Marks, J. D. et al. (1991), J. Mol. Biol., 222:581; Kang, A.S. et al. (1991) Proc. Natl. Acad. Sci. USA, 88:8363) libraries on phage have been used for screening millions of polypeptides or oligopeptides for ones with specific binding properties (Smith, G. P. (1991) Current Opin. Biotechnol., 2:668). Sorting phage libraries of random mutants requires a strategy for constructing and propagating a large number of variants, a procedure for affinity purification using the target receptor, and a means of evaluating the results of binding enrichments. U.S. Patent Nos. 5,223,409, 5,403,484, 5,571,689, and 5,663,143.

[0131] Although most phage display methods have used filamentous phage, lambdoid phage display systems (WO 95/34683; U.S. 5,627,024), T4 phage display systems (Ren et al., Gene, 215: 439 (1998); Zhu et al., Cancer Research, 58(15): 3209-3214 (1998); Jiang et al., Infection & Immunity, 65(11): 4770-4777 (1997); Ren et al., Gene, 195(2):303-311 (1997); Ren, Protein Sci., 5: 1833 (1996); Efimov et al., Virus Genes, 10: 173 (1995)) and T7 phage display systems (Smith and Scott, Methods in Enzymology, 217: 228-257 (1993); U.S. 5,766,905) are also known.

[0132] Many other improvements and variations of the basic phage display concept have now been developed. These improvements enhance the ability of display systems to screen peptide libraries for binding to selected target molecules and to display functional proteins with the potential of screening these proteins for desired properties. Combinatorial reaction devices for phage display reactions have been developed (WO 98/14277) and phage display libraries have been used to analyze and control bimolecular interactions (WO 98/20169; WO 98/20159) and properties of constrained helical peptides (WO 98/20036). WO 97/35196 describes a method of isolating an affinity ligand in which a phage display library is contacted with one solution in which the ligand will bind to a target molecule and a second solution in which the affinity ligand will not bind to the target molecule, to selectively isolate binding ligands. WO 97/46251 describes a method of biopanning a random phage display library with an affinity purified antibody and then isolating binding phage, followed by a micropanning process using microplate wells to isolate high

affinity binding phage. The use of *Staphlylococcus aureus* protein A as an affinity tag has also been reported (Li et al. (1998) Mol Biotech., 9:187). WO 97/47314 describes the use of substrate subtraction libraries to distinguish enzyme specificities using a combinatorial library which may be a phage display library. A method for selecting enzymes suitable for use in detergents using phage display is described in WO 97/09446. Additional methods of selecting specific binding proteins are described in U.S. Patent Nos. 5,498,538, 5,432,018, and WO 98/15833.

[0133] Methods of generating peptide libraries and screening these libraries are also disclosed in U.S. Patent Nos. 5,723,286, 5,432,018, 5,580,717, 5,427,908, 5,498,530, 5,770,434, 5,734,018, 5,698,426, 5,763,192, and 5,723,323.

[0134] In aspect, the present invention concerns ligands for the LYPD5 polypeptide. Figure 32 demonstrates this showing untransfected COS cells (A) and COS cells transfected with GLG-1 and stained with LYPD5-Fc protein. In one embodiment, the ligand for LYPD5 is the golgi complex localized glycoprotein 1 (GLG-1) or E-selectin 1 (ESL-1) polypeptide as shown in SEQ ID NOS:18, 20, 22, or 24, encoded by the nucleic acid shown as SEQ ID NOS: 17, 19, 21, or 23, respectively. In another embodiment, the polynucleotide encoding a GLG-1 polypeptide comprises at least 15, at least 25, at least, at least 50, at least 100, at least 250, at least 500, at least 750, at least 1000, at least 1250, at least 1500, at least 1750, at least 2000, at least 2040, at least 2090, at least 2150, at least 2200, at least 2300, at least 2400, at least 2500, at least 2600, at least 2700, at least 2800, at least 2900, at least 3000, at least 3100, at least 3200, at least 3300, at least 3400, at least 3500, at least 3600, at least 3700, or at least 3720 contiguous nucleotides of SEQ ID NOs 17, 19, 21, or 23, or the polynucleotide encoding a GLG-1 comprises SEQ ID NOs 17, 19, 21, or 23. In one embodiment, a polynucleotide that binds a polynucleotide encoding a GLG-1 (SEQ ID NOs:17, 19, 21, or 23), or fragment thereof, has at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 99% or 100% sequence identity with the GLG-1 polypeptide or fragment thereof. In one embodiment, the GLG-1 polypeptide comprises at least 10, at least 25, at least 50, at least 75, at least 100, at least 125, at least 150, at least 175, at least 200, at least 225, at least 250, at least 275, at least 300, at least 325, at least 350, at least 400, at least 450, at least 500, at least 550, at least 600, at least 650, at least 700, at least 750, at least 800, at least 850, at least 900, at least 950, at least 1000, at least 1050, at least 1100, at least 1150, or at least 1200 contiguous amino acids of SEQ ID NOs:18, 20, 22, or 24, or the GLG-1 polypeptide comprises SEQ ID NOs:18, 20, 22, or 24. GLG-1 or ESL-1 is expressed on neutrophils, believe to be involved in extravasation of neutrophils into tissues, and thought to play an important role in inflammation (see Hidalgo et al. (2007) Immunity, 26(4): 477-489). GLG-1 or ESL-1 has 14 cysteine rich GLG1 domains. The extracellular domain (ECD) is lengthy and as described below, variants or fragments of the GLG-1 ECD were found to have the ability to bind LYPD5.

[0135] In another embodiment, the LYPD5 ligand is a variant or fragment a GLG-1 or ESL-1 molecule described herein. As shown in Figure 33A-B, GLG-1 or ESL-1 may be viewed as fragments 1, 2, 3, and 4 and as described in Example 11, any one of the 4 fragments are sufficient for LYPD5 binding.

[0136] In another embodiment, the LYPD5 ligand is a variant or fragment of GLG-1 or ESL-1 that is a single GLG-1 domain. As shown in Figure 34A-B, GLG-1 is made up of multiple GLG-1 domains and as described in Example 11, single GLG-1 domains are sufficient for LYPD5 binding.

[0137] In another embodiment, the LYPD5 ligand is a variant or fragment of GLG-1 or ESL-1 that is specific for LYPD5. As shown in Figure 35A-B, GLG-1 includes domains 26-114, domain 115, and domain 150 and as described in Example 11, domain 115 binds LYPD5 but domains 26-114 does not bind LYPD5.

[0138] The present invention contemplates variants of GLG-1 in the same manner it contemplates variants for LY6 family members.

C. <u>Polypeptide Variants</u>

[0139] In addition to the polypeptides, antibodies and LY6 binding polypeptides described herein, it is contemplated that variants of such molecules can be prepared for use with the invention herein. Such variants can be prepared by introducing appropriate nucleotide changes into the encoding DNA, and/or by synthesis of the desired antibody or polypeptide. Those skilled in the art will appreciate that amino acid changes may alter post-translational processes of these molecules, such as changing the number or position of glycosylation sites or altering the membrane anchoring characteristics.

[0140] Variations in amino acid sequence can be made, for example, using any of the techniques and guidelines for conservative and non-conservative mutations set forth, for instance, in U.S. Patent No. 5,364,934. Variations may be a substitution, deletion or insertion of one or more codons encoding the amino acid sequence that results in a change in the amino acid sequence as

compared with the native sequence. Optionally the variation is by substitution of at least one amino acid with any other amino acid in one or more of the domains of the amino acid sequence of interest. Guidance in determining which amino acid residue may be inserted, substituted or deleted without adversely affecting the desired activity may be found by comparing the sequence of the amino acid sequence of interest with homologous known protein molecules and minimizing the number of amino acid sequence changes made in regions of high homology. Amino acid substitutions can be the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, *i.e.*, conservative amino acid replacements. Insertions or deletions may optionally be in the range of about 1 to 5 amino acids. The variation allowed may be determined by systematically making insertions, deletions or substitutions of amino acids in the sequence and testing the resulting variants for activity exhibited by the full-length or mature native sequence.

[0141] Fragments of the various polypeptides are provided herein. Such fragments may be truncated at the N-terminus or C-terminus, or may lack internal residues, for example, when compared with a full length native antibody or protein. Such fragments which lack amino acid residues that are not essential for a desired biological activity are also useful with the disclosed methods.

[0142] The above polypeptide fragments may be prepared by any of a number of conventional techniques. Desired peptide fragments may be chemically synthesized. An alternative approach involves generating such fragments by enzymatic digestion, e.g., by treating the protein with an enzyme known to cleave proteins at sites defined by particular amino acid residues, or by digesting the DNA with suitable restriction enzymes and isolating the desired fragment. Yet another suitable technique involves isolating and amplifying a DNA fragment encoding the desired fragment fragment by polymerase chain reaction (PCR). Oligonucleotides that define the desired termini of the DNA fragment are employed at the 5' and 3' primers in the PCR. Preferably, such fragments share at least one biological and/or immunological activity with the corresponding full length molecule.

[0143] In particular embodiments, conservative substitutions of interest are shown in Table 6 under the heading of preferred substitutions. If such substitutions result in a change in biological activity, then more substantial changes, denominated exemplary substitutions in Table 6, or as further described below in reference to amino acid classes, are introduced and the products screened in order to identify the desired variant.

Table 6

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp; Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser, Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp, Gln	Asp
Gly (G)	Pro; Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Leu
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

[0144] Substantial modifications in function or immunological identity of the LY6 polypeptide are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the

substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

1. (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;
2. (2) neutral hydrophilic: Cys, Ser, Thr; Asn; Gln
3. (3) acidic: Asp, Glu;
4. (4) basic: His, Lys, Arg;
5. (5) residues that influence chain orientation: Gly, Pro; and
6. (6) aromatic: Trp, Tyr, Phe.

[0145] Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Such substituted residues also may be introduced into the conservative substitution sites or, more preferably, into the remaining (non-conserved) sites.

[0146] The variations can be made using methods known in the art such as oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR mutagenesis. Site-directed mutagenesis (Carter et al., Nucl. Acids Res., 13:4331 (1986); Zoller et al., Nucl. Acids Res., 10:6487 (1987)), cassette mutagenesis (Wells et al., Gene, 34:315 (1985)), restriction selection mutagenesis (Wells et al., Philos. Trans. R. Soc. London SerA, 317:415 (1986)) or other known techniques can be performed on the cloned DNA to produce the anti-LY6 molecule.

[0147] Scanning amino acid analysis can also be employed to identify one or more amino acids along a contiguous sequence. Among the preferred scanning amino acids are relatively small, neutral amino acids. Such amino acids include alanine, glycine, serine, and cysteine. Alanine is typically a preferred scanning amino acid among this group because it eliminates the side-chain beyond the beta-carbon and is less likely to alter the main-chain conformation of the variant (Cunningham and Wells, Science, 244:1081-1085 (1989)). Alanine is also typically preferred because it is the most common amino acid. Further, it is frequently found in both buried and exposed positions (Creighton, The Proteins, (W.H. Freeman & Co., N.Y.); Chothia, J. Mol. Biol., 150:1 (1976)). If alanine substitution does not yield adequate amounts of variant, an isoteric amino acid can be used.

[0148] Any cysteine residue not involved in maintaining the proper conformation of the LY6 polypeptide also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) may be added to such a molecule to improve its stability (particularly where the antibody is an antibody fragment such as an Fv fragment).

[0149] A particularly preferred type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g., a humanized or human antibody). Generally, the resulting variant(s) selected for further development will have improved biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Briefly, several hypervariable region sites (*e.g.*, 6-7 sites) are mutated to generate all possible amino substitutions at each site. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (e.g., binding affinity) as herein disclosed. In order to identify candidate hypervariable region sites for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and target polypeptide. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

[0150] Nucleic acid molecules encoding amino acid sequence variants of LY6 polypeptides are prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of a native sequence or an earlier prepared variant.

D. <u>Modifications of Polypeptides</u>

[0151] Polypeptides and/or antibodies that have been covalently modified may also be suitable for use within the scope of this

invention. One type of covalent modification includes reacting targeted amino acid residues of such antibodies and polypeptides with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C- terminal residues of such antibodies and polypeptides. Derivatization with bifunctional agents is useful, for instance, for crosslinking the preceding molecules to a water-insoluble support matrix or surface for use in purification. Commonly used crosslinking agents include, *e.g.*, 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), bifunctional maleimides such as bis-N-maleimido-1,8-octane and agents such as methyl-3-[(p-azidophenyl)dithio]propioimidate.

[0152] Other modifications include deamidation of glutaminyl and asparaginyl residues to the corresponding glutamyl and aspartyl residues, respectively, hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α-amino groups of lysine, arginine, and histidine side chains (T.E. Creighton, Proteins: Structure and Molecular Properties, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)), acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

[0153] Another type of covalent modification of the polypeptides or antibodies comprises altering the native glycosylation pattern of the antibody or polypeptide. "Altering the native glycosylation pattern" is intended for purposes herein to mean deleting one or more carbohydrate moieties found in native sequence (either by removing the underlying glycosylation site or by deleting the glycosylation by chemical and/or enzymatic means), and/or adding one or more glycosylation sites that are not present in the respective native sequence. In addition, the phrase includes qualitative changes in the glycosylation of the native proteins, involving a change in the nature and proportions of the various carbohydrate moieties present.

[0154] Glycosylation of antibodies and other polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-aceylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

[0155] Addition of glycosylation sites may be accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original such antibody or polypeptide (for O-linked glycosylation sites). Such antibody or polypeptide sequence may optionally be altered through changes at the DNA level, particularly by mutating the DNA encoding the preceding amino acid sequences at preselected bases such that codons are generated that will translate into the desired amino acids.

[0156] Another means of increasing the number of carbohydrate moieties is by chemical or enzymatic coupling of glycosides to the polypeptide. Such methods are described in the art, e.g., in WO 87/05330 published 11 September 1987, and in Aplin and Wriston, CRC Crit. Rev. Biochem., pp. 259-306 (1981).

[0157] Removal of carbohydrate moieties may be accomplished chemically or enzymatically or by mutational substitution of codons encoding for amino acid residues that serve as targets for glycosylation. Chemical deglycosylation techniques are known in the art and described, for instance, by Hakimuddin, et al., Arch. Biochem. Biophys., 259:52 (1987) and by Edge et al., Anal. Biochem., 118:131 (1981). Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo-and exo-glycosidases as described by Thotakura et al., Meth. Enzymol., 138:350 (1987).

[0158] Another type of covalent modification comprises linking to one of a variety of nonproteinaceous polymers, *e.g.*, polyethylene glycol (PEG), polypropylene glycol, or polyoxyalkylenes, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337. The LY6 polypeptide may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, 16th edition, Oslo, A., Ed., (1980).

[0159] Modifications forming chimeric molecules results from fusions of one polypeptide to another, heterologous polypeptide or amino acid sequence are contemplated for use with the present methods.

[0160] In one embodiment, such a chimeric molecule comprises a fusion of a polypeptide with a tag polypeptide which provides

an epitope to which an anti-tag antibody can selectively bind. The epitope tag is generally placed at the amino- or carboxyl-terminus of such antibody or polypeptide. The presence of such epitope-tagged forms of such antibodies or polypeptides can be detected using an antibody against the tag polypeptide. Also, provision of the epitope tag enables such antibodies or polypeptide to be readily purified by affinity purification using an anti-tag antibody or another type of affinity matrix that binds to the epitope tag. Various tag polypeptides and their respective antibodies are well known in the art. Examples include poly-histidine (poly-his) or poly-histidine-glycine (poly-his-gly) tags; the flu HA tag polypeptide and its antibody 12CA5 (Field et al., Mol. Cell. Biol., 8:2159-2165 (1988)); the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto (Evan et al., Molecular and Cellular Biology, 5:3610-3616 (1985)); and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody (Paborsky et al., Protein Engineering, 3(6):547-553 (1990)). Other tag polypeptides include the Flag-peptide (Hopp et al., BioTechnology, 6:1204-1210 (1988)); the KT3 epitope peptide (Martin et al., Science, 255:192-194 (1992)); an α-tubulin epitope peptide (Skinner et al., J. Biol. Chem., 266:15163-15166 (1991)); and the T7 gene 10 protein peptide tag (Lutz-Freyermuth et al., Proc. Natl. Acad. Sci. USA, 87:6393-6397 (1990)).

[0161] In an alternative embodiment, the chimeric molecule may comprise a fusion of a polypeptide with an immunoglobulin or a particular region of an immunoglobulin. For a bivalent form of the chimeric molecule (also referred to as an "immunoadhesin"), such a fusion could be to the Fc region of an IgG molecule. The Ig fusions preferably include the substitution of a soluble (transmembrane domain deleted or inactivated) form of a preceding antibody or polypeptide in the place of at least one variable region within an Ig molecule. In a particularly preferred embodiment, the immunoglobulin fusion includes the hinge, CH_2 and CH_3, or the hinge, CH_1, CH_2 and CH_3 regions of an IgG1 molecule. For the production of immunoglobulin fusions see also US Patent No. 5,428,130 issued June 27, 1995.

E. Preparation of Polypeptides

[0162] The description below relates primarily to production of polypeptides by culturing cells transformed or transfected with a vector containing nucleic acid such antibodies, polypeptides and oligopeptides. The term "polypeptides" may include antibodies, polypeptides and oligopeptides. It is, of course, contemplated that alternative methods, which are well known in the art, may be employed to prepare such antibodies, polypeptides and oligopeptides. For instance, the appropriate amino acid sequence, or portions thereof, may be produced by direct peptide synthesis using solid-phase techniques [see, *e.g.*, Stewart et al., Solid-Phase Peptide Synthesis, W.H. Freeman Co., San Francisco, CA (1969); Merrifield, J. Am. Chem. Soc., 85:2149-2154 (1963)]. *In vitro* protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be accomplished, for instance, using an Applied Biosystems Peptide Synthesizer (Foster City, CA) using manufacturer's instructions. Various portions of such antibodies, polypeptides or oligopeptides may be chemically synthesized separately and combined using chemical or enzymatic methods to produce the desired product.

1. Isolation of DNA Encoding a Polypeptide

[0163] DNA encoding a polypeptide may be obtained from a cDNA library prepared from tissue believed to possess such antibody, polypeptide or oligopeptide mRNA and to express it at a detectable level. Accordingly, DNA encoding such polypeptides can be conveniently obtained from a cDNA library prepared from human tissue, a genomic library or by known synthetic procedures (*e.g.*, automated nucleic acid synthesis).

[0164] Libraries can be screened with probes (such as oligonucleotides of at least about 20-80 bases) designed to identify the gene of interest or the protein encoded by it. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures, such as described in Sambrook et al., Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Laboratory Press, 1989). Alternatively, PCR methodology may be used. [Sambrook *et al.,* supra; Dieffenbach et al., PCR Primer: A Laboratory Manual (Cold Spring Harbor Laboratory Press, 1995)].

[0165] Techniques for screening a cDNA library are well known in the art. The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized. The oligonucleotide is preferably labeled such that it can be detected upon hybridization to DNA in the library being screened. Methods of labeling are well known in the art, and include the use of radiolabels like ^{32}P-labeled ATP, biotinylation or enzyme labeling. Hybridization conditions, including moderate stringency and high stringency, are provided in Sambrook *et al.,* supra.

[0166] Sequences identified in such library screening methods can be compared and aligned to other known sequences

deposited and available in public databases such as GenBank or other private sequence databases. Sequence identity (at either the amino acid or nucleotide level) within defined regions of the molecule or across the full-length sequence can be determined using methods known in the art and as described herein.

[0167] Nucleic acid having protein coding sequence may be obtained by screening selected cDNA or genomic libraries using the deduced amino acid sequence disclosed herein for the first time, and, if necessary, using conventional primer extension procedures as described in *Sambrook et al.,* supra, to detect precursors and processing intermediates of mRNA that may not have been reverse-transcribed into cDNA.

2. Selection and Transformation of Host Cells

[0168] Host cells are transfected or transformed with expression or cloning vectors described herein for LY6 polypeptide production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences. The culture conditions, such as media, temperature, pH and the like, can be selected by the skilled artisan without undue experimentation. In general, principles, protocols, and practical techniques for maximizing the productivity of cell cultures can be found in Mammalian Cell Biotechnology: A Practical Approach, M. Butler, ed. (IRL Press, 1991) and Sambrook *et al.,* supra.

[0169] Methods of eukaryotic cell transfection and prokaryotic cell transformation are known to the ordinarily skilled artisan, for example, $CaCl_2$, $CaPO_4$, liposome-mediated and electroporation. Depending on the host cell used, transformation is performed using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in Sambrook *et al.,* supra, or electroporation is generally used for prokaryotes. Infection with *Agrobacterium tumefaciens* is used for transformation of certain plant cells, as described by Shaw et al., Gene, 23:315 (1983) and WO 89/05859 published 29 June 1989. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, Virology, 52:456-457 (1978) can be employed. General aspects of mammalian cell host system transfections have been described in U.S. Patent No. 4,399,216. Transformations into yeast are typically carried out according to the method of Van Solingen et al., J. Bact., 130:946 (1977) and Hsiao et al., Proc. Natl. Acad. Sci. (USA), 76:3829 (1979). However, other methods for introducing DNA into cells, such as by nuclear microinjection, electroporation, bacterial protoplast fusion with intact cells, or polycations, *e.g.*, polybrene, polyornithine, may also be used. For various techniques for transforming mammalian cells, see Keown et al., Methods in Enzymology, 185:527-537 (1990) and Mansour et al., Nature, 336:348-352 (1988).

[0170] Suitable host cells for cloning or expressing the DNA in the vectors herein include prokaryote, yeast, or higher eukaryote cells. Suitable prokaryotes include but are not limited to eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *E. coli.* Various *E. coli* strains are publicly available, such as *E. coli* K12 strain MM294 (ATCC 31,446); *E. coli* X1776 (ATCC 31,537); *E. coli* strain W3110 (ATCC 27,325) and K5 772 (ATCC 53,635). Other suitable prokaryotic host cells include Enterobacteriaceae such as *Escherichia, e.g., E. coli, Enterobacter, Erwinia, Klebsiella, Proteus, Salmonella, e.g., Salmonella typhimurium, Serratia, e.g., Serratia marcescans,* and *Shigella,* as well as *Bacilli* such as *B. subtilis* and *B. licheniformis* (*e.g., B. licheniformis* 41P disclosed in DD 266,710 published 12 April 1989), *Pseudomonas* such as *P. aeruginosa,* and *Streptomyces.* These examples are illustrative rather than limiting. Strain W3110 is one particularly preferred host or parent host because it is a common host strain for recombinant DNA product fermentations. Preferably, the host cell secretes minimal amounts of proteolytic enzymes. For example, strain W3110 may be modified to effect a genetic mutation in the genes encoding proteins endogenous to the host, with examples of such hosts including *E. coli* W3110 strain 1A2, which has the complete genotype *tonA ; E. coli* W3110 strain 9E4, which has the complete genotype *tonA ptr3; E. coli* W3110 strain 27C7 (ATCC 55,244), which has the complete genotype *tonA ptr3 phoA E15 (argF-lac)169 degP ompT kan*^{r}*; E. coli* W3110 strain 37D6, which has the complete genotype *tonA ptr3 phoA E15 (argF-lac)169 degP ompT rbs7 ilvG kan"; E. coli* W3110 strain 40B4, which is strain 37D6 with a non-kanamycin resistant *degP* deletion mutation; and an *E. coli* strain having mutant periplasmic protease disclosed in U.S. Patent No. 4,946,783 issued 7 August 1990. Alternatively, *in vitro* methods of cloning, *e.g.,* PCR or other nucleic acid polymerase reactions, are suitable.

[0171] Full length antibody, antibody fragments, and antibody fusion proteins can be produced in bacteria, in particular when glycosylation and Fc effector function are not needed, such as when the therapeutic antibody is conjugated to a cytotoxic agent (*e.g.,* a toxin) and the immunoconjugate by itself shows effectiveness in tumor cell destruction. Full length antibodies have greater half life in circulation. Production in E. coli is faster and more cost efficient. For expression of antibody fragments and polypeptides in bacteria, see, *e.g.,* U.S. 5,648,237 (Carter et. al.), U.S. 5,789,199 (Joly et al.), and U.S. 5,840,523 (Simmons et al.) which describes translation initiation region (TIR) and signal sequences for optimizing expression and secretion. After expression, the antibody is isolated from the *E. coli* cell paste in a soluble fraction and can be purified through, *e.g.*, a protein A or

G column depending on the isotype. Final purification can be carried out similar to the process for purifying antibody expressed in suitable cells (*e.g.*, CHO cells).

[0172] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for vectors encoding desired polypeptides. *Saccharomyces cerevisiae* is a commonly used lower eukaryotic host microorganism. Others include *Schizosaccharomyces pombe* (Beach and Nurse, Nature, 290: 140 [1981]; EP 139,383 published 2 May 1985); *Kluyveromyces* hosts (U.S. Patent No. 4,943,529; Fleer et al., Bio/Technology, 9:968-975 (1991)) such as, *e.g., K. lactis* (MW98-8C, CBS683, CBS4574; Louvencourt et al., J. Bacteriol., 154(2):737-742 [1983]), *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickeramii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilarum* (ATCC 36,906; Van den Berg et al., Bio/Technology, 8:135 (1990)), *K. thermotolerans,* and *K. marxianus; yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070; Sreekrishna et al., J. Basic Microbiol., 28:265-278 [1988]); *Candida; Trichoderma reesia* (EP 244,234); *Neurospora crassa* (Case et al., Proc. Natl. Acad. Sci. USA, 76:5259-5263 [1979]); *Schwanniomyces* such as *Schwanniomyces occidentalis* (EP 394,538 published 31 October 1990); and filamentous fungi such as, *e.g., Neurospora, Penicillium, Tolypocladium* (WO 91/00357 published 10 January 1991), and *Aspergillus* hosts such as *A. nidulans (*Ballance et al., Biochem. Biophys. Res. Commun., 112:284-289 [1983]; Tilburn et al., Gene, 26:205-221 [1983]; Yelton et al., Proc. Natl. Acad. Sci. USA, 81: 1470-1474 [1984]) and *A. niger* (Kelly and Hynes, EMBO J., 4:475-479 [1985]). Methylotropic yeasts are suitable herein and include, but are not limited to, yeast capable of growth on methanol selected from the genera consisting *of Hansenula, Candida, Kloeckera, Pichia, Saccharomyces, Torulopsis,* and *Rhodotorula*. A list of specific species that are exemplary of this class of yeasts may be found in C. Anthony, The Biochemistry of Methylotrophs, 269 (1982).

[0173] Suitable host cells for the expression of glycosylated polypeptide production are derived from multicellular organisms. Examples of invertebrate cells include insect cells such as Drosophila S2 and Spodoptera Sf9, as well as plant cells, such as cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* have been identified. A variety of viral strains for transfection are publicly available, *e.g.*, the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells.

[0174] However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., J. Gen Virol. 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub et al., Proc. Natl. Acad. Sci. USA 77:4216 (1980)); mouse sertoli cells (TM4, Mather, Biol. Reprod. 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., Annals N.Y. Acad. Sci. 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

[0175] Host cells are transformed with the above-described expression or cloning vectors for desired polypeptide production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

3. <u>Selection and Use of a Replicable Vector</u>

[0176] The nucleic acid (*e.g.,* cDNA or genomic DNA) encoding the respective LY6 polypeptide may be inserted into a replicable vector for cloning (amplification of the DNA) or for expression. Various vectors are publicly available. The vector may, for example, be in the form of a plasmid, cosmid, viral particle, or phage. The appropriate nucleic acid sequence may be inserted into the vector by a variety of procedures. In general, DNA is inserted into an appropriate restriction endonuclease site(s) using techniques known in the art. Vector components generally include, but are not limited to, one or more of a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence. Construction of suitable vectors containing one or more of these components employs standard ligation techniques which are known to the skilled artisan.

[0177] The desired polypeptide may be produced recombinantly not only directly, but also as a fusion polypeptide with a

heterologous polypeptide, which may be a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. In general, the signal sequence may be a component of the vector, or it may be a part of the DNA encoding the mature sequence that is inserted into the vector. The signal sequence may be a prokaryotic signal sequence selected, for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the signal sequence may be, e.g., the yeast invertase leader, alpha factor leader (including *Saccharomyces* and *Kluyveromyces* a-factor leaders, the latter described in U.S. Patent No. 5,010,182), or acid phosphatase leader, the *C albicans* glucoamylase leader (EP 362,179 published 4 April 1990), or the signal described in WO 90/13646 published 15 November 1990. In mammalian cell expression, mammalian signal sequences may be used to direct secretion of the protein, such as signal sequences from secreted polypeptides of the same or related species, as well as viral secretory leaders.

[0178] Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-negative bacteria, the 2μ plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells.

[0179] Expression and cloning vectors will typically contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, *e.g.*, ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical nutrients not available from complex media, *e.g.*, the gene encoding D-alanine racemase for *Bacilli.*

[0180] An example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up nucleic acid encoding the desire protein, such as DHFR or thymidine kinase. An appropriate host cell when wild-type DHFR is employed is the CHO cell line deficient in DHFR activity, prepared and propagated as described by Urlaub et al., Proc. Natl. Acad. Sci. USA, 77:4216 (1980). A suitable selection gene for use in yeast is the *trp*1 gene present in the yeast plasmid YRp7 *[*Stinchcomb et al., Nature, 282:39 (1979); Kingsman et al., Gene, 7:141 (1979); Tschemper et al., Gene, 10:157 (1980)]. The *trp*1 gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1 [Jones, Genetics, 85:12 (1977)].

[0181] Expression and cloning vectors usually contain a promoter operably linked to the nucleic acid sequence encoding the desired amino acid sequence, in order to direct mRNA synthesis. Promoters recognized by a variety of potential host cells are well known. Promoters suitable for use with prokaryotic hosts include the β-lactamase and lactose promoter systems [Chang et al., Nature, 275:615 (1978); Goeddel et al., Nature, 281:544 (1979)], alkaline phosphatase, a tryptophan (trp) promoter system [Goeddel, Nucleic Acids Res., 8:4057 (1980); EP 36,776], and hybrid promoters such as the tac promoter [deBoer et al., Proc. Natl. Acad. Sci. USA, 80:21-25 (1983)]. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding the desired protein sequence.

[0182] Examples of suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase [Hitzeman et al., J. Biol. Chem., 255:2073 (1980)] or other glycolytic enzymes [Hess et al., J. Adv. Enzyme Reg., 7:149 (1968); Holland, Biochemistry, 17:4900 (1978)], such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

[0183] Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in yeast expression are further described in EP 73,657.

[0184] DNA Transcription in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus (UK 2,211,504 published 5 July 1989), adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and Simian Virus 40 (SV40), from heterologous mammalian promoters, *e.g.*, the actin promoter or an immunoglobulin promoter, and from heat-shock promoters, provided such promoters are compatible with the host cell systems.

[0185] Transcription of a DNA encoding the desired polypeptide may be increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp, that act on a promoter to increase its transcription. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the

late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. The enhancer may be spliced into the vector at a position 5' or 3' to the coding sequence of the preceding amino acid sequences, but is preferably located at a site 5' from the promoter.

[0186] Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding the respective antibody, polypeptide or oligopeptide described in this section.

[0187] Still other methods, vectors, and host cells suitable for adaptation to the synthesis of the respective antibody, polypeptide or oligopeptide in recombinant vertebrate cell culture are described in Gething et al., Nature, 293:620-625 (1981); Mantei et al., Nature, 281:40-46 (1979); EP 117,060; and EP 117,058.

4. Culturing the Host Cells

[0188] The host cells used to produce the LY6 polypeptide may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham et al., Meth. Enz. 58:44 (1979), Barnes et al., Anal. Biochem. 102:255 (1980), U.S. Pat. Nos. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Patent Re. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and thymidine), antibiotics (such as GENTAMYCINJ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

5. Detecting Gene Amplification/Expression

[0189] 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 may 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.

[0190] Gene expression, alternatively, may be measured by immunological methods, such as immunohistochemical staining of cells or tissue sections and assay of cell culture or body fluids, to quantitate directly the expression of gene product. Antibodies useful for immunohistochemical staining and/or assay of sample fluids may be either monoclonal or polyclonal, and may be prepared in any mammal. Conveniently, the antibodies suitable for the present method may be prepared against a native sequence polypeptide or oligopeptide, or against exogenous sequence fused to DNA and encoding a specific antibody epitope of such a polypeptide or oligopeptide.

6. Protein Purification

[0191] Polypeptides may be recovered from culture medium or from host cell lysates. If membrane-bound, it can be released from the membrane using a suitable detergent solution (*e.g.* Triton-X 100) or by enzymatic cleavage. Cells employed in expression of the preceding can be disrupted by various physical or chemical means, such as freeze-thaw cycling, sonication, mechanical disruption, or cell lysing agents.

[0192] It may be desireable to purify the preceding from recombinant cell proteins or polypeptides. The following procedures are exemplary of suitable purification procedures: by fractionation on an ion-exchange column; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; protein A Sepharose columns to remove contaminants such as IgG; and metal chelating columns to bind epitope-tagged forms of the desired molecules. Various methods of protein purification may be employed and such methods are known in the art and described for example in Deutscher, Methods in Enzymology, 182 (1990); Scopes, Protein Purification: Principles and Practice, Springer-Verlag, New York (1982). The purification step(s) selected will depend, for example, on the nature of the production process used and the particular antibody, polypeptide or oligopeptide produced for the claimed methods.

[0193] When using recombinant techniques, the LY6 polypeptide can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If such molecules are produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, are removed, for example, by centrifugation or ultrafiltration. Carter et al., Bio/Technology 10:163-167 (1992) describe a procedure for isolating antibodies which are secreted to the periplasmic space of *E. coli.* Briefly, cell paste is thawed in the presence of sodium acetate (pH 3.5), EDTA, and phenylmethylsulfonylfluoride (PMSF) over about 30 min. Cell debris can be removed by centrifugation. Where the antibody is secreted into the medium, supernatants from such expression systems are generally first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants.

[0194] Purification can occur using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being the preferred purification technique. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc domain that is present in the antibody. Protein A can be used to purify antibodies that are based on human γ1, γ2 or γ4 heavy chains *(*Lindmark et al., J. Immunol. Meth. 62:1-13 (1983)). Protein G is recommended for all mouse isotypes and for human γ3 (Guss et al., EMBO J. 5:15671575 (1986)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a C_H3 domain, the Bakerbond ABXJresin (J. T. Baker, Phillipsburg, NJ) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSEJ chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

[0195] Following any preliminary purification step(s), the mixture comprising the antibody of interest and contaminants may be subjected to low pH hydrophobic interaction chromatography using an elution buffer at a pH between about 2.5-4.5, preferably performed at low salt concentrations (*e.g.*, from about 0-0.25M salt).

H. Pharmaceutical Formulations

[0196] Therapeutic formulations ("therapeutic agent") used in accordance with the present invention may be prepared for storage by mixing the therapeutic agent(s) having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington: The Science of Practice of Pharmacy, 20th edition, Gennaro, A. et al., Ed., Philadelphia College of Pharmacy and Science (2000)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as acetate, Tris, phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; tonicifiers such as trehalose and sodium chloride; sugars such as sucrose, mannitol, trehalose or sorbitol; surfactant such as polysorbate; salt-forming counter-ions such as sodium; metal complexes (*e.g.*, Zn-protein complexes); and/or non-ionic surfactants such as TWEEN7, PLURONICS$_7$ or polyethylene glycol (PEG). The antibody preferably comprises the antibody at a concentration of between 5-200 mg/ml, preferably between 10-100 mg/ml.

[0197] The formulations herein may also contain more than one active compound as necessary for the particular indication being

treated, preferably those with complementary activities that do not adversely affect each other. For example, in addition to the preceding therapeutic agent(s), it may be desirable to include in the formulation, an additional antibody, e.g., a second such therapeutic agent, or an antibody to some other target such as a growth factor that affects the growth of the glioma. Alternatively, or additionally, the composition may further comprise a chemotherapeutic agent, cytotoxic agent, cytokine, growth inhibitory agent, anti-hormonal agent, and/or cardioprotectant. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[0198] The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington: The Science and Practice of Pharmacy, *supra*.

[0199] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT$_7$ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid.

[0200] The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Methods for the Diagnosis and/or Treatment of Inflammatory Bowel Disease

[0201] To determine LY6 expression in gastrointestinal tissue or cells of a mammal, such as a mammal experiencing IBD, various diagnostic assays are available. In one embodiment, LY6 polypeptide overexpression may be analyzed by RT-PCR, in-situ hybridization, microarray analysis, and/or immunohistochemistry (IHC). Fresh, frozen and/or parafin embedded tissue sections from a gastrointestinal biopsy (such as from the colon or, more specifically, the sigmoid colon) from a mammal (such as without limitation a human) may be subjected to the RT-PCR, in situ hybridization, microarray analysis and/or IHC assay.

[0202] Alternatively, or additionally, FISH assays such as the INFORM7 (sold by Ventana, Arizona) or PATHVISION $_7$ (Vysis, Illinois) may be carried out on formalin-fixed, paraffin-embedded tissue to determine the extent (if any) of LY6 expression and/or upregulation in a tissue sample or biopsy.

[0203] LY6 expression may be evaluated using an *in vivo* diagnostic assay, *e.g.*, by administering a molecule (such as an antibody, oligopeptide or organic molecule) which binds the molecule to be detected and is tagged with a detectable label (*e.g.*, a radioactive isotope or a fluorescent label) and externally scanning the patient for localization of the label.

[0204] Currently, depending on the stage of the IBD, treatment involves one or a combination of the following therapies: surgery to remove affected bowel tissue, administration of therapeutic agents, including without limitation chemotherapy; dietary changes, and lifestyle management. Therapeutic agents or chemotherapeutic agents useful in the treatment of IBD are known in the art and representative therapeutic and chemotherapeutic agents are disclosed herein.

[0205] In particular, combination therapy with palictaxel and modified derivatives (see, *e.g.*, EP0600517) is contemplated. The preceding antibody, polypeptide, oligopeptide or organic molecule will be administered with a therapeutically effective dose of the chemotherapeutic agent. In another embodiment, such antibody, polypeptide, oligopeptide or organic molecule is administered in conjunction with chemotherapy to enhance the activity and efficacy of the chemotherapeutic agent, *e.g.*, paclitaxel. The Physicians= Desk Reference (PDR) discloses dosages of these agents that have been used in treatment of various cancers. The dosing regimen and dosages of these aforementioned chemotherapeutic drugs that are therapeutically effective will depend on the particular cancer being treated, the extent of the disease and other factors familiar to the physician of skill in the art and can be determined by the physician.

[0206] Therapeutic agents or chemotherapeutic agents are administered to a human patient, in accord with known methods, such as intravenous administration, *e.g.*, as a bolus or by continuous infusion over a period of time, by intracranial,

intracerobrospinal, intra-articular, intrathecal, intravenous, intraarterial, subcutaneous, oral, topical, or inhalation routes.

[0207] The present invention provides methods that involve a diagnostic step and a therapeutic treatment step. In one embodiment, the present invention provides methods of detecting inflammatory bowel disease (IBD) in a mammalian subject that include the steps of (1) detecting the level of expression of a nucleic acid or a gene encoding a LY6 polypeptide (a) in a test sample of tissue or cells obtained from the subject, and (b) in a control sample where a higher level of expression of the LY6 nucleic acid or gene in the test sample, as compared to the control sample, indicates the presence of an IBD in the subject from which the test sample was obtained; and (2) administering to the subject an effective amount of an IBD therapeutic agent. In one embodiment, the IBD therapeutic agent is an antagonist of another IBD-associated molecule. The present invention contemplates various IBD-associated molecules that are differentially expressed in IBD. In one embodiment, the IBD-associated molecule is a molecule that is differentially expressed in an IBD. In another embodiment, the IBD-associated molecule is over-expressed in an IBD. In yet another embodiment, the over-expressed IBD-associate molecule is an integrin. In one other embodiment, the IBD-associated molecule is integrin, beta 7 (ITGB2) (see WO 2006/026759) The term "IBD therapeutic agent" as used herein refers to an antagonist of an IBD-associated molecule. In one embodiment, the IBD therapeutic agent is an antagonist of an integrin. In another embodiment, the IBD therapeutic agent is an antagonist of ITGB7. In yet another embodiment, the IBD therapeutic agent is an antagonist of the polypeptide shown as SEQ ID NO: 69 encoded by the nucleic acid sequence shown as SEQ ID NO: 68.

J. Articles of Manufacture and Kits

[0208] For diagnostic applications, the article of manufacture comprises a container and a label or package insert on or associated with the container indicating a use for detecting and expression of LY6 (such as, without limitation LY6, LYPD1, LYPD3, and/or LYPD5) in a gastrointestinal tissue or cell of a mammal. In one embodiement, the mammal is a human. In one embodiment, the tissue or cell is gastrointestinal tissue or cell. In one embodiment, detecting includes quantitation relative to a contro sample. In an embodiment, the container, label or package insert indicates that the gastrointestinal tissue or cells are from colon of a mammal. In an embodiment, the container, label or package insert indicates that increased LY6 expression relative to a control sample is indicative of IBD, including without limitation CD and/or UC, in the mammal. Suitable containers include, for example, bottles, vials, syringes, *etc.* The containers may be formed from a variety of materials such as glass or plastic. Additionally, the article of manufacture may further comprise a second container comprising a buffer or other reagent (such as detectable label) useful for carrying out the detection. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, and dyes.

[0209] For isolation and purification of LY6 polypeptide, the kit can contain the LY6-binding reagent coupled to beads (*e.g.*, sepharose beads). Kits can be provided which contain such molecules for detection and quantitation of LY6 polypeptide *in vitro, e.g.*, in an ELISA or a Western blot. As with the article of manufacture, the kit comprises a container and a label or package insert on or associated with the container. The container holds a composition comprising at least one such LY6 binding antibody, oligopeptide or organic molecule useable with the invention. Additional containers may be included that contain, *e.g.*, diluents and buffers, control antibodies. The label or package insert may provide a description of the composition as well as instructions for the intended in vitro or diagnostic use.

K. Sense and Anti-Sense LY6-Encoding Nucleic Acids

[0210] Molecules that would be expected to bind to nucleic acids encoding an LY6 gene include sense and antisense oligonucleotides, which comprise a single-stranded nucleic acid sequence (either RNA or DNA) capable of binding to target LY6 mRNA or DNA sequences. Antisense or sense oligonucleotides, according to the present invention, comprise a fragment of the coding region of the LY6 DNA or its complement. The ability to derive an antisense or a sense oligonucleotide, based upon a cDNA sequence encoding a given protein is described in, for example, Stein and Cohen (Cancer Res. 48:2659, 1988) and van der Krol et al. (BioTechniques 6:958, 1988).

[0211] The sense and/or antisense oligonucleotides hybridizable to a LY6 gene are useful, for example, for detecting the presence of LY6 DNA or mRNA in a tissue or cell sample gastrointestinal tissue or cells of mammal according to the invention. The sense and/or antisense compounds used in accordance with this invention may be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, Calif.). Any other means for such synthesis known in the art may additionally or alternatively be employed. It is well known to use similar techniques to prepare oligonucleotides such as the phosphorothioates and alkylated derivatives. The compounds of the invention may also be admixed, encapsulated, conjugated or otherwise

associated with other molecules, molecule structures or mixtures of compounds, as for example, liposomes, receptor targeted molecules, oral, rectal, topical or other formulations, for assisting in uptake, distribution and/or absorption. Patents that teach the preparation of such uptake, distribution and/or absorption assisting formulations include, but are not limited to, U.S. Pat. Nos. 5,108,921; 5,354,844; 5,416,016; 5,459,127; 5,521,291; 5,543,158; 5,547,932; 5,583,020; 5,591,721; 4,426,330; 4,534,899; 5,013,556; 5,108,921; 5,213,804; 5,227,170; 5,264,221; 5,356,633; 5,395,619; 5,416,016; 5,417,978; 5,462,854; 5,469,854; 5,512,295; 5,527,528; 5,534,259; 5,543,152; 5,556,948; 5,580,575; and 5,595,756.

[0212] Sense and antisense oligonucleotides include without limitation primers and probes useful in PCR, RT-PCR, hybridization methods, in-situ hybridization, and the like.

[0213] Other examples of sense or antisense oligonucleotides include those oligonucleotides which are covalently linked to organic moieties, such as those described in WO 90/10048, and other moieties that increases affinity of the oligonucleotide for a target nucleic acid sequence, such as poly-(L-lysine). Further still, intercalating agents, such as ellipticine, and alkylating agents or metal complexes may be attached to sense or antisense oligonucleotides to modify binding specificities of the antisense or sense oligonucleotide for the target nucleotide sequence.

[0214] Antisense or sense RNA or DNA molecules are generally at least about 5 nucleotides in length, alternatively at least about 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, or 1000 nucleotides in length, wherein in this context the term "about" means the referenced nucleotide sequence length plus or minus 10% of that referenced length.

EXAMPLES

[0215] The following nonlimiting examples are provided for illustrative purposes and are not intended to limit the scope of the invention. Commercially available reagents referred to in the examples were used according to manufacturer's instructions unless otherwise indicated. The source of those cell lines identified in the following examples, and/or throughout the specification, by ATCC accession numbers is the American Type Culture Collection, Manassas, VA.

Example 1: Materials and Methods

[0216] *Reagents, cells and mice:* IFNγ, TNFα, and IL1β were obtained from Peprotech™ (Rocky Hill, NJ). IFNα was obtained from Hycult Biotechnology™ (The Netherlands). For crosslinking experiments, anti-KLH control antibody, anti-LY6A (clone E13-161.7 or D7) were obtained from Pharmingen™ (San Diego, CA). Anti-LY6C (clone HK1.4) was obtained from Southern Biotech™ (Birmingham, AL).

[0217] Chronic $CD45RB^{high}$ transfer colitis was induced as described previously in SCID mice on a Balb/c background (Powrie, F. et al., (1994) Immunity 1:553-562). IL10-/- mice (Kuhn, R. et al., (1993) Cell 75:263-274) on a 129 background, which develop spontaneous colitis, were sacrificed between 11 and 13 weeks of age. Colons were snap frozen in OCT until used in experiments as described. Proximal colon, middle colon, distal colon and rectum were scored using a scale of 0-5 (0 = normal bowel, 5 = severe disease). Scores were summed to achieve a total colitis severity score for each animal.

[0218] The young adult mouse colonocyte (YAMC) cell line (provided by Robert Whitehead, Vanderbilt University Medical Center, Nahville, TN) was derived from the Immortomouse™ a transgenic animal containing a temperature-sensitive T-antigen (tsTag) under the control of an interferon-γ-dependent promoter, as previously described (Whitehead, R.H. et al, (1993) Proc Natl Acad Sci U S A 90:587-591). YAMC cells proliferate under permissive conditions of 32°C in the presence of 5 units/ml IF-γ (Peprotech™, New Jersey), but no longer proliferate upon removal of IFN-γ at 37°C (nonpermissive conditions).

[0219] YAMC cells were cultured in RPMI containing 5% FBS, 2mM L-glutamine, penicillin/streptomycin, 5 U/ml IFNγ and N-2 supplement (Invitrogen™, Carlsbad, CA). Cells were cultured under non-permissive conditions for 24 hours prior to experiments, and for the duration of experimentation.

[0220] CMT93 cells were obtained from ATCC (ATCC Number® CCL-223™, ATCC, Manassas, VA) cultured in DMEM containing 10% FBS, 2mM L-glutamine, and penicillin/streptomycin.

[0221] *Laser capture microscopy and RNA purification:* 10-12 µm sections were applied to LCM membrane slides (Molecular Machines™, Glattbrugg, Switzerland). Slides were subjected to an abbreviated H&E stain (total time of about five minutes) before crypt epithelial cells were histologically identified and dissected using an MMI Cellcut™ microscope (Molecular Machines, Glattbrugg, Switzerland). RNA was purified from the dissected cells using the Arcturus™ Picopure™ RNA purification kit and manufacturer's protocols (Arcturus™, Sunnyvale, CA) and quantified using the NanoDrop ND-1000™ Spectrophotometer (NanoDrop Technologies™, Wilmington, DE).

[0222] *Microarray hybridization and data analysis:* The quantity and quality of input total RNA samples was determined using ND-1000 spectrophotometer (NanoDrop™ Technologies, Montchanin, DE) and Bioanalyzer 2100™ (Agilent™ Technologies, Palo Alto, CA), respectively. The method for preparation of Cy-dye labeled cRNA and array hybridization was provided by Agilent™ Technologies (Palo Alto, CA). Briefly, total RNA sample was converted to double-stranded cDNA and then to labeled cRNA using a Low RNA Input Fluorescent Linear Amplification™ Kit (Agilent™, Product# 5184-3523). The labeled cRNA was purified using RNeasy™ mini kit (Qiagen™, San Diego, CA) and then quantified using ND-1000™ spectrophotometer (Nanodrop™ Technologies). Cy-dye incorporation was determined by running the labeled cRNA on a Novex™ TBE-Urea gel (Invitrogen™, Carlsbad, CA) followed by gel scanning on a Typhoon™ scanner (GE Healthcare™, Piscataway, NJ). To determine the amount of Cy-dye fluorescent counts, the gel images were analyzed using ImageQuant™ software (GE Healthcare™). Approximately 500,000 counts of Cy-dye labeled cRNA was fragmented and hybridized to the Agilent's whole mouse genome array as described in Agilent's *In situ* Hybridization kit-plus (Agilent™, Product# 5184-3568). LCM samples were labeled with Cy5 dye and hybridized against Cy3 dye labeled universal mouse reference (Stratagene™, La Jolla, CA). Following hybridization, the arrays were washed, dried with acetonitrile and scanned on the Agilent™ DNA microarray scanner. The array image files were analyzed using Agilent™'s Feature Extraction™ software 7.5 and further data analysis was performed using Resolver™ (Merck™, Seattle, WA).

[0223] Data was analyzed using Rosetta Resolver™ software (Rosetta Biosoftware™, Seattle, WA). Briefly, healthy and colitic samples were grouped separately and probes that passed two-tailed anova ($p<0.05$) were selected. These probes were analyzed further for probes that demonstrated a two fold or greater change in colitic samples versus healthy samples.

[0224] *Real time quantitative RT-PCR:* RT-PCR was performed on extracted RNA using Taqman™ Gold™ RT-PCR kit and reagents (Applied Biosystems™, Foster City, CA). All samples were run with gene specific primers using 5'-FAM and 3'-TAMRA labeled internal probes. Analysis was performed compared to housekeeping gene, SPF31, specific primers by the $2^{-\Delta\Delta}$Ct method as described (Livak, K.J., and T.D. Schmittgen (2001) Methods 25:402-408). Primers and probes were either designed using Primer3™ software (Rozen, S., and H. Skaletsky (2000) Methods Mol Biol 132:365-386) or obtained commercially (Applied Biosystems™). Primers and probes used for these assays were the following, shown in the 5'-3' direction:

LY6A:	
Sense: CTT ACC CAT CTG CCC TCC TA	(SEQ ID NO:39)
Antisense: CCT CCA TTG GGA ACT GCT AC	(SEQ ID NO:40)
Probe: TCC TGT TGC CAG GAA GAC CTC TGC	(SEQ ID NO:41)
LY6C:	
Sense: ACT TCC TGC CCA GCA GTT AC	(SEQ ID NO:42)
Antisense: GGC ACT GAC GGG TCT TTA GT	(SEQ ID NO:43)
Probe:CTG CCG CGC CTC TGA TGG AT	(SEQ ID NO:44)

[0225] *Immunofluorescent staining:* Frozen tissues were cut into 5 µm sections and stained with biotinylated anti-LY6C (Southern Biotech™, Birmingham, AL) or anti-SCA-1 at 2.5 ng/ml (R&D Systems™, Minneapolis, MN). Slides were washed and labeled with Alexa Fluor™ 488 conjugated streptavidin, mounted using Prolong Gold™ with DAPI (Invitrogen™, Carlsbad, CA) and visualized by confocal microscopy.

[0226] *Crosslinking LY6 molecules:* The ability of crosslinked LY6 polypeptide to effect chemokine production was tested by incubating YAMC cells with plate-bound anti-LY6C or anti-KLH (control) antibodies and measuring the production of chemokines CXCL2, CXCL5, and CCL7. Because lipid raft formation in the cell membrane is required for crosslinking, chemokine production was tested under conditions of normal raft formation (non-cholesterol depletion) and under conditions of cholesterol depletion.

[0227] For crosslinking using plate-bound antibody, 100 µl of anti-LY6C or anti-KLH (control) antibody at 5µg/mL concentration was added to a 96 well plate, or 2 mL were added to a 60 mm^2 dish and allowed to bind to the plate for 15 hours at 4 °C. YAMC cells, grown in cholesterol depleting or non-depleting conditions (as provided in Example 5, herein) were incubated with the plate-bound antibodies for 15 hours at 32°C under cholesterol non-depleting conditions RNA was collected and expression levels of CXCL2, CXCL5, and CCL7 were determined. The assay is further described and results are shown in Example 5, herein.

[0228] *siRNA inhibition:* Individual siRNA directed against murine LY6C were obtained from Dharmacon (Lafayette, CO). SiRNA was trasnfected into YAMC cells using lipofectamine 2000 (Invitrogen) and standard protocols. 72 hours after transfection, cells were collected to determine knockdown efficiency. One siRNA was chosen for crosslinking experiments based on superior knockdown efficiency (95% inhibition by quantitative RT-PCR).

[0229] *CXCL5 secretion:* Supernatants were collected at the indicated time point from stimulated cells and cytokine CXCL5 concentrations were determined by ELISA using a commercially available kit from R&D Systems™ and manufacturer's protocols. The level of detection was 15 pg/ml of CXCL5.

[0230] *Cholesterol depletion:* YAMC cells were cultured for 72 hours in serum free medium at 37°C in the presence of 4 µM lovastatin and 250 µM mevalonate (Sigma). Cells were plated and maintained in lovastatin and mevalonate throughout the experiment.

[0231] *Statistics:* Student's t test was used for comparison between groups (* indicates $p<0.05$).

Example 2: Gene expression patterns of IEC are altered during colitis

[0232] Studies have indicated that gene expression patterns of IEC are significantly altered in mouse models of colitis, as well as human IBD (Fahlgren, A., et al. (2004) Clin Exp Immunol 137:379-385; Brand, S. et al. (2006) Am J Physiol Gastrointest Liver Physiol 290:G827-838; Ruiz, P.A. et al. (2005) J Immunol 174:2990-2999). In this example, evaluated gene expression patterns in IEC of healthy and colitic mice were examined in order to illuminate novel genes and pathways altered in IBD.

[0233] The identification of genes involved in the immunopathology of IBD was sought by evaluating intestinal epithelial cells (IEC) from the $CD45RB^{Hi}$ T cell transfer colitis mouse model as well as the $IL10^{-/-}$ mouse model, both of which result from Th1 dysregulation and share many features of human Crohn's disease (Elson, C.O. et al. (2005) Immunol Rev 206:260-276; Bouma, G., and W. Strober (2003) Nat Rev Immunol 3:521-533). Laser capture microdissection (LCM) was used to isolate crypt IEC from the colons of healthy and colitic mice in the two models of murine IBD. RNA was extracted from these samples and analyzed by microarray technology as described herein in Example 1. The gene expression profile of IEC of colitic mice in the transfer colitis model identified 1770 probes with > 2 fold expression changes compared to control mice, while the IL10 -/- model identified 1140 probes. Overlapping in both models, there were 540 probes with >2 fold changes in expression, corresponding to approximately 400 different genes (data not shown).

Example 3: Pathways and genes affected in IEC during colitis

[0234] Of the approximately 400 genes affected in both models, genes involved in antigen presentation, TLR signaling and cell migration were overrepresented (Table 7). In Table 7, numbers represent the mean with standard deviation of the fold change compared to universal standard RNA of colitic mice over healthy mice in either the $IL10^{-/-}$ model of colitis or the $CD45RB^{Hi}$ model of colitis, as indicated. The results indicated that some IEC expressed genes show altered expression patterns in murine models of IBD. Many of these genes, including TLR2, CCL7, CXCL5 and ICAM-1 have been described previously as having increased epithelial expression during colitis (Breider, M.A. et al. (1997) Vet Pathol 34:598-604; Uguccioni, M. et al. (1999) Am J Pathol 155:331-336; Z'Graggen, K. et al. (1997) Gastroenterology 113:808-816; Singh, J.C. et al. (2005) Am JPhysiol Gastrointest Liver Physiol 288:G514-524), suggesting that the gene expression pattern obtained in these microarrays are an accurate reflection of the biology of IEC in colitis.
Table 7

Fold change (p value):		
Cell migration	**IL10 -/- model**	**CD45RBhi model**
CXCL1	+3.89 (<0.0001)	+2.09 (0.00066)
CXCL5	+21.82 (<0.0001)	+23.34 (<0.0001)
CXCL 13	+3.01 (<0.0001)	+2.85 (<0.0001)
CCL6	-3.47 (<0.0001)	-2.5 (<0.0001)
CCL7	+4.2 (<0.0001)	+5.54 (0.00026)
CCL11	-3.43 (<0.0001)	-3.6 (0.00607)
TLR signaling		
TLR2	+2.15 (<0.0001)	+2.68 (<0.0001)
Fos	+3.64 (<0.0001)	+2.03 (<0.0001)
LBP	+2.34 (<0.0001)	+2.57(<0.0001)
NFKBIA	+2.37 (<0.0001)	+2.15 (<0.0001)
Antigen presentation		
H2-D1	+2.77 (<0.0001)	+2.23 (<0.0001)
HLA-A	+2.83(<0.0001)	+2.40(<0.0001)
HLA-B	+2.71(<0.0001)	+2.44(<0.0001)
HLA-E	+2.31(<0.0001)	+2.34(<0.0001)
ICAM-1	+2.51(<0.0001)	+2.587(<0.0001)
PSMB8	+8.10(<0.0001)	+3.09(<0.0001)
PSMB9	+6.61(<0.0001)	+2.72(<0.0001)
TAP1	+4.05(<0.0001)	+4.10(<0.0001)
TAP2	+2.08(<0.0001)	+2.18(<0.0001)

[0235] IEC can function as non-professional APC (Snoeck, V. et al., (2005) Microbes Infect 7:997-1004; and Shao, L et al., (2005) Immunol Rev 206:160-176), and the gene expression pattern obtained in these microarrays indicate that these functions are enhanced during colitis by upregulation in genes associated with antigen processing, such as LMP7 and TAP1, as well as MHC class I and II genes which would serve to enhance presentation of antigens on the surface of the IEC.

[0236] The microarray data supports the concept that colitic IEC attract immune cells to the colon through altered chemokine expression, and may present antigen to infiltrating T cells by upregulating expression of genes associated with antigen presentation.

Example 4: Expression of LY6 family members is strongly upregulated on the surface of colitic IEC

[0237] Members of the mouse LY6 family of molecules were overrepresented in number as well as degree of upregulation in both the transfer colitis mouse model and the IL10-/- mouse model (Figures 23A and 23B). These results were confirmed by real-time quantitative RT-PCR of pooled and amplified IEC RNA in the transfer colitis model (data not shown). Expression of the LY6 family members was unique to the disease state, so no healthy mice expressed appreciable levels of any of these LY6 family members.

[0238] While expression of murine LY6 molecules on the surface of cells of hematopoietic origin is known, expression on IEC has not been previously described (Bamezai, A. (2004) Arch Immunol Ther Exp (Warsz) 52:255-266; and Rock, K.L. et al. (1989) Immunol Rev 111:195-224). Expression of murine LY6A and LY6C is detectable on many non-epithelial cells present within the colon, such as T cells and granulocytes. Immunofluorescent staining was performed for both murine LY6A and LY6C on healthy and colitic colons. Levels of murine LY6A and LY6C were minimal or absent on the surface of healthy IEC (Figure 24A and 24C, respectively). Expression of both murine LY6A and LY6C was detectable on the surface of IEC throughout the colons of colitic mice (Figure 24B and 24D, respectively). There was no evidence of polarization of either LY6A or LY6C, and staining was present on both the apical and basolateral membranes, making LY6 molecules potentially accessible to ligands on either surface. These

results indicate that the microarray analysis results showing upregulation of murine LY6A and LY6C in murine colitic models was not due to the influx of contaminating immune cells.

Example 4: Transcription of LY6 genes is stimulated by inflammatory cytokines

[0239] LY6 expression on T cells is induced and enhanced by both type I and type II IFNs (Khodadoust, M.M., K.D. Khan, and A.L. Bothwell. 1999. Complex regulation of Ly-6E gene transcription in T cells by IFNs. J Immunol 163:811-819). Furthermore, expression of a number of cytokines, is elevated in the colon during active colitis (Niessner, M., and B.A. Volk. 1995. Altered Th1/Th2 cytokine profiles in the intestinal mucosa of patients with inflammatory bowel disease as assessed by quantitative reversed transcribed polymerase chain reaction (RT-PCR). Clin Exp Immunol 101:428-435).

[0240] To determine if cytokines present during colitis affect transcription of LY6 family members in IEC, we treated YAMC cells, a conditionally immortalized murine IEC line, with IL-1β, IFNα, TNFα, IFNγ or the combination of TNFα and IFNγ and analyzed the transcription of all identified murine LY6 genes by real-time quantitative RT-PCR (Table 8). Briefy, mRNA levels of the indicated LY6 family member in IEC was determined by real time quantitative RT-PCR after 15 hours of treatment with the indicated cytokine. Number represents the fold change (determined by 2-ΔΔCt method) versus the untreated, media control. *, $P<0.05$ versus media control. †, $p<0.05$ versus IFNγ treated cells. The following LY6 family members were tested, but not detected in samples, regardless of treatment: LY6K, Lypd3, Lypd4, Lypd5, LY6g5b, Ly6g6d, Ly6g6e, Slurp1. The results indicate that IEC upregulate LY6 family members in response to inflammatory cytokines.

Table 8

	Media	IL1β	TNFα	IFNα	IFNγ	IFNγ & TNFα
Ly6A	1.0	1.8*	2.2*	2.8*	33.1*	65.4*†
Ly6C	1.0	1.6*	1.2*	2.4*	65.6*	63.6*
Ly6D	1.0	2.7*	2.1*	1.5*	1.0	0.9
Ly6E	1.0	1.4*	1.5*	2.1*	1.9*	2.9*†
Ly6F	1.0	2.5*	0.6*	7.1*	108.2*	169.7*†
Ly6H	1.0	1.0	1.1	1.2	3.7*	1.4†
Lypd1	1.0	1.5*	2.1*	1.3*	1.3*	2.9*†
Lypd2	1.0	0.1*	ND	0.4*	0.1*	ND
Ly6g5c	1.1	1.3	0.8	0.9	1.3	1.1
Ly6g6c	1.0	0.7	0.7	0.6	0.6*	0.3*†
Slurp2/Lynx1	1.1	0.7	0.4	0.7	1.5	0.4*

[0241] While many of the LY6 family members were not detected in either the presence or absence of inflammatory cytokines, we detected a strong upregulation in the transcription of murine LY6A, LY6C and LY6F in response to the majority of the cytokines tested, as well as more moderate upregulation of murine LY6E, LY6H and LYPD1 in response to some cytokines tested. However, IFNγ was by far the most potent cytokine in inducing LY6 upregulation. Furthermore, TNFα enhanced the effects of IFNγ on the expression of LY6A, LY6F, LY6E and LYPD1. Similar upregulation of LY6 family members were seen in another murine IEC line, CMT93 (data not shown).

[0242] To examine the surface expression of LY6 family members in response to cytokines, YAMC cells were exposed to the above cytokines and analyzed by flow cytometry for expression of murine LY6A and LY6C, for which commercial antibodies are available, as descriebed herein in Example 1. High levels of murine LY6A was expressed on YAMC cells even in the absence of added cytokines (Figure 25B, media). Expression of murine LY6C (Figure 25A, media) was considerably lower than expression of LY6A.

[0243] IL-1β and TNFα induced slight increases in the surface expression of both murine LY6A and LY6C, in agreement with the RNA expression (Figures 25A and 25B). A more moderate increase in expression was noted when IFNα was added to the cells, while IFNγ induced dramatic increases in surface expression of both LY6A and LY6C (Figure 25A and 25B). Surface protein expression closely mirrored RNA expression. Th2 cytokines, such as IL4, IL10 or IL 13 had no effect on surface expression of either LY6A or LY6C (data not shown).

[0244] Induction of both LY6A (Figure 25D) and LY6C (Figure 25C) by IFNγ was dose dependent. Doses as low as 6.25 units/ml of IFNγ resulted in detectable increases in both LY6 molecules by flow cytometry. Furthermore, the increase in both LY6A (Figure 25F) and LY6C (Figure 25E) surface expression became evident between 2 and 4 hours after IFNγ treatment, and steadily increased for at least 24 hours after IFNγ treatment. This data indicates that relatively low concentrations of IFNγ are sufficient to increase surface expression of LY6 molecules within hours.

[0245] There is evidence that IL-22, which is secreted primarily from activated T cells, functions through the IL-22R complex, present on IEC to promote cytokine production and an inflammatory phenotype (Brand, S.F. et al. Am J Physiol Gastrointest Liver Physiol 290:G827-838 (2006)). Furthermore, IL-22 is involved in the immunopathogenesis of Crohn's Disease. To examine whether IL-22 affects LY6 molecule expression on murine IEC, YAMC cells were cultured in the presence of IL-22 and analyzed for expression of LY6C (Figure 25G) and LY6A ((Figure 25H). Both LY6 molecules were substantially increased in the presence of IL-22 at comparable levels to the induction seen after treatment with IFNγ.

[0246] To ensure that the upregulation of LY6 molecules was not specific to the YAMC cell line, RNA levels of murine LY6A and LY6C in the murine colonic epithelial tumor cell line CMT93 was examined. Levels of both murine LY6A and murine LY6C were upregulated upon treatment with IFNγ (Figure 25I). Though the levels of upregulation of LY6 molecules were more modest in CMT93 cells, flow cytometry analysis indicated that levels were quite high even in untreated cells (data not shown), which is likely a result of the tumor phenotype of CMT93 cells.

[0247] This data supports the data obtained by real time quantitative RT-PCR in confirming that IEC upregulate LY6 family members in response to inflammatory cytokines.

Example 5: LY6 stimulation of IEC is associated with lipid raft formation

[0248] As GPI-anchored proteins, LY6 family members do not possess a unique intracellular domain associated with traditional outside-in signaling. Rather, they are present within lipid raft microdomains (Bohuslav, J. et al. Eur J Immunol 23:825-831(1993)). However, it has been suggested that cross-linking of LY6 family members on the surface of cells results in redistribution of other cell surface molecules as well as reorganization of lipid raft structures, suggesting a mechanism by which LY6 molecules can affect signal transduction and downstream cellular functions (Simons, K. et al., Nat Rev Mol Cell Biol 1:31-39 (2000)).

[0249] Few ligands for LY6 proteins have been identified to date, and no ligand for LY6A or LY6C is currently known (Paret, C. et al., (2005) Int J Cancer 115:724-733; Apostolopoulos, J. et al., (2000) Immunity 12:223-232; and Classon, B.J. (2001) Trends Immunol. 22:126-127). Cholesterol is required to maintain lipid raft integrity. (Simons, K., et al. J Clin Invest 110:597-603 (2002)), and depletion of cholesterol is often used to inhibit lipid raft biosynthesis *in vitro* (von Tresckow, B. et al. J Immunol 172:4324-4331 (2004)).

[0250] To analyze whether lipid raft reorganization occurs in IEC in response to LY6 crosslinking, YAMC cells were grown in cholesterol-depleting conditions (conditions under which lipid rafts are depleted from cells) and cholesterol non-depleting conditions (conditions permissive for lipid raft formation). For cholesterol depleting conditions, YAMC cells were grown in the absence of serum and in the presence of 4μM lovastatin and 0.25 mM mevalonate (Sigma Chemical Co., St. Louis, MO) for 72 hours at 37 °C. The same growth conditions were use for YAMC cells under cholesterol non-depleting conditions, except that no lovastatin or mevalonate were added to the growth medium. Cells were then lifted and LY6C was crosslinked as described above in Example 1. RNA was collected and expression levels of CXCL2, CXCL5, and CCL7 were determined.

[0251] The results of these studies indicated that lipid raft depletion results in an inhibition of LY6C-mediated chemokine production. Figures 26A-26C show that cholesterol depleted (dark bars) YAMC cells produced less chemokine than cells that were not depleted of cholesterol (open bars). Cholesterol depletion affected chemokine production in control anti-KLH stimulated groups, irrespective of LY6C stimulation, however the response was minimal and not in a consistent direction. To examine whether cholesterol depletion globally affected cell viability, we measured cell death, by 7AAD exlusion, and determined that cholesterol depletion did not significantly affect the viability of the YAMC cells (92% viability versus 86% in the cholesterol depleted cells, data not shown). Surface expression of both LY6A (Figure 26D) and LY6C (Figure 26E) were both significantly lower in cholesterol depleted YAMC cells, suggesting that plasma membrane cholesterol levels and lipid raft integrity affect the levels of LY6 expression on the surface of cells. This data suggests that lipid raft integrity, influenced by cholesterol biosynthesis, allows for the expression of LY6 molecules on the surface, and is potentially involved in the LY6C mediated induction of chemokines. Thus, the enhancement of chemokine production mediated by interaction of LY6C polypeptides in the cell

membrane requires the presence of lipid rafts on the cell surface.

Example 6: Crosslinking LY6C results in increased surface expression of LY6 molecules

[0252] It has been reported that crosslinking LY6C on the surface of T cells results in shedding of LY6C (Jaakkola, I. et al. (2003) J Immunol 170:1283-1290). However, unlike T cells, when murine LY6C was crosslinked on the surface of IEC, no shedding of either LY6A or LY6C occurred (Figure 27A and 27B, respectively). To the contrary, in the absence of IFNγ, surface expression levels of both LY6A and LY6C were increased on IEC with crosslinked LY6C, but not LY6A. When IEC were preincubated with IFNγ, much of this effect was abolished (Figure 27C), however a slight upregulation of LY6A was still detected (Figure 27D).

[0253] These data indicate a positive feedback loop whereby stimulation through LY6C on IEC results in increased surface expression of LY6 molecules.

Example 7: Stimulation of LY6A results in increased secretion of chemokines

[0254] Functions for LY6 molecules have not been fully elucidated. To examine the role of LY6 molecues in the immunopathology of colitis, stimulation of LY6 molecules was studied for affects on the transcription and secretion of chemokines from IEC.

[0255] To analyze production of chemokines from IEC in response to crosslinking of murine LY6 molecules, YAMC cells, either pretreated with IFNγ or untreated, were cultured on plates coated with either anti-KLH control antibody, anti-LY6A or anti-LY6C. Twenty four hours later, mRNA from these cells was obtained and analyzed by quantitative RT-PCR for expression of CCL2, CCL4, CCL5, CCL7, CCL8, CCL25, CXCL1, CXCL2, CXCL5, CXCL10, CXCL12 and CX3CL1, which are chemokines that have been implicated in colitis (Table 9) (Papadakis, K.A. (2004) Curr Allergy Asthma Rep 4:83-89; Banks, C. et al., (2003) J Pathol 199:28-35; and Papadakis, K.A., and S.R. Targan (2000) Inflamm Bowel Dis 6:303-313). The assay was performed under non-permissive growth conditions (37°C in the absence of IFNγ) to rule out the possibility of increased proliferation of IEC in response to IFNγ stimulation.

Table 9

Pretreatment >	Media			IFN		
Crosslink >	Anti-KLH	Anti-LY6A	Anti-LY6C	Anti-KLH	Anti-LY6A	Anti-LY6C
CCL2	1.01 (0.14)	-1.38 (0.13)	8.81 (0.72)	3.53 (0.21)	2.83 (0.21)	16.12 (0.56)
CCL4	1.31 (1.14)	1.83 (0.49)	3.15 (1.17)	5.35 (0.48)	3.65 (0.69)	16.12 (0.56)
CCL5	1.00 (0.10)	1.09 (0.02)	3.31 (0.15)	2.73 (0.13)	2.76 (0.23)	10.13 (0.27)
CCL7	1.00 (0.11)	1.06 (0.08)	3.37 (0.15)	2.82 (0.44)	1.39 (0.33)	5.81 (0.51)
CCL8	1.03 (0.30)	2.05 (0.37)	12.78 (3.14)	74.22 (8.94)	74.44 (9.81)	110.44 (3.36)
CCL25	1.01 (0.16)	1.06 (0.11)	1.16 (0.00)	1.38 (0.36)	1.32 (0.15)	1.46 (0.19)
CXCL1	1.00 (0.07)	-3.17 (0.11)	11.58 (0.12)	-1.13 (0.10)	-1.64 (0.17)	13.36 (0.35)
CXCL2	1.33 (1.10)	ND	21.81 (3.13)	14.30 (3.30)	10.95 (3.05)	113.20 (16.23)
CXCL5	1.08 (0.53)	ND	118.45 (65.14)	1.70 (1.15)	ND	150.99 (55.50)
CXCL10	1.00 (0.05)	1.02 (0.06)	5.11 (0.19)	5.68 (0.31)	5.22 (0.22)	12.22 (0.51)
CXCL12	1.01 (0.14)	1.12 (0.05)	-1.99 (0.14)	-1.11 (0.05)	-1.23 (0.21)	-3.02 (0.06)
CX3CL1	1.00 (0.08)	-1.18 (0.15)	1.92 (0.07)	2.21 (0.11)	1.87 (0.16)	3.22 (0.42)

[0256] Cells pretreated with IFNγ showed upregulation of many of these chemokine genes (see Media, Anti-KLH group versus IFNγ, Anti-KLH group of Table 9). However, with the exception of an upregulation of CCL8 and a downregulation of CXCL1, anti-LY6A stimulated YAMC cells showed similar gene expression patterns as anti-KLH stimulated YAMC cells. However, YAMC cells stimulated with anti-LY6C showed increased expression of all chemokines analyzed except for CCL25, which remained essentially unchanged, and CXCL12, which was downregulated in response to LY6C stimulation. While the increased gene expression of chemokines induced by LY6C crosslinking was not dependent upon IFNγ, cells pretreated with IFNγ showed increased expression

of chemokines versus cells that had not been pretreated with IFNγ.

[0257] To analyze the kinetics of chemokine induction induced by murine LY6C stimulation, 96 well plates were coated with anti-KLH antibody or either anti-LY6A or anti-LY6C monoclonal antibodies. YAMC cells, either pretreated or not with IFNγ, were added for 24, 48 or 72 hours. At the indicated time point RNA was collected for quantitative RT-PCR analysis and supernatants were collected for ELISA.

[0258] Within 24 hours, a spike in transcription of both CXCL5 and CCL7 was detected on cells with crosslinked LY6C, but not LY6A (Figure 28A). Increased expression of CXCL5 and CCL7 diminished over time but was still detectable after 72 hours in culture. Though IFNγ was not required to enhance chemokine transcription, IFNγ acted synergistically with LY6C stimulation in inducing transcription of both CXCL5 and CCL7 at early time points.

[0259] In parallel with the gene expression, supernatants of LY6C, but not LY6A, crosslinked cells contained significantly higher concentrations of CXCL5 at 48 hours (Figure 28B). The effect was dose dependent, and detectable with as little as 1 µg/ml of coated anti-LY6C. Like transcription, secretion of CXCL5 was enhanced when cells were pretreated with IFNγ, but IFNγ was not required for the effect. Increased secretion of CXCL5 was noted at both the 24 and 72 hour time points as well.

[0260] To ensure that LY6C was involved in the observed upregulation of chemokines, we used siRNA to knockdown LY6C. LY6C transcript was inhibited by 95% in the absence of IFNγ and about 90% in the presence of IFNγ by real time quantitative RT-PCR which corresponded to significantly lower levels of LY6C on the surface of the YAMC cells (data not shown). Cells with decreased levels of LY6C on the surface showed a diminished response to LY6C crosslinking with regard to transcription of chemokines (Figure 28C). Secretion of CXCL5 was markedly inhibited by knocking down LY6C as well (data not shown).

[0261] These results indicate that crosslinking of LY6C, but not LY6A, on the surface of IEC results in increased secretion of chemokines.

Example 8: IEC in vivo show a similar chemokine gene expression to LY6C stimulated cells

[0262] The above data establishes a model whereby IEC stimulated through murine LY6C significantly upregulate expression of chemokine genes.

[0263] Analyzing the microarray data from laser capture microdissected IEC in murine models of colitis, the expression of the same 12 chemokine genes in healthy and colitic mice in the two murine models of colitis was examined to determine if the chemokines stimulated by LY6C crosslinking *in vitro* correlate with the chemokines secreted by IEC *in vivo* (Figures 29A and 29B). Though the expression pattern is not identical to the upregulation of chemokines resulting from LY6C stimulation, expression of CXCL5, which was the most highly upregulated chemokine gene in *in vitro* studies, was also the highest upregulated chemokine in murine models of colitis. We saw significant upregulation in expression of CXCL1, CXCL10, CCL5 and CCL7 in both models of colitis. In addition, we saw upregulation of CCL4 and CCL8 in the transfer colitis model or the IL10 -/- model, respectively.

[0264] Interestingly, the only chemokine that was down-regulated as a result of murine LY6C stimulation *in vitro,* CXCL12 was also the only one of these chemokines downregulated *in vivo.*

Example 9: Expression of human LY6 genes in colon cells

[0265] Expression of human LY6H, LYPD1, LYPD3, and LYPD5 in a human colon cell line, Colo 205 cells (a cell line derived from human colon carcinoma, ATCC™ accession number CCL-222™), was examined. Human Colo 205 cells were treated with the cytokines IFN-r, LPS, TNFα, IFN-r + TNFα, IFN-r + LPS, or LPS + TNFα (all at 100 ng/ml, except LPS at 1 ug/ml) for 18 hours (LYPD3) or 24 hours (LY6H or LYPD5). RNA was collect and purified and expression of the indicated LY6 family member was determined by quantitative RT-PCR using reagents from Applied Biosystems™ according to manufacturer's instructions. Primers and probes used for RT-PCR analysis were the following:

LYPD 1:	
Sense: CAT GAT CCT CCG AAT CTG GT	(SEQ ID NO:59)
Antisense: AGC ACA GAA CAG AGG GGC TA	(SEQ ID NO:60)
Probe: ATA CGG CCA ATG TCA CAA CA	(SEQ ID NO:61)

LYPD3:	
Sense: ACT TCC TGT TCC CAC CAC TG	(SEQ ID NO:62)
Antisense: AGA GGA CAA GCG GAG AGA CA	(SEQ ID NO:63)
Probe: TTC TGG CAG GGG TGT TCT AG	(SEQ ID NO:64)
LY6H:	
Sense: AGC AGC AGC AGG AAG GAT	(SEQ ID NO:65)
Antisense: AAA AGT GCC GCT TAA CGA AG	(SEQ ID NO:66)
Probe: CAA GAT GTG TGC TTC CTC CTG CGA	(SEQ ID NO:67)

[0266] LYPD5 primers and probes were purchased from Applied Biosysems™ (catalog number HS00289062_m1).

[0267] The results plotted in Figures 30A-30C indicate fold increases in expression of these human LY6 genes relative to human B-actin control. Significant increases in expression of human LY6H, LYPD3, and LYPD5 were observed following treatment with the indicated cytokines.

Example 10: Expression of human LY6 genes in colon biopsy tissue

[0268] To further investigate the source of the increased LYPD1 and LYPD5 expression in the colon of patients with CD and UC, was undertaken in a cohort of biopsies of patients with UC, CD and controls. Microarray analysis for LYPD1 expression using RNA extracted from colon biopsies showed statistically increased expression in inflamed colon tissue of CD patients (Figure 31A). In the UC and CD biopsies taken from the colon, statistically increased LYPD5 expression was observed in inflamed UC and CD patients (Figure 31B). This was not observed in the non-inflamed control biopsies.

[0269] Expression of human LY6H in terminal ileum biopsies of inflamed IBD tissue was analyzed relative to control (non-IBD) terminal ileum biopsies using RT-PCR (Taqman™) analysis. Human LY6H expression was at least 1.5 fold greater in inflamed IBD biopsies relative to control.

[0270] Human LYPD3 expression in inflamed UC colon biopsies was upregulated and less than 2 fold greater in inflamed IBD biopsies relative to control.

[0271] The results of these examples demonstrated expression of LY6 molecules on the surface of IEC, and further indicated that expression is unique to IEC in the context of inflammation. Furthermore, surface expression levels of LY6A and LY6C were high on IEC of colitic mice, and nearly universal throughout the colon. As molecules both specific to the diseased state, and ubiquitously expressed during disease, detection of human LY6 gene or polypeptide expression, particularly human LY6H, LYPD1, LYPD3, and LYPD5, is a useful method for detecting IBD, including UC and/or CD in humans. Additionally, the method of detecting human LY6 expression is useful for diagnosing IBD, UC and/or CD in a human and monitoring response to IBD therapeutic agents.

[0272] In the Examples disclosed herein, the functional significance of LY6 expression in IEC was demonstrated. YAMC cells were strongly positive for LY6A, and expressed lower levels of LY6C. However, upon stimulation with a number of cytokines present within the colon during colitis, including IL-1β, TNFα, IFNα, and in particular IL-22 and IFNγ, expression levels of both LY6 molecules were greatly enhanced. YAMC cells pretreated with IFNγ to upregulate expression of LY6 molecules, were a useful *in vitro* model to analyze functional significance for LY6 expression.

[0273] The conditionally immortalized nature of the YAMC cells comes from MHC II promoter driven expression of the SV40 large T antigen; low levels (2.5-5 U/ml) of IFNγ are used to drive proliferation of these cells (Whitehead, R.H. et al. (1993) Proc Natl Acad Sci U S A 90:587-591 ; Whitehead, R.H., and J.L. Joseph. (1994) Epithelial Cell Biol 3:119-125). YAMC cells are often used as an in vitro model for cytokine treatments of murine IEC (Mei, J.M. et al. (2000) Faseb J 14:1188-1201; Yan, F., and D.B. Polk (2002) J Biol Chem 277:50959-50965). The SV40 large T antigen that these cells contained is temperature sensitive, and non-functional at 37°C. All experiments performed herein involved IFNγ treatment under these non-permissive conditions. In addition, YAMC cells were serum starved (and IFNγ starved) at 37°C for 24 hours prior to experiments. Under such conditions, effects indicating residual T antigen expression, such as proliferation of cells, were not observed. As a result, effects of IFNγ treatment were due to inherent effects of IFNγ rather than effects stemming from driving expression of the T antigen. Furthermore, the

upregulation of LY6 family members was detected in a second murine cell line, CMT93, confirming that this is effect is broadly applicable to IEC.

[0274] Furthermore, IFNγ was not unique among cytokines for inducing LY6 molecules as modest upregulation of LY6 expression was noted after treatment with TNFα, IL-1β and, IL-22. The upregulation of LY6 molecules on IEC in response to IL-22 is interesting in light of recent data demonstrating a potential role for IL-22 in Crohn's Disease (Wolk, K., et al. J Immunol 178:5973-5981 (2007)). Though homology between mouse and human LY6 molecules are often complicated, there is evidence to suggest that the upregulation of LY6 molecules is not restricted to mice. Previous studies in rats have suggested upregulation of LY6 molecules in the small intestine in colitis models, and it has been suggested that such expression is involved in inflammation, cell/cell interactions as well as signaling within the rat IEC (Baksheev, L. et al. J Gastroenterol 41:1041-1052 (2006)).

[0275] The data described above indicates that there is a possibility that lipid raft integrity is involved in LY6C mediated signal transduction in IEC. This implies that disruption of lipid rafts might serve to attenuate downstream affects of LY6C stimulation both by downregulating LY6C expression and disrupting the structural components of LY6C signaling. Recently, it has been determined that cholesterol depletion of IEC with statins inhibits proinflamamtory gene expression through NF-κB modulation (Lee, J. et al., Int Immunopharmacol 7:241-248 (2007)). Furthermore, statins have been effective therapeutics in murine models of colitis (Naito, Y., et al. Int J Mol Med 17:997-1004 (2006)). The mechanism linking lipid raft motility and NF-κB blockade remain undetermined, but our data suggests that activation through LY6C could be one hypothesis to explain the mechanism of action.

[0276] In this study, we identify LY6 molecules as a potential upstream switch in the expression of chemokine genes. Crosslinking of the LY6C receptor with monoclonal antibodies resulted in dramatic upregulation of nearly all chemokines analyzed, including CXCL5. We further confirmed that CXCL5 secretion is greatly enhanced in LY6C crosslinked IEC. It is interesting that even though both LY6A and LY6C are anchored to the cell surface by a GPI moiety, and despite higher levels of expression of LY6A than LY6C on the surface of IEC, that the downstream effects on chemokine secretion are seen with LY6C crosslinking and not consistently with LY6A crosslinking.

Example 11: Identification of a ligand for LYPD5

[0277] In this study, a search for ligands of LYPD5 was performed through techniques well known to those of ordinary skill in the art, namely by expression cloning of about 14,000 human genes under CMV promoter into COS cells. Pools of 100 genes were transfected into the COS cells grown in 140 wells on 12 well plates. Following transfection, the cells were stained with LYPD5-Fc protein (see Figure 32). Wells with positive staining were identified and individual clones were transfected into COS cells. A single well expressing a single protein, GLG-1 (ESL-1) was identified as a ligand for LYPD5. GLG-1 is characterized by a lengthy extracellular domain (ECD), a transmembrane domain and a cytoplasmic domain. A series of co-immunoprecipitation studies were conducted using techniques known to those of ordinary skill in the art to assess the ability of various regions of the GLG-1 ECD to bind LYPD5. It was found that variants or fragments of the GLG-1 ECD (see Figures 33-35) were able to serve as a ligand for LYPD5. Figure 33B shows the results of co-immunoprecipitation studies using Fragments 1, 2, 3, or 4 as depicted in Figure 33A and demonstrates that any one of the fragments is sufficient for LYPD5 binding.

[0278] In addition, a GLG-1 ECD domain by itself was found to be sufficient for LYPD5 binding. As shown in Figure 33, GLG-1 is made up of multiple GLG-1 domains and single GLG-1 domains can bind LYPD5. Figure 34B shows the results of a co-immunoprecipitation demonstrating that Fragments 1, 2, 3, and 4, as well as single GLG-1 domains 115, 150, 215, 538, 609, 670, 729, and 858 (as shown in Figure 34A) were able to bind LYPD5.

[0279] Through another co-immunoprecipitation study, binding was shown to be specific based on fragments of LYPD5 (see Figure 35A) in which LYPD5 was found not to bind BAP negative control, an FN14 negative control was not found to bind GLG-1 fragment 2, the human GLG-1 domain 115 binds LYPD5, domain 115 is not always expressed at detectable levels but still pulls down LYPD5, and the fraction of human GLG-1 fragment 1 that lacks domain 115 (residues 26-114) does not bind LYPD5 (Figure 35B).

[0280] The "*" in Figures 34A and 35A indicates a potential fucosylation site.

[0281] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention.

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Patentkrav

1. Fremgangsmåde til detektering af et terapeutisk lægemiddelrespons hos et pattedyr behandlet med et terapeutisk middel mod IBD, hvor fremgangsmåden omfatter at bestemme og sammenligne LY6-ekspressionsniveauer i en testprøve omfattende gastrointestinalt væv eller gastrointestinale celler fra pattedyret før og efter behandling med det terapeutiske middel mod IBD, hvor et lavere niveau af LY6-ekspression i testprøven efter behandlingen indikerer et respons fra pattedyret på det terapeutiske middel.

2. Fremgangsmåde ifølge krav 1, hvor testprøven er fra pattedyrets colon.

3. Fremgangsmåde ifølge krav 1, hvor testprøven er fra pattedyrets terminale ileum.

4. Fremgangsmåde ifølge et hvilket som helst af kravene 1-3, omfattende:
(a) at bringe testprøven i kontakt med et detekterbart middel, som specifikt binder et polynukleotid, der koder for LY6-polypeptid eller fragment deraf; og
(b) at detektere dannelsen af et kompleks mellem midlet og polynukleotidet i testprøven; eventuelt hvor fremgangsmåden er *in situ*-hybridiseringsanalyse eller realtids polymerasekædereaktions- (RT-PCR-) analyse.

5. Fremgangsmåde ifølge krav 4, hvor polynukleotidet omfatter nukleinsyresekvensen ifølge SEQ ID NO: 8, 9, 1, 3, 4, 6 eller et fragment deraf omfattende mindst 15 sammenhængende nukleotider ifølge SEQ ID NO: 8, 9, 1, 3, 4 eller 6.

6. Fremgangsmåde ifølge krav 4, hvor det detekterbare middel er et andet polynukleotid, der hybridiserer til et polynukleotid med sekvensen SEQ ID NO: 8, 9, 1, 3, 4, 6 eller dets komplement.

7. Fremgangsmåde ifølge krav 6, hvor det andet polynukleotid omfatter et detekterbart mærke eller er fastgjort til et fast underlag, hvor det detekterbare mærke er direkte detekterbart eller indirekte detekterbart, og eventuelt hvor

det detekterbare mærke er et fluorescerende mærke.

8. Fremgangsmåde ifølge et hvilket som helst af kravene 1 til 3, omfattende: (a) at bringe testprøven i kontakt med et detekterbart middel, der specifikt binder et LY6-polypeptid eller fragment deraf; og (b) at detektere dannelsen af et kompleks mellem midlet og polypeptidet i testprøven.

9. Fremgangsmåde ifølge krav 8, hvor LY6-polypeptidet omfatter SEQ ID NO: 10, 2, 5 eller 7 eller et fragment deraf omfattende mindst 10 sammenhængende aminosyrer ifølge SEQ ID NO: 10, 2, 5 eller 7.

10. Fremgangsmåde ifølge krav 8, hvor det detekerbare middel er et antistof eller bindende fragment deraf, eventuelt hvor antistoffet eller det bindende fragment deraf omfatter et detekterbart mærke, hvor det detekterbare mærke er direkte detekterbart eller indirekte detekterbart, og eventuelt hvor det detekterbare mærke er et fluorescerende mærke eller et radioaktivt mærket stof.

11. Fremgangsmåde ifølge et hvilket som helst af kravene 1-10, hvor pattedyret har ulcerøs kolitis (UC).

12. Fremgangsmåde ifølge et hvilket som helst af kravene 1-10, hvor pattedyret har Crohns sygdom (CD).

DRAWINGS

cgcgtctgcggctgcgttccccgaaagacgaggctgcgcccggattccggtccgcagggagaccgaagggcacagctccccgcgccg
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Figure 1A

MLPAAMKGLGLALLAVLLCSAPAHGLWCQDCTLTTNSSHCTPKQCQPSDTVCASVRIT
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GHSPWALAGGLLLSLGPALLWAGP

Figure 1B

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Figure 2A

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Figure 2B

MWVLGIAATFCGLFLLPGFALQIQCYQCEEFQLNNDCSSPEFIVNCTVNVQDMCQKEV
MEQSAGIMYRKSCASSAACLIASAGYQSFCSPGKLNSVCISCCNTPLCNGPRPKKRGSSA
SALRPGLRTTILFLKLALFSAHC

Figure 2C

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Figure 3A

MDPARKAGAQAMIWTAGWLLLLLLRGGAQALECYSCVQKADDGCSPNKMKTVKCAP
GVDVCTEAVGAVETIHGQFSLAVRGCGSGLPGKNDRGLDLHGLLAFIQLQQCAQDRCN
AKLNLTSRALDPAGNESAYPPNGVECYSCVGLSREACQGTSPPVVSCYNASDHVYKGC
FDGNVTLTAANVTVSLPVRGCVQDEFCTRDGVTGPGFTLSGSCCQGSRCNSDLRNKTYF
SPRIPPLVRLPPPEPTTVASTTSVTTSTSAPVRPTSTTKPMPAPTSQTPRQGVEHEASRDEE
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Figure 3B

gatccgcttt gcgcatccca gtgattcttg ggttccgcgt gtagtttcgg aaggagacat cgaagcaggg cgaggcgcag
agggcgttgc ggactcatgc cccagtcggc agtgcggggt cccaagccct gcagtgctac agctttgagc acacctactt
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cagtactgga agaaactgga cacagtcacc agcatccccg gggagggcaa aacagccatg tcgtgccccg atgaagagca
attctgatca cagctgttac tcactgagca ccagccaggc accaggcacc ccataacacg gcttcctgtg ctctccctcc
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gccctaacag ttagtaggcc aggctctgga gctaagctgc atgggttcaa atcccagctc caccattcag cctgcagaga
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aataggatta gtgcccttat aaaagaggcc ctggagagct gccttgcccc ttccaccaca tgagaacaca gccagcaggt
gcctataagc aagaaagtgg gttctcacca gccatcgaat ctgctggtgc attgattgca gacttcccag actccagagc
tatgagacat aaatttctgt tgtgtataag ccaaaaaaaa aaaaaaaaaa

Figure 4A

gattcttggg ttccgcgcgt agtttcggaa ggagacatcg aagcagggcg aggcgcagag ggcgttgcgg actcatgccc
cagtcggcag tgcggggtcc caagccctgc agtgctacag CtttgagCaC acctactttg gccccttga cctcagggcc
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acaaaggtct gagggaggaa ggaacacaag gagtgcagga cactttcata actgcatgaa cttcatagag atgggatcct
ttagcatgtt ctctgtgcac atgcttgacc atgttctttc acatgctttt tgccacttga tctttccagc aactcagtga gagaagcaaa
aaagtaagtt gcatcctg

Figure 4B

MKLPSISCPHECFEAILSLDTGYRAPVTLVRKGCWTGPPAGQTQSNADALPPDYSVVRG
CTTDKCNAHLMTHDALPNLSQAPDPPTLSGAECYACIGVHQDDCAIGRSRRVQCHQDQ
TACFQGNGRMTVGNFSVPVYIRTCHRPSCTTEGTTSPWTAIDLQGSCCEGYLCNRKSMT
QPFTSASATTPPRALQVLALLLPVLLLVGLSA

Figure 4C

ctgagaggaa gttttatctg tgcagcccct ctctgaggat ggacacttct cacactacaa agtcctgttt gctgattctt cttgtggccc tactgtgtgc agaaagagct cagggactgg agtgttacca gtgctatgga gtcccatttg agacttcttg cccatcaatt acctgccccc accctgatgg agtctgtgtt actcaggagg cagcagttat tgtggattct caaacaagga aagtaaagaa caatctttgc ttacccatct gccctcctaa tattgaaagt atggagatcc tgggtactaa ggtcaacgtg aagacttcct gttgccagga agacctctgc aatgtagcag ttcccaatgg aggcagcacc tggaccatgg caggggtgct tctgttcagc ctgagctcag tcctcctgca gaccttgctc tgatggtcct cccaatgacc tccacccttg tccttttatc ctcatgtgca acaattcttc ctggagccct ctagtgatga attatgagtt atagaagctc caaggtggga gtagtgtgtg aaataccatg ttttgccttt atagcccctg ctgggtaggt aggtgctcta atcctctcta gggctttcaa gtctgtactt cctagaatgt cattttgttg tggattgctg ctcatgaccc tggaggcaca cagccagcac agtgaagagg cagaattcca aggtattatg ctatcaccat ccacacataa gtatctgggg tcctgcaatg ttcccacatg tatcctgaat gtcccccctgt tgagtccaat aaaccctttg ttctcccaaa aaaaaaaaaa aa

Figure 5A

MDTSHTTKSCLLILLVALLCAERAQGLECYQCYGVPFETSCPSITCPYPDGVCVTQEAAV
IVDSQTRKVKNNLCLPICPPNIESMEILGTKVNVKTSCCQEDLCNVAVPNGGSTWTMAG
VLLFSLSSVLLQTLL

Figure 5B

gctccggcca gccgcggtcc agagcgcgcg aggttcgggg agctccgcca ggctgctggt acctgcgtcc gcccggcgag caggacaggc tgctttggtt tgtgacctcc aggcaggacg gccatcctct ccagaatgaa gatcttcttg ccagtgctgc tggctgccct tctgggtgtg gagcgagcca gctcgctgat gtgcttctcc tgcttgaacc agaagagcaa tctgtactgc ctgaagcccga ccatctgctc cgaccaggac aactactgcg tgactgtgtc tgctagtgcc ggcattggga atctcgtgac atttggccac agcctgagca agacctgttc cccggcctgc cccatcccag aaggcgtcaa tgttggtgtg gcttccatgg gcatcagctg ctgccagagc tttctgtgca atttcagtgc ggccgatggc gggctgcggg caagcgtcac cctgctgggt gccgggctgc tgctgagcct gctgccggcc ctgctgcggt ttggcccctg accgcccaga ccctgtcccc cgatccccca gctcaggaag gaaagcccag ccctttctgg atcccacagt gtatgggagc ccctgactcc tcacgtgcct gatctgtgcc cttggtccca ggtcaggccc acccccctgca cctccacctg ccccagcccc tgcctctgcc caagtgggcc agctgccctc acttctgggg tggatgatgt gaccttcctt gggggactgc ggaagggacg agggttccct ggagtcttac ggtccaacat cagaccaagt cccatggaca tgctgacagg gtccccaggg agaccgtgtc agtagggatg tgtgcctggc tgtgtacgtg ggtgtgcagt gcacgtgaga gcacgtggcg gcttctgggg gccatgtttg gggagggagg tgtgccagca gcctggagag cctcagtccc tgtagccccc tgccctggca cagctgcatg cacttcaagg gcagcctttg ggggttgggg tttctgccac ttccgggtct aggccctgcc caaatccagc cagtcctgcc ccagcccacc cccacattgg agccctcctg ctgctttggt gcctcaaata aatacagatg tcccc

Figure 6A

MKIFLPVLLAALLGVERASSLMCFSCLNQKSNLYCLKPTICSDQDNYCVTVSASAGIGNL
VTFGHSLSKTCSPACPIPEGVNVGVASMGISCCQSFLCNFSAADGGLRASVTLLGAGLLL
SLLPALLRFGP

Figure 6B

ccagtctgtc gccacctcac ttggtgtctg ctgtccccgc caggcaagcc tggggtgaga gcacagagga gtgggccggg
accatgcggg ggacgcggct ggcgctcctg gcgctggtgc tggctgcctg cggagagctg gcgccggccc tgcgctgcta
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ggccctatgc ggcccagccc cgaatgcctt gaagaagtgc ccctgcacc aggaaaaaaa aaaaaaaaaa

Figure 7A

MRGTRLALLALVLAACGELAPALRCYVCPEPTGVSDCVTIATCTTNETMCKTTLYSREI
VYPFQGDSTVTKSCASKCKPSDVDGIGQTLPVSCCNTELCNVDGAPALNSLHCGALTLL
PLLSLRL

Figure 7B

aagatggcgg cgtgtggacg tgtacggagg atgttccgct tgtcggcggc gctgcatctg ctgctgctat tcgcggccgg
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cggaggacat ccgcttggag

Figure 8A

ccagatctat acgaagcctg caagagtgac atcaaaaact tctgttccgc tgtgcaatat ggcaacgctc agattatcga atgtctgaaa gaaaacaaga agcagctaag cacccgctgc caccaaaaag tatttaagct gcaggagaca gagatgatgg acccagagct agactacacc ctcatgaggg tctgcaagca gatgataaag aggttctgtc cggaagcaga ttctaaaacc atgttgcagt gcttgaagca aaataaaaac agtgaattga tggatcccaa atgcaaacag atgataacca agcgccagat cacccagaac acagattacc gcttaaaccc catgttaaga aaagcctgta aagctgacat tcctaaattc tgtcacggta tcctgactaa ggccaaggat gattcagaat tagaaggaca agtcatctct tgcctgaagc tgagatatgc tgaccagcgc ctgtcttcag actgtgaaga ccagatccga atcattatcc aggagtccgc cctggactac cgcctggatc ctcagctcca gctgcactgc tcagacgaga tctccagtct atgtgctgaa gaagcagcag cccaagagca gacaggtcag gtggaggagt gcctcaaggt caacctgctc aagatcaaaa cagaattgtg taaaaaggaa gtgctaaaca tgctgaagga aagcaaagca gacatctttg ttgacccggt acttcatact gcttgtgccc tggacattaa acaccactgc gcagccatca ccctggccg cgggcgtcaa atgtcctgtc tcatggaagc actggaggat aagcgggtga ggttacagcc cgagtgcaaa aagcgcctca atgaccggat tgagatgtgg agttacgcag caaaggtggc cccagcagat ggcttctctg atcttgccat gcaagtaatg acgtctccat ctaagaacta cattctctct gtgatcagtg ggagcatctg tatattgttc ctgattggcc tgatgtgtgg acggatcacc aagcgagtga cacgagagct caaggacagg tagagccacc ttgaccacca aaggaactac ctatccagtg cccagtttgt acagccctct tgtatagcat ccccactcac ctcgctcttc tcagaagtga caccaacccc gtgttagagc attagcagat gtccactgcg ttgtcccatc cagcctccac tcgtgtccat ggtgtcctcc tcctcctcac cgtgcagcag cagcagctgg tcgctggggt tactgccttt gtttggcaaa cttgggttta cctgcctgta gacaagtctc tctcatacca acagaacttc cggtacttcc agaaccaact cacctgacct gcaactcaaa ggctttttta agaaaaccac caaaaaaaaa a

Figure 8A (continued)

MAACGRVRRMFRLSAALHLLLLFAAGAEKLPGHGVHSQGQGPGANFVSFVGQAGGGG
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SVAREVCKSTITEIKECADEPVGKGYMVSCLVDHRGNITEYQCHQYITKMTAIIFSDYRLI
CGFMDDCKNDINILKCGSIRLGEKDAHSQGEVVSCLEKGLVKEAEEREPKIQVSELCKK
AILRVAELSSDDFHLDRHLYFACRDDRERFCENTQAGEGRVYKCLFNHKFEESMSEKCR
EALTTRQKLIAQDYKVSYSLAKSCKSDLKKYRCNVENLPRSREARLSYLLMCLESAVHR
GRQVSSECQGEMLDYRRMLMEDFSLSPEIILSCRGEIEHHCSGLHRKGRTLHCLMKVVR
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MEHLYTEKMVEDCEHRLLELQYFISRDWKLDPVLYRKCQGDASRLCHTHGWNETSEF
MPQGAVFSCLYRHAYRTEEQGRRLSRECRAEVQRILHQRAMDVKLDPALQDKCLIDLG
KWCSEKTETGQELECLQDHLDDLVVECRDIVGNLTELESEDIQIEALLMRACEPIIQNFC
HDVADNQIDSGDLMECLIQNKHQKDMNEKCAIGVTHFQLVQMKDFRFSYKFKMACKE
DVLKLCPNIKKKVDVVICLSTTVRNDTLQEAKEHRVSLKCRRQLRVEELEMTEDIRLEP
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LMRVCKQMIKRFCPEADSKTMLQCLKQNKNSELMDPKCKQMITKRQITQNTDYRLNP
MLRKACKADIPKFCHGILTKAKDDSELEGQVISCLKLRYADQRLSSDCEDQIRIIIQESAL
DYRLDPQLQLHCSDEISSLCAEEAAAQEQTGQVEECLKVNLLKIKTELCKKEVLNMLKE
SKADIFVDPVLHTACALDIKHHCAAITPGRGRQMSCLMEALEDKRVRLQPECKKRLND
RIEMWSYAAKVAPADGFSDLAMQVMTSPSKNYILSVISGSICILFLIGLMCGRITKRVTRE
LKDR

Figure 8B

gcgtcgagct cgccgcggac tcaagatggc ggcgtgtgga cgtgtacgga ggatgttccg cttgtcggcg gcgctgcatc
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gtggtatcat gcttggagaa aggcctggtg aaagaagcag aagaaagaga acccaagatt caagtttctg aactctgcaa
gaaagccatt ctccgggtgg ctgagctgtc atcggatgac tttcacttag accggcattt atattttgct tgccgagatg
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ttcggttttc

Figure 8C

ttacaagttt aaaatggcct gcaaggagga cgtgttgaag ctttgcccaa acataaaaaa gaaggtggac gtggtgatct gcctgagcac gaccgtgcgc aatgacactc tgcaggaagc caaggagcac agggtgtccc tgaagtgccg caggcagctc cgtgtggagg agctggagat gacggaggac atccgcttgg agccagatct atacgaagcc tgcaagagtg acatcaaaaa cttctgttcc gctgtgcaat atggcaacgc tcagattatc gaatgtctga aagaaaacaa gaagcagcta agcacccgct gccaccaaaa agtatttaag ctgcaggaga cagagatgat ggacccagag ctagactaca ccctcatgag ggtctgcaag cagatgataa agaggttctg tccggaagca gattctaaaa ccatgttgca gtgcttgaag caaaataaaa acagtgaatt gatggatccc aaatgcaaac agatgataac caagcgccag atcacccaga acacagatta ccgcttaaac cccatgttaa gaaaagcctg taaagctgac attcctaaat tctgtcacgg tatcctgact aaggccaagg atgattcaga attagaagga caagtcatct cttgcctgaa gctgagatat gctgaccagc gcctgtcttc agactgtgaa gaccagatcc gaatcattat ccaggagtcc gccctggact accgcctgga tcctcagctc cagctgcact gctcagacga gatctccagt ctatgtgctg aagaagcagc agcccaagag cagacaggtc aggtggagga gtgcctcaag gtcaacctgc tcaagatcaa aacagaattg tgtaaaaagg aagtgctaaa catgctgaag gaaagcaaag cagacatctt tgttgacccg gtacttcata ctgcttgtgc cctggacatt aaacaccact gcgcagccat caccctggc cgcgggcgtc aaatgtcctg tctcatggaa gcactggagg ataagcgggt gaggttacag cccgagtgca aaaagcgcct caatgaccgg attgagatgt ggagttacgc agcaaaggtg gccccagcag atggcttctc tgatcttgcc atgcaagtaa tgacgtctcc atctaagaac tacattctct ctgtgatcag tgggagcatc tgtatattgt tcctgattgg cctgatgtgt ggacggatca ccaagcgagt gacacgagag ctcaaggaca ggctacaata caggtcagag acaatggctt ataaaggttt agtgtggtct caggatgtga caggcagtcc agcctgacct ttctgcacac tccagacaaa cttcccagac aagctccttt gtgcctctac gtggagaggg tgtggaaagt tatcacatta aaagatggag gatttaaaaa aaaaaaaaaa aaaaaaaaaa aaagaaaaaa aaaaaaaaa

Figure 8C (continued)

MAACGRVRRMFRLSAALHLLLLFAAGAEKLPGQGVHSQGQGPGANFVSFVGQAGGGG
PAGQQLPQLPQSSQLQQQQQQQQQQQQPQPPQPPFPAGGPPARRGGAGAGGGWKLAE
EESCREDVTRVCPKHTWSNNLAVLECLQDVREPENEISSDCNHLLWNYKLNLTTDPKFE
SVAREVCKSTITEIKECADEPVGKGYMVSCLVDHRGNITEYQCHQYITKMTAIIFSDYRLI
CGFMDDCKNDINILKCGSIRLGEKDAHSQGEVVSCLEKGLVKEAEEREPKIQVSELCKK
AILRVAELSSDDFHLDRHLYFACRDDRERFCENTQAGEGRVYKCLFNHKFEESMSEKCR
EALTTRQKLIAQDYKVSYSLAKSCKSDLKKYRCNVENLPRSREARLSYLLMCLESAVHR
GRQVSSECQGEMLDYRRMLMEDFSLSPEIILSCRGEIEHHCSGLHRKGRTLHCLMKVVR
GEKGNLGMNCQQALQTLIQETDPGADYRIDRALNEACESVIQTACKHIRSGDPMILSCL
MEHLYTEKMVEDCEHRLLELQYFISRDWKLDPVLYRKCQGDASRLCHTHGWNETSEF
MPQGAVFSCLYRHAYRTEEQGRRLSRECRAEVQRILHQRAMDVKLDPALQDKCLIDLG
KWCSEKTETGQELECLQDHLDDLVVECRDIVGNLTELESEDIQIEALLMRACEPIIQNFC
HDVADNQIDSGDLMECLIQNKHQKDMNEKCAIGVTHFQLVQMKDFRFSYKFKMACKE
DVLKLCPNIKKKVDVVICLSTTVRNDTLQEAKEHRVSLKCRRQLRVEELEMTEDIRLEP
DLYEACKSDIKNFCSAVQYGNAQIIECLKENKKQLSTRCHQKVFKLQETEMMDPELDYT
LMRVCKQMIKRFCPEADSKTMLQCLKQNKNSELMDPKCKQMITKRQITQNTDYRLNP
MLRKACKADIPKFCHGILTKAKDDSELEGQVISCLKLRYADQRLSSDCEDQIRIIIQESAL
DYRLDPQLQLHCSDEISSLCAEEAAAQEQTGQVEECLKVNLLKIKTELCKKEVLNMLKE
SKADIFVDPVLHTACALDIKHHCAAITPGRGRQMSCLMEALEDKRVRLQPECKKRLND
RIEMWSYAAKVAPADGFSDLAMQVMTSPSKNYILSVISGSICILFLIGLMCGRITKRVTRE
LKDRLQYRSETMAYKGLVWSQDVTGSPA

Figure 8D

caaaaacttc tgttccgctg tgcaatatgg caacgctcag attatcgaat gtctgaaaga aaacaagaag cagctaagca
cccgctgcca ccaaaaagta tttaagctgc aggagacaga gatgatggac ccagagctag actacaccct catgagggtc
tgtaagcaga tgataaagag gttctgtccg gaagcagatt ctaaaaccat gttgcagtgc ttgaagcaaa ataaaaacag
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tgttaagaaa agcctgtaaa gctgacattc ctaaattctg tcacggtatc ctgactaagg ccaaggatga ttcagaatta
gaaggacaag tcatctcttg cctgaagctg agatatgctg accagcgcct gtcttcagac tgtgaagacc agatccgaat
cattatccag gagtccgccc tggactaccg cctggatcct cagctccagc tgcactgctc agacgagatc tccagtctat
gtgctgaaga agcagcagcc caagagcaga caggtcaggt ggaggagtgc ctcaaggtca acctgctcaa gatcaaaaca
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aaggtggccc cagcagatgg cttctctgat cttgccatgc aagtaatgac gtctccatct aagaactaca ttctctctgt gatcagtggg
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gagccacctt gaccaccaaa ggaactacct atccagtgcc cagtttgtac agccctcttg tatagcatcc ccactcacct
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cacagccatg ggtatttctg tggtctcctc gcttcattga agcaaagcat gagccttcct agacaagggc agctggggag
gggaagggac cggaagtttg tgaagttgaa cagtccatcc atctgcactg agaggctgga tcctgagtcc cggggcagca
ggatcccagg aaccttcctc ctccagggca gcacaggact cagccgtgtc tggaccggcc ctgctgaggc tacagtcact
ctggaagctc tgcgcttcat caggaggcag gactgtggcg ggaggggtcc ttgaagatgg gtgtggggag cagtgggtca
ggaagtggga gccagaggtt tgactcactt tgctttattt ttcaggctac aatacaggtc agagacaatg gcttataaag gtttagtgtg
gtctcaggat gtgacaggca gtccagcctg acctttctgc acactccaga caaacttccc agacaagctc ctttgtgcct
ctacgtggag agggtgtgga aagttatcac attaaaagat ggaggattaa aaaaaaaaaa aaaaaaaaaa aaaaaaaa

Figure 8E

MMDPELDYTLMRVCKQMIKRFCPEADSKTMLQCLKQNKNSELMDPKCKQMITKRQIT
QNTDYRLNPMLRKACKADIPKFCHGILTKAKDDSELEGQVISCLKLRYADQRLSSDCED
QIRIIIQESALDYRLDPQLQLHCSDEISSLCAEEAAAQEQTGQVEECLKVNLLKIKTELCK
KEVLNMLKESKADIFVDPVLHTACALDIKHHCAAITPGRGRQMSCLMEALEDKRVRLQ
PECKKRLNDRIEMWSYAAKVAPADGFSDLAMQVMTSPSKNYILSVISGSICILFLIGLMC
GRITKRVTRELKDR

Figure 8F

aatttagcca gctgtggtgg cacatacctg taatcccagc tacttgagag actgaggcag gagaatcact tgaaccggga gatggagttt gcagtgagcc gagatggtgc cactgtactc cagcctgggt gacagagcaa gactctgtct ccaaaaaaaa aaaaaaaaaa aaaaaaaaga catttcaagc tggaagattt ggttccctaa ctttgagcct agctctttca ttaaagtaat aataaaagta gaactctaca tttatataat ggttttgact ttccaaagtg attttcacat ctcagcagtc ctgtgaagga ctaaataagg tgtttcaggg tagacttggc attgtgtttt gcaaagaagg tccaaggcca tgcagctatt tggtgacaga attgaaagta aagcctgatt ctcttgctgc aaggcgactt tgctatctag aagccagggt cactagacaa gatgcagtca acaaataagt ctccagaaca tatgacatct ccagcctaaa ccaagctcac ctttccatgc tggctccctc atgcagacgg aggacatccg cttggagcca gatctatacg aagcctgcaa gagtgacatc aaaaacttct gttccgctgt gcaatatggc aacgctcaga ttatcgaatg tctgaaagaa aacaagaagc agctaagcac ccgctgccac caaaaagtat ttaagctgca ggagacagag atgatggacc cagagctaga ctacaccctc atgagggtct gcaagcagat gataaagagg ttctgtccgg aagcagattc taaaaccatg ttgcagtgct tgaagcaaaa taaaaacagt gaattgatgg atcccaaatg caaacagatg ataaccaagc gccagatcac ccagaacaca ggtaagatct tggcttggct ctcctggccc cgtggagtat ctgaaaagga attcagtggc tgtagagtga cctgctcaaa ctcccagggc tttgttgcct gggaatttta agggaggagt ctgagtgtaa gcagggcctt cctcctttga ggagcatcca gaaaaatgga gggagagtca ggggagagag gaggccacaa gaaccagaaa actgccctaa aagaacgttc agaaggaatc aggccggcag tccttggaaa gaaaaatcta gaaattcaat aaaacttcat gagtgtgcca ggagaatgta cgggtaatct gattcggaac agaaacattt cacctctgag ttggaagacc tcgtaagtta atggtcacag tgagttggat attgtatttc tttttcagtg ttctcaaaag tgtctgttat ggggaaggtt gctgatgtcc ccttgatttt tctgaggact ccttagagta ttggagtctg cacaaaaccc cgcagagtag aaagattcct gaggacctcc agaagtactc gttaacaagt catattgctg attaaaaaca gtgtagtgag agctcagtaa atgtttattg aatagataaa tccatggttg tagtcatgat cattgacata atatgctccc tttaggaagg tggatatcta aaaatgtgtg aatcaggtgg aatgttttgt cacatgctca ctgctttcta ctctagatta ccgcttaaac cccatgttaa gaaaagcctg taaagctgac attcctaaat tctgtcacgg tatcctgact aaggccaagg atgattcaga attagaagga caagtcatct cttgcctgaa gctgagatat gctgaccagc gcctgtcttc agactgtgaa gaccagatcc gaatcattat ccaggagtcc gccctggact accgcctgga tcctcagctc cagctgcact gctcagacga ggtgggattt gcgtgcaaaa ctggttacgc acagagctgc tcagagaagt ttccactgga gaaaagttgt ttactttctc tcccttcagc cgtgaatgat ctggtgaatt gaaggccatc ttctaggctc tccatggtct gcattcctgt tctttgtaac actgaattca acttggcatt agtcctgaca ctctaaagcg ttgttccata tttctctgtt gaacaagggt gttctttcat tatagctctc tgtaaatttg ttcttccctt cttcttattc tggatggtaa acccaagacc tgccagaaag ataaaagtgc tttcagctgg gcacggtggc tcacgcctgt aatcccaaca ctttgggagg ccaaggaggg tggatcatct gaggtcagga gttcaagacc agcctggcta acatggagaa atctgtctct actaaaaata caaaaaatta gccaggcgtg gtggcgtgca ccagtaatct cagctactca ggaggctgag gcaggagaat cacttgaacc cgggaggcgg tggttgcagt gagctgagat catgccactg caccccagcc tgggcgacag aggaagactc tgtctc

Figure 8G

MQTEDIRLEPDLYEACKSDIKNFCSAVQYGNAQIIECLKENKKQLSTRCHQKVFKLQETE
MMDPELDYTLMRVCKQMIKRFCPEADSKTMLQCLKQNKNSELMDPKCKQMITKRQIT
QNTGKILAWLSWPRGVSEKEFSGCRVTCSNSQGFVAWEF

Figure 8H

ctgagaggaa gttttatctg tgcagcccct ctctgaggat ggacacttct cacactacaa agtcctgttt gctgattctt cttgtggccc tactgtgtgc agaaagagct cagggactgg agtgttacca gtgctatgga gtcccatttg agacttcttg cccatcaatt acctgccccc acccctgatgg agtctgtgtt actcaggagg cagcagttat tgtggattct caaacaagga aagtaaagaa caatctttgc ttacccatct gccctcctaa tattgaaagt atggagatcc tgggtactaa ggtcaacgtg aagacttcct gttgccagga agacctctgc aatgtagcag ttcccaatgg aggcagcacc tggaccatgg caggggtgct tctgttcagc ctgagctcag tcctcctgca gaccttgctc tgatggtcct cccaatgacc tccacccttg tccttttatc ctcatgtgca acaattcttc ctggagccct ctagtgatga attatgagtt atagaagctc caaggtggga gtagtgtgtg aaataccatg ttttgccttt atagcccctg ctgggtaggt aggtgctcta atcctctcta gggctttcaa gtctgtactt cctagaatgt cattttgttg tggattgctg ctcatgaccc tggaggcaca cagccagcac agtgaagagg cagaattcca aggtattatg ctatcaccat ccacacataa gtatctgggg tcctgcaatg ttcccacatg tatcctgaat gtccccctgt tgagtccaat aaacccttttg ttctcccaaa aaaaaaaaaa aa

Figure 9A

MDTSHTTKSCLLILLVALLCAERAQGLECYQCYGVPFETSCPSITCPYPDGVCVTQEAAV
IVDSQTRKVKNNLCLPICPPNIESMEILGTKVNVKTSCCQEDLCNVAVPNGGSTWTMAG
VLLFSLSSVLLQTLL

Figure 9B

atctgacaga acttgccact gtgcctgcaa ccttgtctga gaggaaccct tctctgagga tggacacttc tcacactaca aagtcctgtg tgctcattct tcttgtggcc ctactgtgtg cagaaagagc tcagggactg cagtgctacg agtgctatgg agtgccaatt gagacttcct gcccagcagt tacctgccgc gcctctgatg gattctgcat tgctcaaaac atagaattga ttgaggactc tcaaagaagg aaactaaaga cccgtcagtg cctttctttc tgccctgctg gtgtgccaat cagggatcct aacatcaggg agaggacttc ctgttgcagc gaagacctct gcaatgcagc agttcccact gcaggtagca cctggaccat ggcaggggtg cttctgttca gcctgagctc agtcgtcctg cagaccttgc tctgatggtc cttccaatga ccccaccct tttccttta tcttcatgtg caaccactct ttcctggagt cctctagtga caaattatat gttatagaag gtccaatgtg gggatagtgt gtggaacacc ctgtttcacc tttatagccc ctgctgggta agtgcccgac tcctctctag ggctttcaaa tctgtacttc ttgcaatgcc atttagttgt ggatttctat tcttggccct ggaggcatgt ggccagcaca tgcaacaggc agtattccaa ggtattatag tatcaccatc cacacataag tatctggggt cctgcagggt tcccatgtat gcctgtcaat gacccctgtt gagtccaata aaagctttgt tctcccagcc aaaaaaaaaa aaaaaaaaaa aa

Figure 10A

MDTSHTTKSCVLILLVALLCAERAQGLQCYECYGVPIETSCPAVTCRASDGFCIAQNIELI
EDSQRRKLKTRQCLSFCPAGVPIRDPNIRERTSCCSEDLCNAAVPTAGSTWTMAGVLLFS
LSSVVLQTLL

Figure 10B

gcccaccccc gcccagcccg tgcctataag gccttggcaa tgcaggggcc cgcactgctc ccagacgaca tcagagatga
ggacagcatt gctgctcctt gcagccctgg ctgtggctac agggccagcc cttaccctgc gctgccacgt gtgcaccagc
tccagcaact gcaagcattc tgtggtctgc ccggccagct ctcgcttctg caagaccacg aacacagtgg agcctctgag
ggggaatctg gtgaagaagg actgtgcgga gtcgtgcaca cccagctaca ccctgcaagg ccaggtcagc agcggcacca
gctccaccca gtgctgccag gaggacctgt gcaatgagaa gctgcacaac gctgcaccca cccgcaccgc cctcgcccac
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gcccctcatg cctttccttc cctttctctg gggattccac acctctcttc cccagccgca acgggggtgc caggagcccc
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ccccacagag gatgaagcca ccccacagag gatgcagccc ccagctgcat ggaaggtgga ggacagaagc cctgtggatc
cccggatttc acactccttc tgttttgttg ccgtttattt ttgtactcaa atctctacat ggagataaat gatttaaacc agaaaa

Figure 11A

MRTALLLLAALAVATGPALTLRCHVCTSSSNCKHSVVCPASSRFCKTTNTVEPLRGNLV
KKDCAESCTPSYTLQGQVSSGTSSTQCCQEDLCNEKLHNAAPTRTALAHSALSLGLALS
LLAVILAPSL

Figure 11B

gctccggcca gccgcggtcc agagcgcgcg aggttcgggg agctccgcca ggctgctggt acctgcgtcc gcccggcgag caggacaggc tgctttggtt tgtgacctcc aggcaggacg gccatcctct ccagaatgaa gatcttcttg ccagtgctgc tggctgccct tctgggtgtg gagcgagcca gctcgctgat gtgcttctcc tgcttgaacc agaagagcaa tctgtactgc ctgaagccga ccatctgctc cgaccaggac aactactgcg tgactgtgtc tgctagtgcc ggcattggga atctcgtgac atttggccac agcctgagca agacctgttc cccggcctgc cccatcccag aaggcgtcaa tgttggtgtg gcttccatgg gcatcagctg ctgccagagc tttctgtgca atttcagtgc ggccgatggc gggctgcggg caagcgtcac cctgctgggt gccgggctgc tgctgagcct gctgccggcc ctgctgcggt ttggccccctg accgcccaga ccctgtcccc cgatccccca gctcaggaag gaaagcccag ccctttctgg atcccacagt gtatgggagc ccctgactcc tcacgtgcct gatctgtgcc cttggtccca ggtcaggccc acccccctgca cctccacctg ccccagcccc tgcctctgcc caagtgggcc agctgccctc acttctgggg tggatgatgt gaccttcctt gggggactgc ggaagggacg agggttccct ggagtcttac ggtccaacat cagaccaagt cccatggaca tgctgacagg gtccccaggg agaccgtgtc agtagggatg tgtgcctggc tgtgtacgtg ggtgtgcagt gcacgtgaga gcacgtggcg gcttctgggg gccatgtttg gggagggagg tgtgccagca gcctggagag cctcagtccc tgtagccccc tgccctggca cagctgcatg cacttcaagg gcagcctttg ggggttgggg tttctgccac ttccgggtct aggccctgcc caaatccagc cagtcctgcc ccagcccacc cccacattgg agccctcctg ctgctttggt gcctcaaata aatacagatg tcccc

Figure 12A

MKIFLPVLLAALLGVERASSLMCFSCLNQKSNLYCLKPTICSDQDNYCVTVSASAGIGNL
VTFGHSLSKTCSPACPIPEGVNVGVASMGISCCQSFLCNFSAADGGLRASVTLLGAGLLL
SLLPALLRFGP

Figure 12B

actgtgcctg caacctggtc agagaggaag taaggactgg tgtcaggagg gagctgctag gtttgatctg tgcagcccct ctccaaggat ggacagttgt cacactacaa agtcctgtgt actcatcctt cttgtggtcc tattgtgtgc agaaagagct caggggctgg agtgctataa ctgcctggga gtttcacttg gaattgcctg caaatcaatt acctgccccc accctgatgc agtctgcatt tctcagcagg tagaacttat tgtggactct caaagaagga aagtaaagaa caaactctgc tttcctttct gccctgctaa tcttgaaaat atggagatcc tgggtactac tgtcaacgtg aatacttcct gttgcaagga agacctctgc aatgcaccat tttccactgg aggcagcacc tggaccatga caagggtgct tctgttaaat ctgggctcgg tcttcctgca gaccttgctg taaaaggtcc ttccaaggac ctccaccctt gttgttttat cctcatttgc aactattcct tcctggagcc ctctagtgat gaattatgag atattgaagc tccaaggtgg gagtagtgtt tgtggaatac gttgtttcaa ctttatagcc cctgcttggt aaatgcccca ctcctctcta ggaatttcaa atatgtactt cctagaatgc cattttgttg tggcttgcta atcttggccc tggaggcccg tggctagcag agggtagagg cagaattcca aggtattaag ccatcaccat ccacacataa gtgtctgagg ttctgcagga ttctcatgta tgcggcttta tgtccccttg ttgagtccaa taaacccttt gttctcc

Figure 13A

MDSCHTTKSCVLILLVVLLCAERAQGLECYNCLGVSLGIACKSITCPYPDAVCISQQVELI
VDSQRRKVKNKLCFPFCPANLENMEILGTTVNVNTSCCKEDLCNAPFSTGGSTWTMTR
VLLLNLGSVFLQTLL

Figure 13B

ctgcagccag gtctgagagg aagtaaggac tggtgtcagg agggagctgc taggtgacaa agggaagaac cctcaggata gggctgtggt gggagtgaga ttaggaaaga agagctgggt gggtggtgga tgagagaagt aggcagacat gtattcctca gggaaagctg tgtagagggt tggagggagg gaatattgga tggctgagcc gtgtgagagc ccaggggtgt gatcaggggt ctattaactg gctccaactt ccaaggtttt atctgtgcag cccttctcca aggatggaca cttctcacga gataaagtcc tgtgtgctga tccttcttgt gaccctactc tgtgcagaaa gagctcaggg actggagtgt taccagtgct atggagtccc atttgagact tcttgcccat catttacctg cccctaccct gatggattct gtgttgctca ggaggaagaa tttattgcaa actctcaaag aaagagagta aagagccgtt cttgccatcc tttctgccct gatgaaattg aaaagaagtt tatcctggat cctaacacca agatgaatat ttcctgttgc caggaagacc tctgcaatgc agcagttccc actggaggca gctcctggac cacggcaggg gtgcttctgt tcagcctggg ctcagtcctc ctgcagaccc tgatgtgatg gtccccaccc

Figure 14A

MDTSHEIKSCVLILLVTLLCAERAQGLECYQCYGVPFETSCPSFTCPYPDGFCVAQEEEFI
ANSQRKRVKSRSCHPFCPDEIEKKFILDPNTKMNISCCQEDLCNAAVPTGGSSWTTAGV
LLFSLGSVLLQTLM

Figure 14B

gttatcagag gtgagcccgt gctcttcagc ggagaagatc ccctacctgg ccgccggcca ctttctgtgg gccgtggggt cctcaaggag acggcccttg ggctcagggg ctgcgtttcc acacgcgcct ttcccagggc tcccgcgccc gttcctgcct ggccgccggc cgctccaaca gcagcacaag gcgggactca gaaccggcgt tcagggccgc cagcggccgc gaggccctga gatgaggctc caaagacccc gacaggcccc ggcgggtggg aggcgcgcgc cccggggcgg gcggggctcc ccctaccggc cagacccggg gagaggcgcg cggaggctgc gaaggttcca gaagggcggg gaggggggcgc cgcgcgctga ccctccctgg gcaccgctgg ggacgatggc gctgctcgcc ttgctgctgg tcgtggccct accgcgggtg tggacagacg ccaacctgac tgcgagacaa cgagatccag aggactccca gcgaacggac gagggtgaca atagagtgtg gtgtcatgtt tgtgagagag aaaacacttt cgagtgccag aacccaagga ggtgcaaatg gacagagcca tactgcgtta tagcggccgt gaaaatattt ccacgttttt tcatggttgc gaagcagtgc tccgctggtt gtgcagcgat ggagagaccc aagccagagg agaagcggtt tctcctggaa gagcccatgc ccttctttta cctcaagtgt tgtaaaattc gctactgcaa tttagagggg ccacctatca actcatcagt gttcaaagaa tatgctggga gcatgggtga gagctgtggt gggctgtggc tggccatcct cctgctgctg gcctccattg cagccggcct cagcctgtct tgagccacgg gactgccaca gactgagcct tccggagcat ggactcgctc cagaccgttg tcacctgttg cattaaactt gttttctgtt gattacctct tggtttgact tcccagggtc ttgggatggg agagtgggga tcaggtgcag ttggctctta accctcaagg gttctttaac tcacattcag aggaagtcca gatctcctga gtagtgattt tggtgacaag tttttctctt tgaaatcaaa ccttgtaact catttattgc tgatggccac tcttttcctt gactccccte tgcctctgag ggcttcagta ttgatgggga gggaggccta agtaccactc atggagagta tgtgctgaga tgcttccgac ctttcaggtg acgcaggaac actgggggag tctgaatgat tggggtgaag acatccctgg agtgaaggac tcctcagcat gggggggcagt ggggcacacg ttagggctgc ccccattcca gtggtggagg cgctgtggat ggctgctttt cctcaacctt tcctaccaga ttccaggagg cagaagataa ctaattgtgt tgaagaaact tagacttcac ccaccagctg gcacaggtgc acagattcat aaattcccac acgtgtgtgt tcaacatctg aaacttaggc caagtagaga gcatcagggt aaatggcgtt catttctctg ttaagatgca gccatccatg gggagctgag aaatcagact caaagttcca ccaaaaacaa atacaagggg acttcaaaag ttcacgaaaa aattgaatta aaagataaaa attaa

Figure 15A

MRLQRPRQAPAGGRRAPRGGRGSPYRPDPGRGARRLRRFQKGGEGAPRADPPWAPLGT
MALLALLLVVALPRVWTDANLTARQRDPEDSQRTDEGDNRVWCHVCERENTFECQNP
RRCKWTEPYCVIAAVKIFPRFFMVAKQCSAGCAAMERPKPEEKRFLLEEPMPFFYLKCC
KIRYCNLEGPPINSSVFKEYAGSMGESCGGLWLAILLLLASIAAGLSLS

Figure 15B

ggagagagca ggacacagct atggatgccg ccaggagagg agatacacag ccagtgatgt ggaccaccgg atggctgttg ctgctgccgc ttctgctgtg tgaaggagcg caagccctggagtgctacag ctgcgtgcag aaggcggacg atggatgctc tccgcacagg atgaagacagtcaaatgtgg tcccgggggtg gacgtctgta ccgaggccgt gggagcggta gagaccatccacgggcaatt ctctgtggcg gtgcggggct gcggttccgg aatcccgggc aagaacgaccgcggactgga ccttcacggg ctcctggcct tctttcagct acagcagtgc tccgaggaccgatgcaacgc caaactcaac ctcactttgc gaggcctcaa ccctgcaggc aatgagagtgcatatgagcc taacggtgca gagtgctaca gctgtgtggg tctgagccgc gagaagtgccagggctccat gccgccggtc gtgaactgct acaacgccag tggccgtgtc tacaagggctgcttcgatgg taacgtcacc ctgacggcag ccaacgtgac cgtgtcctta cctgtccgaggctgcgtcca ggacgagacc tgcacccggg atggggtgac gggtccagga ttcacactcagcggctcttg ctgtcagggc cccgctgta acgccgacct tcgcaacaag acctatttctcccctcgaat cccacccctа gtcctgctgc ccctccaac caccgcagcc ccatccactcgggcccagaa ctcctccagc acgacctcta cagcagcccc aaccacgacc acctccatcatcaagcccac cacagcccaa gccagccaca cttctcccca tgaaatggat ctcgaagtcatacaggaaga gggggcgtcg ttgagtggag gtgctgcggg ccatggaggt actgcgggccatggaggtgc tgcgggccac caagaccgca gcaatatgga gaagtatcca ggaaagggtg gggcccagat cccagctaaa ggaggctctg gcactctagg gtcctggttg tctgcagttc tgttgactgt ggttgctggc gcgatgctgt gaatgtctca tctcgaaaag tccatctcac tttgtttccc tggccccgtg gtaccaactc tttccatttc tcacttgact ggactggctc cgccccccatc cttcagcatt ctcagttccg actgcactgg tttgcagctt cggaaaacag tcctctgttg taaatattcc gctcgggtgg ccctactttt ttgatgcggc cacagcattc cccctgatgg tgaccaggac agagggaaga gacgtctact ggctgagaga ggcccagaga gtccacggca agcctcctct tcccgttttc ctgaccaggc tggaagatga ccaggcaggt agacaatgga tccatcctcc gagcactgtg cttgcctggc acattgtgcg gaaatctggt cgcctgtctt ccttaggaga ctgtgaacaa ctctacaaca gggtcttgtc tctggcctct ctatgtgttc tgtctggcac aggaaggtgt caataaagat ttagttactt tgtatagtga gttaactaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaa

Figure 16A

MDAARRGDTQPVMWTTGWLLLLPLLLCEGAQALECYSCVQKADDGCSPHRMKTVKC
GPGVDVCTEAVGAVETIHGQFSVAVRGCGSGIPGKNDRGLDLHGLLAFFQLQQCSEDR
CNAKLNLTLRGLNPAGNESAYEPNGAECYSCVGLSREKCQGSMPPVVNCYNASGRVY
KGCFDGNVTLTAANVTVSLPVRGCVQDETCTRDGVTGPGFTLSGSCCQGPRCNADLRN
KTYFSPRIPPLVLLPPPTTAAPSTRAQNSSSTTSTAAPTTTTSIIKPTTAQASHTSPHEMDLE
VIQEEGASLSGGAAGHGGTAGHGGAAGHQDRSNMEKYPGKGGAQIPAKGGSGTLGSW
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Figure 16B

tcgctccccg cgccgtgccc gccgctgagc ccggagtgcg gacaccccag ggatgcctgc gccccagagg accccgcct gcagcccccg cgcctctttc aggccctatc ggagcatgctgcctgcagcc atgaagagcc tcggtctggc gctgctggcc ttgcttctct gcccctcgccggccccatggc ctgtggtgcc aggactgcac cctggccaat tccagccatt gcgctccgaagcagtgccag cccaccgata ccgtttgtgc cagcgtgcgg atcaccgacc ccagcagcagcaggaaggat cattctgtga acaagatgtg tgcttcctcc tgcgacttcg ttaagcggcacttttctca gactatctga tggggttcat taactctggg atcttaaaag tcgacgtggactgctgcgag aaagatttgt gcaacggggc atcggtcgca ggacgcagcc cctgggccctggctgggggg ctcctgctca gcctggggcc tgctcttctc tgggctgggc cctaagaccccctccctccct cctgctgggc tttggagctt gtcccctaag cctgttgctg cccctccccagcctggcctg gctggggctg ggacagcaag ggtttggcat caaggtctga ggctctcaacctccctagat gtgagtgagc cttctccgtt tctccaccag ctccatatcc caagcagctgaatatctcca ggagtccaga catcctggca ggaagctggg gtaggggga ggggagggcaagggactga gaccctccag gtctccaagg ggagggaggt caagccaggg acagcccaacagcctggcct gaggggcatt aactacagag aaataaagtc acttctgagt cttgtgaaaaaaaaaaaaaa aaaaaaaaa aaaaaaaaaa aaaa

Figure 17A

MPAPQRTPACSPRASFRPYRSMLPAAMKSLGLALLALLLCPSPAHGLWCQDCTLANSSH
CAPKQCQPTDTVCASVRITDPSSSRKDHSVNKMCASSCDFVKRHFFSDYLMGFINSGILK
SRRGLLRERFVQRGIGRRTQPLGPGWGAPVQLGAWLFFGLGPKTPPSLLVGFGACPLSL
LVPLPSLAWLGLGQQGFGIKV

Figure 17B

agaagaggcg agacttttt gggtgctccg gatcgccagt agttcttcaa gcctcagcag ccaactcctc cggaggcgct gcgctccgcc ccagggagcg cgaatccaag gagcctgggaccagcctctg ggagcccccg gcgcgggcga tgcgggcgcc gcgggcgaca cctgcggctcctctcggtgg cagccgtcgc ttgggcggca gcagcgcgag cctcggcagc ctcggcagctactgtcgccg cggccagaac agcctccgct gcggtcgtgg tctctgatgc tcttgcccgctcccggccct gccgatccgg gaggatgtgg gttctcggca tcgcagcaac ttttgcggattgttctggc ttccagggct ggcgctgcaa attcagtgct accagtgtga agaattccagctgaacaacg attgctcatc ccctgagttc atcgtaaatt gcaccgtgaa cgttcaagacatgtgtcaga aagaagtgat ggagcaaagt gctgggatca tgtaccggaa gtcgtgtgcatcgtcagcag cctgtctcat tgcttcagct gggtaccagt ccttctgttc ccctgggaaactgaactccg tgtgcatcag ctgctgcaac accctctttt gcaatgggcc gaggcccaagaagagaggca gctctgcctc ggccatcagg ccagggcttc tcaccactct cctgttcttccacttagccc tctgcttggc acactgctga agctaaagga gatgccaacc cctgctgcctcacctgtctg gcccttcgtc tctcaccttc ccgagtctct tctgggtgtc cttttattctgggtagacaa gggagtcttt ttgttccctc ctttcaagta acgcaagatt gccgtgcacaaatacttttg taagctctga accaattcat tctgaatttc tgtgtgtagt tgaagaaaaaagcatggagc agaaagtcca gaccctccca tcccaatctg gttaaccacc gccaaggcta gcctggaaga accagccctt agaagtcatt gagatacgca tctgcctttc ccaaagcctt gagcttccat tctgtcccag taggagtcac agtctattca gagactgctg ctgcgtgaag gtaactttgc ttttgcggga ggggagagcc agtttcggct caaggcttct gaacttgcca ttcatacttc ctgctcctgt aaactatttt ctggggtgga cccagctggt ttggtctctg agccagtctg tggtgactca ggactcaagg gctggggctt agcctctcca ggcttggcct cagtctgaaa agtgcttaag aaaaccttgt tagttctcct ggaggaagag ttactgcgcc gggaggctag gaagatgagg gggctgcggg ctgagctggt gctgtccttg gtggagatga agcgggcacg ctggcgtttc tcttggttgg catgctgcag agtcaggcgg cagcagagca cctgccagaa caccttccgg aactgctgag aggacacgtt gtagaggaga gggttgacca cagagctgag gtagaagaag gtatcagaga agggcaggag gatcatgtat gccctgaagt acgttctggt ccagtcatgt ttgggttttg ctgcagccat gatccgtcgg atctgattgg gcatccaaca cacggccaac gtcaccacaa tcagtcctgg caggcaagaa caggagagaa aaggagacgg ggagagaaac agcatgagaa caaaaataaa taaataaaaa cccataaaat attaagcccc ttggttctgt tgcttactgg ccgagaaacg gtaccaatct ttcagctctg tgcttgtcgg cttcttttg ccactggcaa aggagaattt aatgctgctt caagctcagg ggacttggct atgttaaaaa gcgttaaatg ctttcgacag tgtatttata cttacggctg cctgttaatt ttcaaaatgt tttcattgtt gctcgtgtat ccagaaaata tctcacgttg gccaaaa

Figure 18A

MWVLGIAATFCGLFWLPGLALQIQCYQCEEFQLNNDCSSPEFIVNCTVNVQDMCQKEV
MEQSAGIMYRKSCASSAACLIASAGYQSFCSPGKLNSVCISCCNTPLCNGPRPKKRGSSA
SAIRPGLLTTLLFFHLALCLAHC

Figure 18B

ggcaggcctg agtgaggacc tcgaccatgc aggggacctg gatggtgctg ttggcactga tattgggcac cttcggggag cttgctatgg ccttacagtg ctacacctgt gcgaatcctgtgagtgcatc caactgtgtc accaccaccc actgccacat caatgaaacc atgtgcaagactacgctcta ctcccctggag attgttttcc ctttcctggg ggactccacg gtgaccaagtcctgcgccag caagtgtgag ccttcggatg tggatggcat tgggcaaacc cggccagtgtcctgctgcaa ttctgaccta tgcaacgtgg atggggcacc cagcctgggc agtcctggtggcctgctcct tgccctggca cttttcttgc tcttgggtgt cctgctgtaa agccatggccatctagctcc actcccttgt ccctgacatc ccagttccct aatgcctaga agaaatacaatggccatctg caaaaaaaaa aaaaaaaaaa aaaaaa

Figure 19A

MQGTWMVLLALILGTFGELAMALQCYTCANPVSASNCVTTTHCHINETMCKTTLYSLEI
VFPFLGDSTVTKSCASKCEPSDVDGIGQTRPVSCCNSDLCNVDGAPSLGSPGGLLLALAL
FLLLGVLL

Figure 19B

atgcttttta tggcaggccc tgcagccagc tggtccctga ggcccctggg actccatggc gtcccccaag ccttgtgtgc tgtcctctta acagtgctgg tcatgaagac cttggttctcggtgatacca agctcgagga ccttcacccct cagtccctcc cactaaacaa gtacctgaattgctaccgat gtctgctgga gaccgaagag ctggggtgcc tcctggggtc tgacacctgcctgacacctc tgggcagcag ctgtgtcacc ctgcacataa agaacagcag cggttttaatgtcatggtga gcgactgcta cagcaaggag cagatggtcc attgttcata tacccgtgcttccccggtgt ttggcttttg gatattctat cagtgttgct tcctggattt ctgcaacaatccggacaaca gaaagaatag catgcactag

Figure 20A

MLFMAGPAASWSLRPLGLHGVPQALCAVLLTVLVMKTLVLGDTKLEDLHPQSLPLNKY
LNCYRCLLETEELGCLLGSDTCLTPLGSSCVTLHIKNSSGFNVMVSDCYSKEQMVHCSY
TRASPVFGFWIFYQCCFLDFCNNPDNRKNSMH

Figure 20B

agagctggag acctgggaat ctgctgtcaa ctgctggggc tgtggacatt ctcaggaccc tcaccatgaa acacctcctg ttgctcaccc tgtctgccct actctactgc tgggtctcagctgatactcg atgtcactcc tgctacaaag tccctgtgct gggctgtgtg gatcgccagtcctgccgcct ggagccgggc cacaaatgcc tgacaacaaa cgtgtacctt gggaagatgtgggttttctc taacctgcgc tgcggcacac cagaagagcc ttgtcgggag gtcttcaacgaaaccaacca taagctgggc ctgaactaca acaccacctg ctgtgacaag gataactgtaacagcccggc tccacggccc acacccgcac tggccctcat ctccctcacc tccttggctggcctcggcct ctggttattg cattgagact agctccatgg ctacaatctt accacctgctatagcctgag cctttctccc tgtgtcctca gagctccagc tttccagaat cttctctcctcccaccccct tcttctgaag atcatgtccc tagtcctata ccatttattt catgggactgtacctggagt ggcctttcta gccaccgctc ctctccctca cttgtcacct tccactccattccacccaca cacagacaca cagacacaca gacacaaaga cacacacaca cacacacacacacacacaca cccagtcctt tcccatttcc ttctagaaca ctctacctcc tccactggccactgaaaggc tcccctcctt ggacgcacac tgctgtgcct ctgggatcta agtctggaagaactcctgtc ttgtctccag ggagtgattc caaaaggcgc tggcctcatt gcatgggcctggcttaccag accctctgct tgtccccttc tatcttgaga aataaacatc agtgtctaat

Figure 21A

MKHLLLLTLSALLYCWVSADTRCHSCYKVPVLGCVDRQSCRLEPGHKCLTTNVYLGK
MWVFSNLRCGTPEEPCREVFNETNHKLGLNYNTTCCDKDNCNSPAPRPTPALALISLTS
LAGLGLWLLH

Figure 21B

gcctacttgg cctgcctgcg atgcggtacc aacaccgcac gaagtgtgta cagattccca gttagacagc aggagggacc
tgggagcggc caggggatg ttttatctct aagagaccaagagctcaggc agggcttctg tgccctgctt cctccctggc
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gagcgcgcat gcacatgtgc actcagtgca ggctacagag cccaaaaggc agcactggct gtggtgtccc ctgaggccca
ggcaagatgc taggaggaag ccaatgctgc cccacctga gctcacatgg aacatgcaca ccaccagcag cagcagcaag
cattgagact gacctgtgga cgccataggg cactggcaag gagggtcaga ggcgggtccc tgactcagtg ggtgaggccc
gggaaacatt atcctgttac cctgcgtgtg caagatcatt gtccccagct

Figure 22A

agatggcgtc ctcaaccaaa actgagagga gccccagttc aggtcctccc tcctaccaca agggggtggt gtggaggagg cttgattgcc cttggagaag caccggtact gcagagctgg gggccagctt ctttcatctg tgtctagaca ccgaccagat aggccccaca gtggcaacac tgccacacag ccctacaaga agccctgtgc ctagctagca cagagcccca aaaggtgctc aattaataca gggccaagcc tgccagtggg ggggatgcag attaggggaa cagacccaga tggcctgtcc tgaaccctgt ctggggtggt gtgatgagca tctgtctagc ccactgcagg tggctctaca cactccacaa cagttctgca aaagtgtatg aggtggtcat tactgcgccc ctctcacagg taaaggcact gaggcacgga ggagtgaggc acttcatttt cctgggccat tcaactttcc aggaccaaca cattcaacta tgggtactac tccaatagct ggggttcttt gaggctgggc cccctgaaga tgatagtggc ttcatcaacc agagaatttc agagtgcagt gttgtaggag cctatgaacc tgaaatgtca gaactggagg tttgaggggc tgaggggtag gccaggggtg tctggcccct tgtgtggaga cagagagaga gggaacatgg gatggggtag tagagagaag tgcaaaggag cgtcagcctt tctcagggct aatgctgtca gggacgaggg ctcaagcctg tgagtgttct cacactgtga taaacagtgg ccctcaaca cagacggtgt ccagagtggc cggcagtggt tatctagagt tgcaatctgg aagcctcttg gtagtcactg gagagaggcc gcttgatggg acagcaccaa atgtgtgtgc ttctgtggga tgtgaggaag ctgggtcagc gcatgaagcc aaagcgtcct tcagagcaga ggggtggctg gtctagtcca ccagagacaa gctatccagt gagagtcata ctctgccacc gtctctgtga ttaccttacc ccaaagcaga cggggacggg atgcagagca cccgtgtctt catcttctgc ggcaagcacg tgagttcaca ttctgaaact ctagaaagat ttccaggagt ggggtgtgcc tttgctttgg tgcatggtta cttcctggca agcaccgtgg catcccgcag cactgagtga cctgggctcc tcaagccatc tcattggtga aatgacagtg ccagtaccct ctcagctggc tcttggaggc ctgtgcatgg ggtctgcaca gaggaggccc ccaaactatg catggacgga cacgtgatgc ctagcacttc ccttggttgt gtctctgcca accccaggct ctcacccagc aaggaaatga aatccacttt tatgacacat ctccctcccc cagccagctc cattcaccta tatgccaggg tggtcccttt caatgtctgt cccccattgg atgaataaac aagcgaagga ca

Figure 22A (continued)

MTHLLTVFLVALMGLPVAQALECHVCAYNGDNCFKPMRCPAMATYCMTTRTYFTPYR
MKVRKSCVPSCFETVYDGYSKHASATSCCQYYLCNGAGFATPVTLALVPALLATFWSL
L

Figure 22B

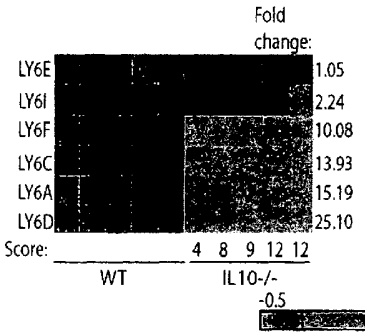


Figure 23 A

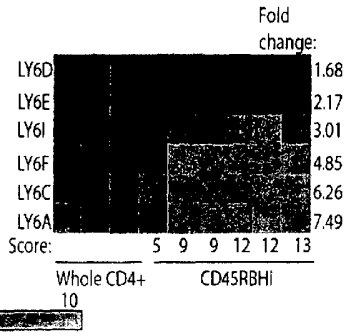


Figure 23 B

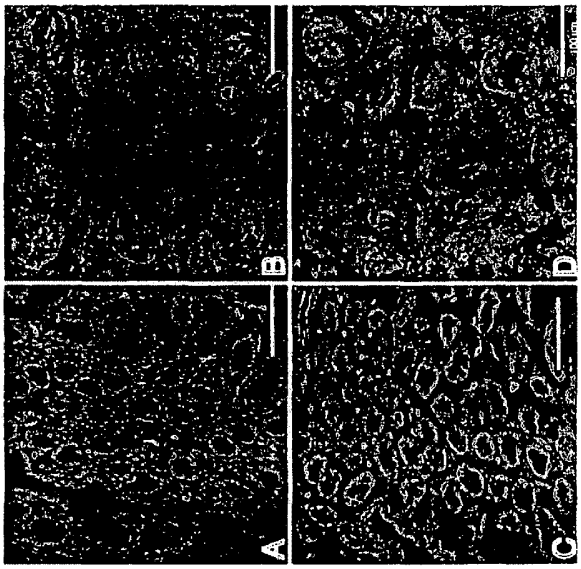


Figure 24

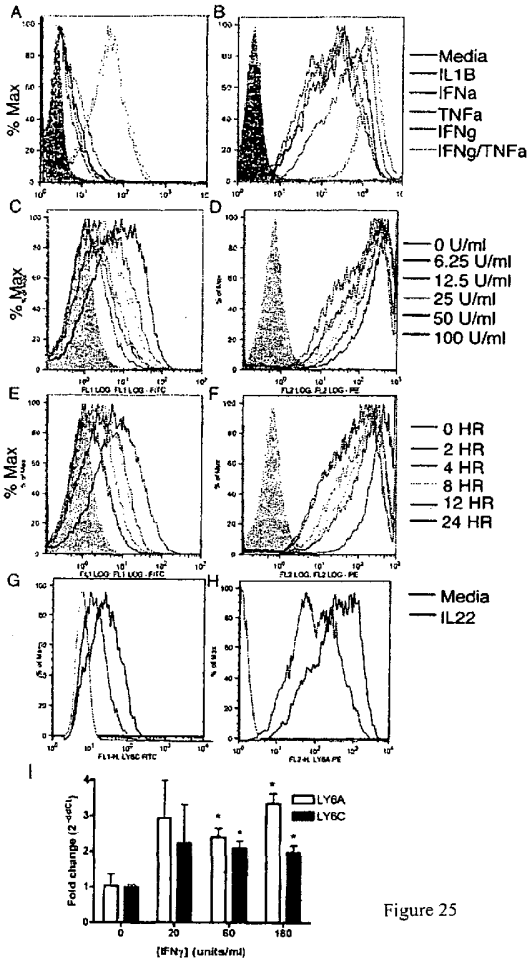
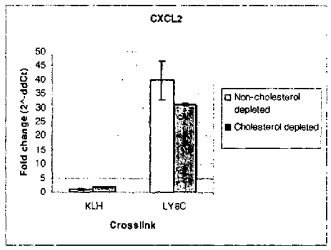
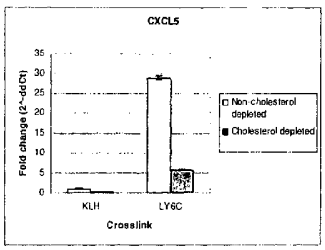

A
B
% Max
Media
IL1B
IFNa
TNFa
IFNg
IFNg/TNFa
C
D
% Max
0 U/ml
6.25 U/ml
12.5 U/ml
25 U/ml
50 U/ml
100 U/ml
E
F
% Max
0 HR
2 HR
4 HR
8 HR
12 HR
24 HR
G
H
Media
IL22
I
LY6A
LY6C
Fold change (2^-ddCt)
0
20
60
180
[IFNγ] (units/ml)

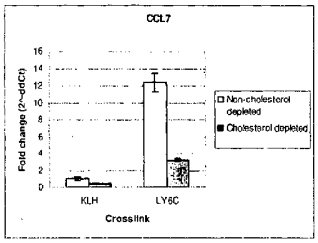
Figure 25



A



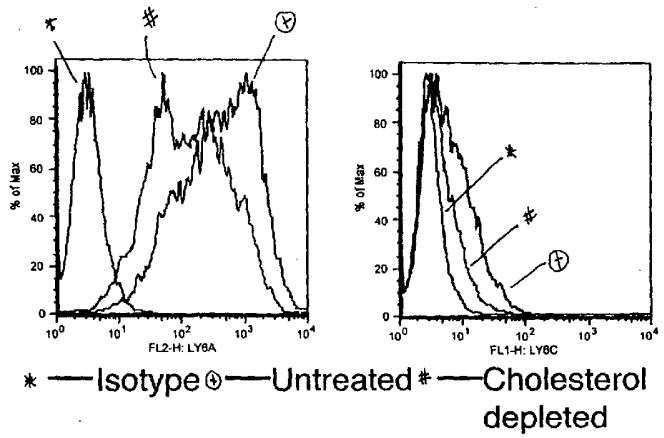
B



C

Figure 26

D



E

Figure 26 (cont)

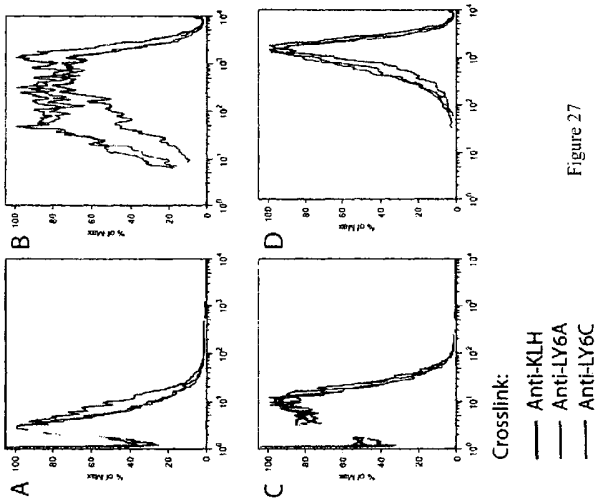


Figure 27

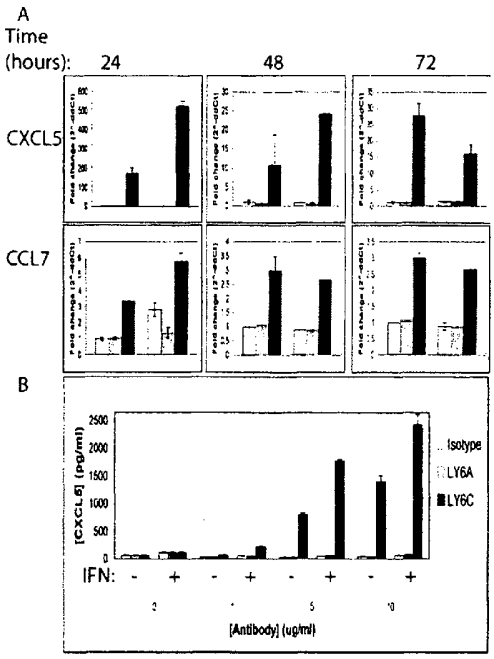

A
Time
(hours):
24
48
72
CXCL5
CCL7
B
[CXCL5] (pg/ml)
2500
2000
1500
1000
500
0
Isotype
LY6A
LY6C
IFN: - + - + - + - +
2
5
10
[Antibody] (ug/ml)

Figure 28

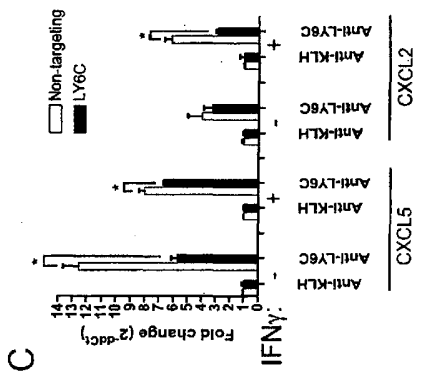


Figure 28 (cont)

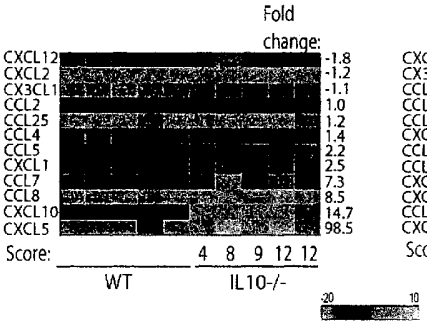


Figure 29 A

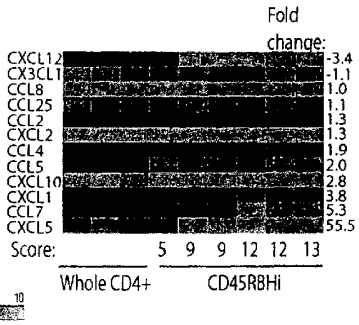


Figure 29 B

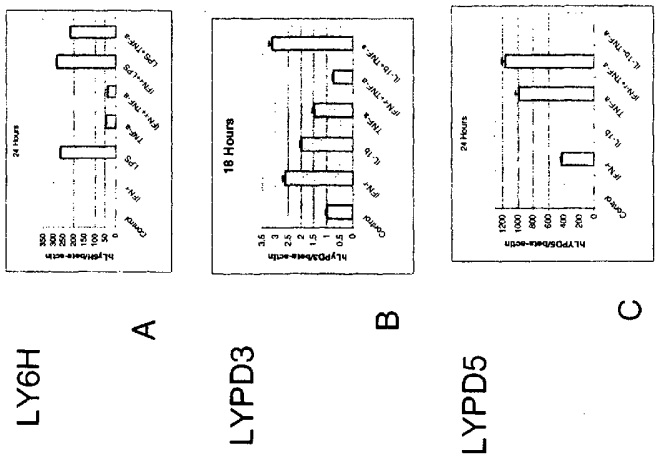


Figure 30

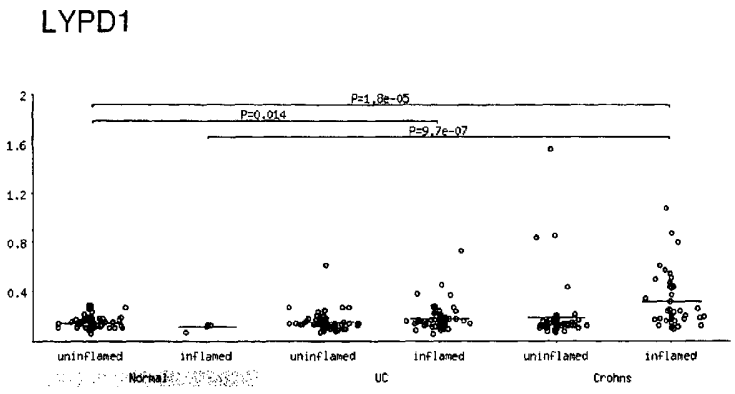

LYPD1
P=1.8e-05
P=0.014
P=9.7e-07
2
1.6
1.2
0.8
0.4
uninflamed
inflamed
uninflamed
inflamed
uninflamed
inflamed
Normal
UC
Crohns

Figure 31 A

LYPD5

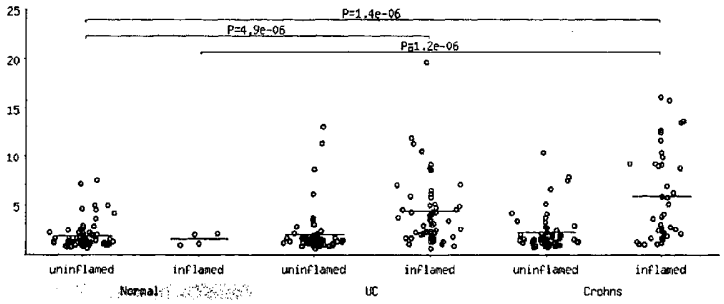


Figure 31 B

Figure 32A

Figure 32B

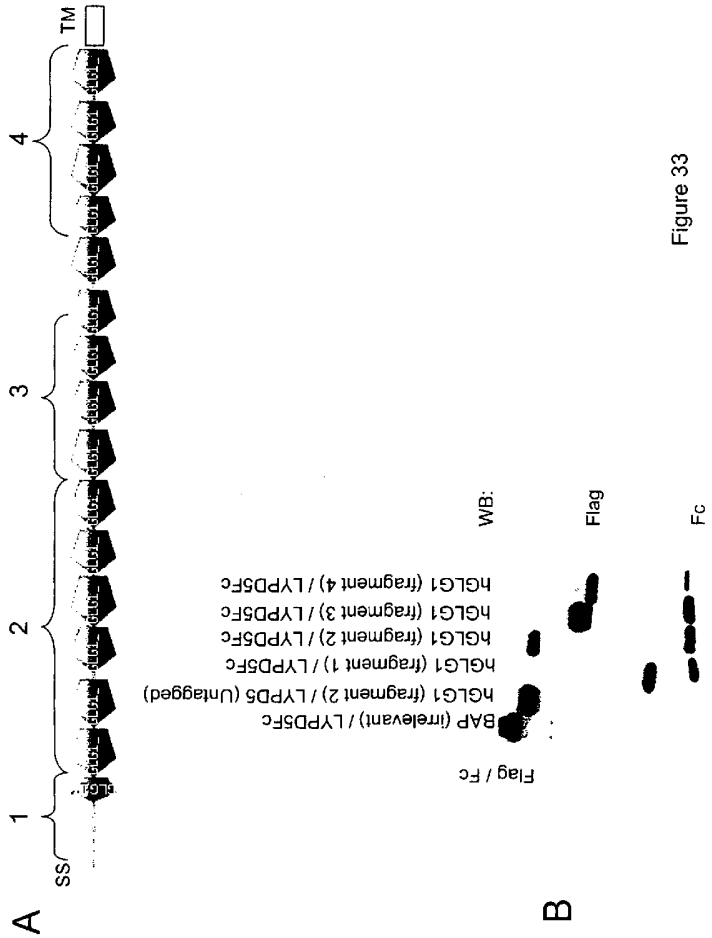

A
SS
1
2
3
4
TM
B
WB:
Flag / Fc
BAP (irrelevant) / LYPD5Fc
hGLG1 (fragment 2) / LYPD5 (Untagged)
hGLG1 (fragment 1) / LYPD5Fc
hGLG1 (fragment 2) / LYPD5Fc
hGLG1 (fragment 3) / LYPD5Fc
hGLG1 (fragment 4) / LYPD5Fc
Flag
Fc

Figure 33

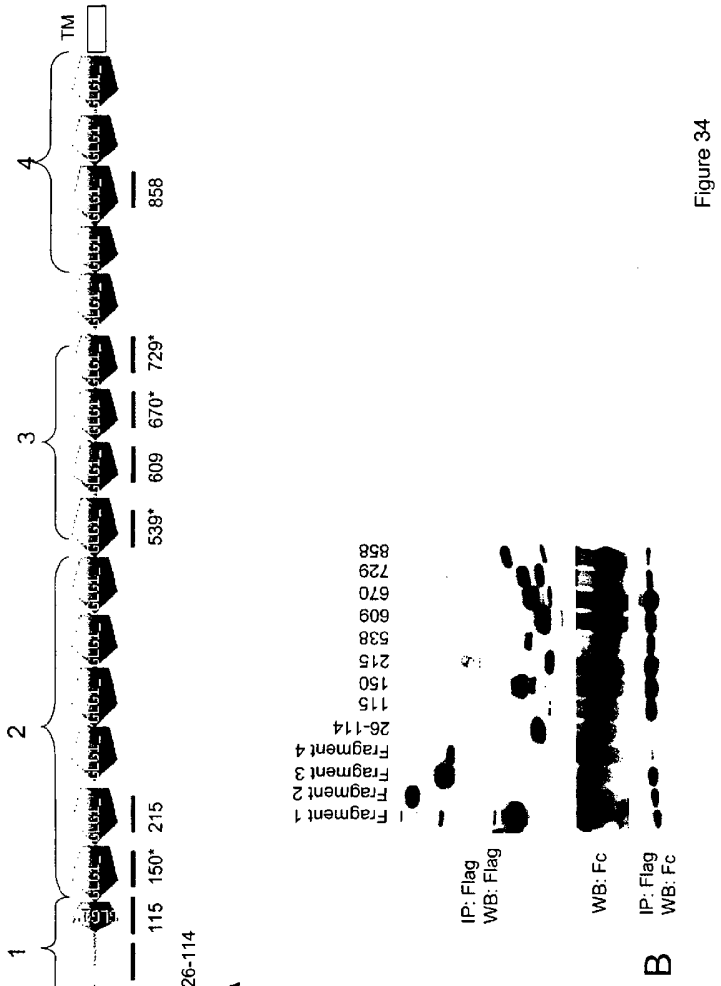

A
SS
1
2
3
4
TM
26-114
115
150*
215
539*
609
670*
729*
858
B
IP: Flag
WB: Flag
WB: Fc
IP: Flag
WB: Fc
Fragment 1
Fragment 2
Fragment 3
Fragment 4
26-114
115
150
215
538
609
670
729
858

Figure 34

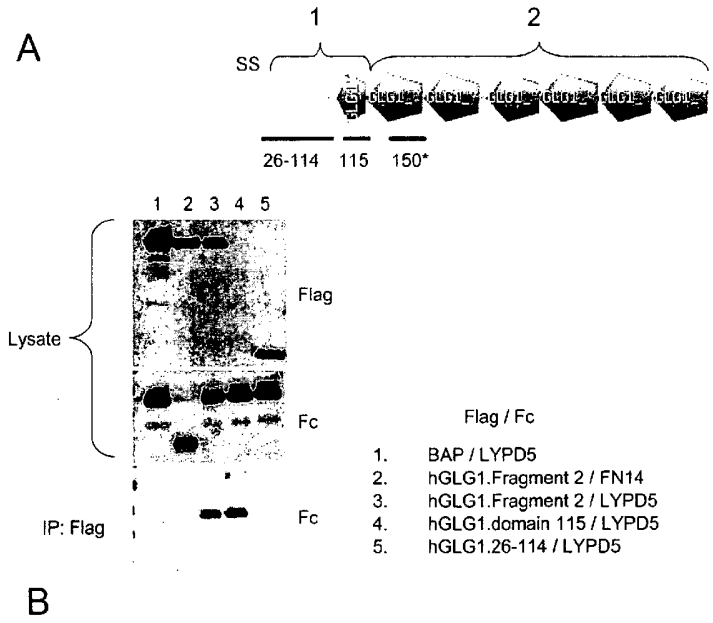

A
1
2
SS
26-114
115
150*
B
1 2 3 4 5
Lysate
Flag
Fc
IP: Flag
Fc
Flag / Fc
1. BAP / LYPD5
2. hGLG1.Fragment 2 / FN14
3. hGLG1.Fragment 2 / LYPD5
4. hGLG1.domain 115 / LYPD5
5. hGLG1.26-114 / LYPD5

Figure 35

cgttgctgtc gctctgcacg cacctatgtg gaaactaaag cccagagaga aagtctgact
tgccccacag ccagtgagtg actgcagcag caccagaatc tggtctgttt cctgtttggc
tcttctacca ctacggcttg ggatctcggg catggtggct ttgccaatgg tccttgtttt
gctgctggtc ctgagcagag gtgagagtga attggacgcc aagatcccat ccacagggga
tgccacagaa tggcggaatc ctcacctgtc catgctgggg tcctgccagc cagccccctc
ctgccagaag tgcatcctct cacaccccag ctgtgcatgg tgcaagcaac tgaacttcac
cgcgtcggga gaggcggagg cgcggcgctg cgcccgacga gaggagctgc tggctcgagg
ctgcccgctg gaggagctgg aggagccccg cggccagcag gaggtgctgc aggaccagcc
gctcagccag ggcgcccgcg gagagggtgc cacccagctg gcgccgcagc gggtccgggt
cacgctgcgg cctggggagc cccagcagct ccaggtccgc ttccttcgtg ctgagggata
cccggtggac ctgtactacc ttatggacct gagctactcc atgaaggacg acctggaacg
cgtgcgccag ctcgggcacg ctctgctggt ccggctgcag gaagtcaccc attctgtgcg
cattggtttt ggttcctttg tggacaaaac ggtgctgccc tttgtgagca cagtaccctc
caaactgcgc cacccctgcc ccacccggct ggagcgctgc cagtcaccat tcagctttca
ccatgtgctg tccctgacgg gggacgcaca agccttcgag cgggaggtgg ggcgccagag
tgtgtccggc aatctggact cgcctgaagg tggcttcgat gccattctgc aggctgcact
ctgccaggag cagattggct ggagaaatgt gtcccggctg ctggtgttca cttcagacga
cacattccat acagctgggg acgggaagtt gggcggcatt ttcatgccca gtgatgggca
ctgccacttg gacagcaatg gcctctacag tcgcagcaca gagtttgact acccttctgt
gggtcaggta gcccaggccc tctctgcagc aaatatccag cccatctttg ctgtcaccag
tgccgcactg cctgtctacc aggagctgag taaactgatt cctaagtctg cagttgggga
gctgagtgag gactccagca acgtggtaca gctcatcatg gatgcttata atagcctgtc
ttccaccgtg acccttgaac actcttcact ccctcctggg gtccacattt cttacgaatc
ccagtgtgag ggtcctgaga agagggaggg taaggctgag gatcgaggac agtgcaacca
cgtccgaatc aaccagacgg tgactttctg ggtttctctc caagccaccc actgcctccc
agagccccat ctcctgaggc tccgggccct tggcttctca gaggagctga ttgtggagtt
gcacacgctg tgtgactgta attgcagtga cacccagccc caggctcccc actgcagtga
tggccaggga cacctacaat gtggtgtatg cagctgtgcc cctggccgcc taggtcggct
ctgtgagtgc tctgtggcag agctgtcctc cccagacctg gaatctgggt gccgggctcc
caatggcaca gggcccctgt gcagtggaaa gggtcactgt caatgtggac gctgcagctg
cagtggacag agctctgggc atctgtgcga gtgtgacgat gccagctgtg agcgacatga
gggcatcctc tgcggaggct ttggtcgctg ccaatgtgga gtatgtcact gtcatgccaa
ccgcacgggc agagcatgcg aatgcagtgg ggacatggac agttgcatca gtcccgaggg
agggctctgc agtgggcatg gacgctgcaa atgcaaccgc tgccagtgct tggacggcta
ctatggtgct ctatgcgacc aatgcccagg ctgcaagaca ccatgcgaga gacaccggga
ctgtgcagag tgtggggcct tcaggactgg cccactggcc accaactgca gtacagcttg
tgcccatacc aatgtgaccc tggccttggc ccctatcttg gatgatggct ggtgcaaaga
gcggaccctg gacaaccagc tgttcttctt cttggtggag gatgacgcca gaggcacggt
cgtgctcaga gtgagacccc aagaaaaggg agcagaccac acgcaggcca ttgtgctggg
ctgcgtaggg ggcatcgtgg cagtgggggt ggggctggtc ctggcttacc ggctctcggt
ggaaatctat gaccgccggg aatacagtcg ctttgagaag gagcagcaac aactcaactg
gaagcaggac agtaatcctc tctacaaaag tgccatcacg accaccatca atcctcgctt

Figure 36A

tcaagaggca gacagtccca ctctctgaag gagggaggga cacttaccca aggctcttct
ccttggagga cagtgggaac tggagggtga gaggaagggt gggtctgtaa gaccttggta
ggggactaat tcactggcga ggtgcggcca ccaccctact tcattttcag agtgacaccc
aagagggctg cttcccatgc ctgcaacctt gcatccatct gggctacccc acccaagtat
acaataaagt cttacctcag aaaaaaaaaa aaaaaaaa

Figure 36A (continued)

MVALPMVLVLLLVLSRGESELDAKIPSTGDATEWRNPHLSMLGSCQPAPSCQKCILSHP
SCAWCKQLNFTASGEAEARRCARREELLARGCPLEELEEPRGQQEVLQDQPLSQGARG
EGATQLAPQRVRVTLRPGEPQQLQVRFLRAEGYPVDLYYLMDLSYSMKDDLERVRQL
GHALLVRLQEVTHSVRIGFGSFVDKTVLPFVSTVPSKLRHPCPTRLERCQSPFSFHHVLSL
TGDAQAFEREVGRQSVSGNLDSPEGGFDAILQAALCQEQIGWRNVSRLLVFTSDDTFHT
AGDGKLGGIFMPSDGHCHLDSNGLYSRSTEFDYPSVGQVAQALSAANIQPIFAVTSAAL
PVYQELSKLIPKSAVGELSEDSSNVVQLIMDAYNSLSSTVTLEHSSLPPGVHISYESQCEG
PEKREGKAEDRGQCNHVRINQTVTFWVSLQATHCLPEPHLLRLRALGFSEELIVELHTLC
DCNCSDTQPQAPHCSDGQGHLQCGVCSCAPGRLGRLCECSVAELSSPDLESGCRAPNGT
GPLCSGKGHCQCGRCSCSGQSSGHLCECDDASCERHEGILCGGFGRCQCGVCHCHANR
TGRACECSGDMDSCISPEGGLCSGHGRCKCNRCQCLDGYYGALCDQCPGCKTPCERHR
DCAECGAFRTGPLATNCSTACAHTNVTLALAPILDDGWCKERTLDNQLFFFLVEDDAR
GTVVLRVRPQEKGADHTQAIVLGCVGGIVAVGLGLVLAYRLSVEIYDRREYSRFEKEQQ
QLNWKQDSNPLYKSAITTTINPRFQEADSPTL

Figure 36B