(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 October 2011 (27.10.2011)

PCT

(10) International Publication Number
WO 2011/133931 A1

(51) International Patent Classification:
A61K 39/395 (2006.01) *C07K 16/24* (2006.01)
A61P 37/00 (2006.01)

(21) International Application Number:
PCT/US2011/033667

(22) International Filing Date:
22 April 2011 (22.04.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/327,028 22 April 2010 (22.04.2010) US

(71) Applicant *(for all designated States except US)*: **GENENTECH, INC.** [US/US]; 1 DNA Way, South San Francisco, CA 94080-4990 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **COX, Jennifer, H.** [US/US]; 157 Ashbury Street, San Francisco, CA 94117 (US). **GHILARDI, Nico, P.** [US/US]; 840 Brookside Lane, Millbrae, CA 94030 (US). **DIEHL, Lauri** [US/US]; 1460 Holidale Court, Los Altos, CA 94024 (US).

(74) Agents: **ZHOU, Jie** et al.; Morrison & Foerster LLP, 755 Page Mill Road, Palo Alto, CA 94304-1018 (US).

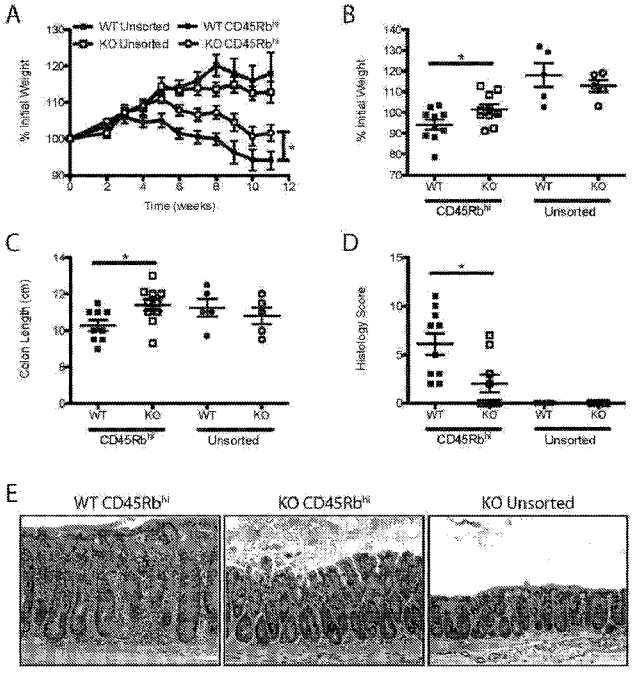
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: USE OF IL-27 ANTAGONISTS FOR TREATING INFLAMMATORY BOWEL DISEASE

Figure 1



(57) Abstract: This invention relates to methods of treating the autoimmune disorder inflammatory bowel disease (including ulcerative colitis and Crohn's disease) with IL-27 antagonists, as well as articles of manufacture comprising IL-27 antagonists.

WO 2011/133931 A1

Published:

— *with international search report (Art. 21(3))*

USE OF IL-27 ANTAGONISTS FOR TREATING INFLAMMATORY BOWEL DISEASE

Cross-Reference to Related Applications

[0001] This application claims the priority benefit of U.S. provisional application serial no. 61/327,028, filed April 22, 2010, which is incorporated herein by reference in its entirety.

Field of the Invention

[0002] This invention relates to compositions and methods for using IL-27 antagonists to treat inflammatory bowel disease.

Background of the Invention

[0003] Interleukin (IL)-27 is a heterodimeric cytokine formed by association of the subunit proteins IL-27p28 and Epstein Bar virus induced protein 3 (Ebi3) (Pflanz et al., *Immunity* 16:779-790 (2002)). It is predominantly expressed by myeloid cells and signals through a heterodimeric receptor that consists of *Il*27ra (WSX-1, TCCR) and gp130 (Pflanz et al., *J Immunol* 172:2225-2231 (2004)) and is expressed throughout the immune system. Most of the studies on IL-27 have been conducted in T-cells, where receptor ligation results in activation of the TH1 transcription factors T-bet and STAT1, and subsequent upregulation of the IL-12Rβ2 chain. Despite this apparent TH1 inducing signaling profile, mice deficient in EBI3 (Ebi3-/-) or IL-27Rα (Il27ra-/-) do not display major defects in the ability to mount TH1 responses, even though TH1 responses are somewhat delayed in a limited number of infectious scenarios (Batten et al., *J Mol Med* 85:661-672 (2007)). Instead, these mice exhibit exacerbated inflammation in response to a wide variety of immune challenges, including pathogens that elicit TH1 and TH2 responses and inflammatory models of disease that rely on TH2 and TH17 activity (Batten et al., *J Mol Med* 85:661-672 (2007); Kastelein et al., *Annu Rev Immunol* 25:221-242 (2007)). Several possible mechanisms for this immunomodulatory activity have been identified: IL-27 is known to antagonize TH17 development (Batten et al., 2006, *Nat Immunol* 7:929-936; Stumhofer et al., 2006, *Nat Immunol* 7:937-945), induce IL-10 production (Awasthi et al., *Nat Immunol* 8:1380-1389 (2007); Batten et al., *J Immunol* 180:2752-2756 (2008); Fitzgerald et al., *Nat Immunol* 8:1372-1379 (2007); Stumhofer et al., *Nat Immunol* 8:1363-1371(2007)), and suppress IL-6-induced T cell proliferation (Batten et al., *Nat Immunol* 7:929-936 (2006)). Nevertheless, IL-27

plays a pro-inflammatory role in some situations. For example, *Il*27ra-/- mice are protected from proteoglycan induced arthritis (PGIA) (Cao et al., *J Immunol* 180:922-930 (2008)), and deletion of the *Il*27ra in the MRL/lpr model of lupus results in lower Th1 cytokine production, diminished anti-dsDNA antibodies, and enhanced survival (Shimizu et al., *J Immunol* 175:7185-7192 (2005)).

[0004] Colitis occurs when tolerance to microbial antigens is broken, resulting in mucosal inflammation. In a recent genome wide association study (GWAS), IL-27p28 was found to be associated specifically with human early onset inflammatory bowel disease (IBD) (Imielinski et al., *Nat Genet 41*:1335-1340 (2009)). Consistent with a proposed immunoregulatory function of IL-27, the risk allele was found to result in lower expression of IL-27 by donor derived lymphoblastoid cell lines. However, two other studies found transcripts for IL-27p28 (Schmidt et al., *Inflamm Bowel Dis* 11:16-23 (2005)) and EBI (Omata et al., *Inflamm Bowel Dis* 7:215-220 (2001)) to be overexpressed in biopsy samples from IBD patients, which would be consistent with either a pro-inflammatory or an ineffective protective role of IL-27 in IBD. Thus, the pathophysiological relevance of IL-27 in human IBD remains unresolved.

[0005] All references cited herein, including patent applications and publications, are hereby incorporated by reference in their entirety.

Summary of the Invention

[0006] The invention provides methods for treating or preventing inflammatory bowel disease (such as Crohn's disease and ulcerative colitis) in an individual comprising administering to the individual an effective amount of an IL-27 antagonist. In certain embodiments, the individual is a human. In some embodiments, the individual has inflammatory bowel disease or is at risk of developing inflammatory bowel disease. In certain embodiments, T cells (such as $CD4^+$ T cells) are detected in a biopsy sample from the intestinal lesion site in the individual.

[0007] In certain embodiments, the IL-27 antagonist inhibits IL-27 signal transduction. In certain embodiments, the IL-27 antagonist inhibits the production of IL-10 (for example, IL-27-induced IL-10 production). In certain embodiments, the IL-27 antagonist inhibits the production of IL-21 (for example, IL-27-induced IL-21 production).

[0008] In certain embodiments, the IL-27 antagonist is an anti-IL-27 antibody that specifically binds to IL-27. In certain embodiments, the IL-27 antagonist is an antibody that specifically binds to the Epstein Barr virus induced protein 3 ("Ebi3") subunit of IL-27 ("IL-27Ebi3"). In

certain embodiments, the anti-IL-27Ebi3 antibody specifically binds to the Ebi3 subunit of IL-27 and blocks its dimerization with the p28 subunit of IL-27. In certain embodiments, the IL-27 antagonist is an antibody that specifically binds to the p28 subunit of IL-27 ("IL-27p28"). In certain embodiments, the anti-IL-27p28 antibody specifically binds to the p28 subunit of IL-27 and blocks its dimerization with the Ebi3 subunit of IL-27.

[0009] In certain embodiments, the IL-27 antagonist is an anti-IL-27 receptor antibody that specifically binds to IL-27Ra.

[0010] In certain embodiments, the antibodies described herein are monoclonal antibodies. In certain other embodiments, the antibodies are antibody fragments selected from the group consisting of Fab, Fab'-SH, Fv, scFv, and $(Fab')_2$ fragments. In certain embodiments, the antibodies are humanized antibodies. In certain embodiments, the antibodies are human antibodies.

[0011] In certain embodiments, the IL-27 antagonist is a small molecule that inhibits binding between IL-27 and its receptor. In certain embodiments, the IL-27 antagonist is a polypeptide that inhibits binding between IL-27 and its receptor. In certain embodiments, the IL-27 antagonist is a short interfering RNA ("siRNA") that inhibits expression of one or both subunits of IL-27, or IL-27Ra. In certain embodiments, the IL-27 antagonist is an RNA or DNA aptamer that binds to IL-27, one or both subunits of IL-27, or to IL-27Ra.

[0012] In certain embodiments, the IL-27 antagonist is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. In certain embodiments, the IL-27 antagonist is used for treating or preventing IBD. In certain embodiments, the IL-27 antagonist is used for treating or preventing ulcerative colitis. In certain embodiments, wasting disease associated with the ulcerative colitis is treated or prevented. In certain embodiments, the IL-27 antagonist is used for treating or preventing Crohn's disease.

[0013] The invention also provides a pharmaceutical composition comprising an IL-27 antagonist for use in treating or preventing inflammatory bowel disease (such as Crohn's disease and ulcerative colitis). The invention also provides use of an IL-27 antagonist in the manufacture of a medicament for treating or preventing inflammatory bowel disease (such as Crohn's disease and ulcerative colitis).

[0014] The invention also provides an article of manufacture comprising an IL-27 antagonist and instructions for using the IL-27 antagonist to treat or prevent inflammatory bowel disease (such as Crohn's disease and ulcerative colitis). Any IL-27 antagonists descried herein may be included in the article of manufacture.

[0015] It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the present invention. These and other aspects of the invention will become apparent to one of skill in the art.

Brief Description of the Drawings

[0016] **Figure 1** shows that the severity of $CD45RB^{hi}$ colitis is decreased in the absence of T cell-derived IL-27R. (A) Relative weight loss following transfer of $CD4^+CD45Rb^{hi}$ or unsorted $CD4^+$ cells from WT or IL-$27Ra^{-/-}$ mice into CB17-SCID recipients. (B) Weight loss relative to initial weight at 12 weeks following transfer of $CD4^+CD45Rb^{hi}$ or unsorted $CD4^+$ cells from WT or IL-27Ra KO mice. (C) Colon length measurements at 12 weeks post transfer. (D) Histological scoring of colitis severity (E) Representative H&E staining of colons from mice transferred with WT $CD45Rb^{hi}$, IL-$27Ra^{-/-}$ $CD45Rb^{hi}$, or IL-$27Ra^{-/-}$ unsorted cells. Data are from one experiment representing three individual experiments. *, $P < 0.05$.

[0017] **Figure 2** shows that transfer of IL-$27Ra^{-/-}$ $CD45Rb^{hi}$ cells reduced TH1 and enhanced TH17 polarization. (A) Representative IFN-γ and IL-22 intracellular cytokine staining of lamina propria isolates stimulated with PMA, ionomycin, and brefeldin A. Samples are gated on $CD4^+$ cells. (B) Quantitative analysis of intracellular IFN-γ, (C) IL-17A, and (D) IL-22 staining in splenocytes (SPLEEN), mesenteric lymph nodes (MLN), and colonic lamina propria lymphocytes (LPL) from CB17-SCID mice transferred with WT or IL-$27Ra^{-/-}$ $CD45Rb^{hi}$ cells. *, $P < 0.05$; * *, $P < 0.01$; * * *, $P < 0.001$. Data are representative of three individual experiments.

[0018] **Figure 3** shows the neutrophil content in the lamina propria of WT and $IL27Ra^{-/-}$ $CD45Rb^{hi}$ recipient mice. (A) Representative flow cytometry of colonic lamina propria isolates 12 weeks post-transfer of $CD45Rb^{hi}$ cells. Lamina propria leukocytes were surface-stained with anti-Gr1 and anti-CD11b. Quantification of percentage (B) and absolute number (C) of $Gr1^{hi}CD11b^+$ cells. Data are from one single experiment.

[0019] **Figure 4** shows that IL-$27Ra^{-/-}$ $CD4^+CD25^+$ cells have normal suppressive function in vitro and in vivo. (A) Proliferation of $CD4^+CD25^-$ responder T cells (T_{eff}) from WT mice

cultured alone or at the indicated ratios with $CD4^+CD25^+$ regulatory T cells (T_{reg}) in the presence of irradiated APCs and soluble anti-CD3. (B) Rescue of wasting disease, measured as percentage of initial weight, in WT $CD45Rb^{hi}$-transferred $Rag2^{-/-}$ recipients either without further treatment, or with administration of WT or IL-$27Ra^{-/-}$ $CD4^+CD25^+$ cells 7 weeks after $CD45Rb^{hi}$ transfer. (C) Terminal percentage of initial weight in untreated $Rag2^{-/-}$ mice and mice treated with WT or IL-$27Ra^{-/-}$ $CD4^+CD25^+$ cells. All groups received $CD45Rb^{hi}$ cells at time 0 to initiate disease. Flow cytometry analysis of Foxp3 expression, gated on $CD4^+$ cells, in the spleen (D) and mesenteric lymph nodes (E) is shown. *, $P < 0.05$. Data in panel A is representative of two experiments and panels B-E are from one single experiment.

[0020] **Figure 5** shows the normal development of nT_{regs} in IL-$27Ra^{-/-}$ mice. Flow cytometry of splenic MACS-purified $CD4^+$ cells (top panels) and FACS-sorted $CD4^+CD45Rb^{hi}$ cells (bottom panels) from WT and IL-$27Ra^{-/-}$ mice. Cells were surfaced-stained with anti-CD4 and stained intracellularly with anti-Foxp3. Data are from one single experiment representative of two different experiments.

[0021] **Figure 6** shows that IL-$27Ra^{-/-}$ $CD45Rb^{hi}$ cells preferentially assume a FoxP3 positive phenotype. (A) Time course of the percentage of $Foxp3^+$ cells relative to $CD4^+$ cells in peripheral blood of mice transferred with $CD45Rb^{hi}$ cells from WT or IL-$27Ra^{-/-}$ donor mice. (B) Representative Foxp3 staining of splenocytes, gated on $CD4^+$ cells, at 12 weeks post-transfer of $CD45Rb^{hi}$ cells. (C and D) Quantification of Foxp3-expressing $CD4^+$ T cells (C) and total $CD4^+$ cells (D), obtained from the blood, spleen, MLN, and lamina propria (LPL) at 12 weeks following transfer. *, $P < 0.05$; * *, $P < 0.01$; * * *, $P < 0.001$. Data are representative of three individual experiments.

[0022] **Figure 7** shows $Il27ra^{-/-}$ $CD45Rb^{hi}$ cells preferentially assume a Foxp3 positive phenotype. (A and B) Frequencies of Foxp3-expressing $CD4^+$ T cells (A) and total $CD4^+$ cells (B), obtained from the spleen, mLN, and lamina propria at 12 weeks following transfer. (C) Absolute number of $CD4^+$ T cells in spleen, mLN, and lamina propria at 12 weeks following transfer. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Data are representative of three individual experiments.

[0023] **Figure 8** shows that IL-27 inhibits the induction of T regulatory cells in vitro. (A) Foxp3 and CD25 staining of FACS-sorted $CD4^+CD25^-$ cells stimulated with αCD3/αCD28 and TGF-β, in the presence or absence of IL-2 and IL-27. (B) Quantification of Foxp3-expressing

cells following 3 days stimulation of WT or IL-27Ra$^{-/-}$ CD4^{+}CD25^{-} cells with increasing amounts of IL-27. (C and D) DO11.10 CD4^{+} cells from WT or IL-27Ra$^{-/-}$ mice were incubated with CD11c^{+}MHCII^{+} dendritic cells isolated from the colonic lamina propria (LPDC) in the presence of $OVA_{323-339}$, TGF-β, with or without IL-27 and stained for Foxp3 and CD25 (C) and CD69 (D). Data are representative of 2 individual experiments.

[0024] **Figure 9** shows that IL-27Ra$^{-/-}$ DO11.10^{+} T cells convert more readily to T regulatory cells in vivo. (A) DO11.10^{+}Rag2$^{-/-}$ CD4^{+} T cells were isolated and confirmed to be Foxp3^{-}CD25^{-}. (B) Representative Foxp3 staining of DO11.10^{+} cells following transfer to *balb/c* mice and oral administration of 1.5% ovalbumin in water for 5 days. (C) Quantitative analysis of percentage Foxp3^{+} cells in the mesenteric lymph nodes (MLN) and spleens of mice transferred with WT or IL-27Ra$^{-/-}$ DO11.10^{+} T cells and fed 1.5% ovalbumin. *, P = 0. 0249 by 2 way ANOVA (diet and genotype). Data is from a single experiment representing two individual experiments.

[0025] **Figure 10** shows Il27ra$^{-/-}$ DO11.10^{+} T cells convert more readily to T regulatory cells in vivo. (A) Quantitative analysis absolute numbers of Foxp3^{+} cells in the mesenteric lymph nodes and spleens of mice transferred with WT or Il27ra$^{-/-}$ DO11.10^{+} T cells and fed 1.5% ovalbumin or control water. (B) Intracellular cytokine staining analysis of IL-2 and IFN-γ (C) in the mesenteric lymph nodes from ovalbumin-fed or control mice. *, P = 0.0249 by 2 way ANOVA (diet and genotype). Data is from a single experiment representing two individual experiments.

[0026] **Figure 11** shows ovalbumin-induced generation of Foxp3^{+} T_{regs} is increased in the absence of *IL-27Ra* in mice with colitis. CB17-SCID mice received 3 × 10^{5} CD45Rb^{hi} cells to induce colitis and 4 weeks later were transferred with 2 ×10^{6} CD4^{+}DO11.10^{+} cells from WT or IL-27Ra$^{-/-}$ mice. Some mice remained on normal water (control) and the remainder were given 1.5% ovalbumin in water (OVA) for 5 days. Absolute numbers of Foxp3^{+} DO11.10^{+} cells were determined from the mesenteric lymph nodes. *, P < 0.05. Data is from one experiment.

[0027] **Figure 12** shows cytokine expression profiles in the colons of mice receiving either WT or IL27Ra$^{-/-}$ CD45Rb^{hi} cells. Colons were collected 12 weeks after cell transfer in RNA later (Ambion) and homogenized with a Tissuelyser (Qiagen). Total RNA from colons was isolated with the RNeasy kit (Qiagen) using DNA-free DNAse I digestion (Ambion). Taqman quantitative RT-PCR was performed with a 7500 Real Time PCR System according to the instructions of the manufacturer (Applied Biosystems). (A) Expression of the gene of interest

was normalized to expression of Rpl19 mRNA. Arbitrary relative expression units were calculated by division of expression of the gene of interest by Rpl19 mRNA expression and multiplication of the result by 1000. Results are from one experiment (n = 5 mice per group). (B) Primer and probe sequences for Taqman analysis in 5' to 3' direction. IL27p28 assay was from Applied Biosystems.

[0028] **Figure 13** shows reduced TH1 and enhanced TH17 polarization following transfer of $Il27ra^{-/-}$ $CD45Rb^{hi}$ cells. (A) Representative IFN-γ and IL-22 intracellular cytokine staining of lamina propria isolates stimulated with PMA, ionomycin, and brefeldin A. Samples are gated on $CD4^+$ cells. (B) Quantitative analysis of intracellular IFN-γ, IL-17A (C), IL-22 (D), and IL-13 (E) staining in splenocytes, mesenteric lymph nodes, and colonic lamina propria lymphocytes from CB17-SCID mice transferred with WT or $Il27ra^{-/-}$ $CD45Rb^{hi}$ cells. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Data are representative of three individual experiments.

[0029] **Figure 14** shows naïve $IL27Ra^{-/-}$ mice have unaltered levels of $Foxp3^+$ T_{regs} and decreased levels of IFN-γ producing $CD4^+$ cells. (A) Spleens, mesenteric lymph nodes (mLN), and colonic lamina propria (cLP) cells were isolated from naïve WT or $IL-27Ra^{-/-}$ mice and analyzed for intracellular Foxp3 levels. (B-D) Alternatively, cells were stimulated with PMA, ionomycin, and brefeldin A and analyzed for intracellular IFN-γ (B), IL-17 (C), and IL-22 (D). ***, $P < 0.001$. Data are representative of two individual experiments.

[0030] **Figure 15** shows transfer of IFN-$\gamma^{-/-}$ $CD45Rb^{hi}$ cells results in increased wasting disease and decreased colitis. (A) CB17-SCID mice were transferred with FACS-sorted $CD45Rb^{hi}$ cells from either WT or IFN-γ KO donors (Jackson Labs, Balb/c background) and monitored for weight loss, which is reported relative to initial weight. (B) Terminal percentage of initial weight at time of 5.5 weeks and (C) histological analysis of colitis. Quantification of frequencies of $Foxp3^+$ cells (D) and absolute numbers of $CD4^+$ T cells (E) in the spleen and mesenteric lymph nodes. *, $P < 0.05$; ***, $P < 0.001$. Data are from one experiment.

[0031] **Figure 16** shows neutrophil content in the lamina propria of WT and $IL27Ra^{-/-}$ CD45Rbhi recipient mice. (A) Representative flow cytometry of colonic lamina propria isolates 12 weeks posttransfer of $CD45Rb^{hi}$ cells. Lamina propria leukocytes were surface-stained with anti-Gr1 and anti-CD11b. Quantification of percentage (B) and absolute number (C) of $Gr1^{hi}CD11b^+$ cells. Data are from one single experiment.

Detailed Description of the Invention

I. General techniques

[0032] The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized methodologies described in Sambrook et al., *Molecular Cloning: A Laboratory Manual* 3d edition (2001) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; *Current Protocols in Molecular Biology* (F.M. Ausubel, et al. eds., (2003)); the series *Methods in Enzymology* (Academic Press, Inc.): *PCR 2: A Practical Approach* (M.J. MacPherson, B.D. Hames and G.R. Taylor eds. (1995)), Harlow and Lane, eds. (1988) *Antibodies, A Laboratory Manual*, and *Animal Cell Culture* (R.I. Freshney, ed. (1987)); *Oligonucleotide Synthesis* (M.J. Gait, ed., 1984); *Methods in Molecular Biology*, Humana Press; *Cell Biology: A Laboratory Notebook* (J.E. Cellis, ed., 1998) Academic Press; *Animal Cell Culture* (R.I. Freshney), ed., 1987); *Introduction to Cell and Tissue Culture* (J.P. Mather and P.E. Roberts, 1998) Plenum Press; *Cell and Tissue Culture: Laboratory Procedures* (A. Doyle, J.B. Griffiths, and D.G. Newell, eds., 1993-8) J. Wiley and Sons; *Handbook of Experimental Immunology* (D.M. Weir and C.C. Blackwell, eds.); *Gene Transfer Vectors for Mammalian Cells* (J.M. Miller and M.P. Calos, eds., 1987); *PCR: The Polymerase Chain Reaction*, (Mullis et al., eds., 1994); *Current Protocols in Immunology* (J.E. Coligan et al., eds., 1991); *Short Protocols in Molecular Biology* (Wiley and Sons, 1999); *Immunobiology* (C.A. Janeway and P. Travers, 1997); *Antibodies* (P. Finch, 1997); *Antibodies: A Practical Approach* (D. Catty., ed., IRL Press, 1988-1989); *Monoclonal Antibodies: A Practical Approach* (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); *Using Antibodies: A Laboratory Manual* (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); *The Antibodies* (M. Zanetti and J. D. Capra, eds., Harwood Academic Publishers, 1995); and *Cancer: Principles and Practice of Oncology* (V.T. DeVita et al., eds., J.B. Lippincott Company, 1993).

II. Definitions

[0033] "Inflammatory bowel disease" or "IBD" refers to the group of disorders that cause the intestines to become inflamed, generally manifested with symptoms including abdominal cramps and pain, diarrhea, weight loss and intestinal bleeding. The main forms of IBD are ulcerative colitis (UC) and Crohn's disease.

[0034] "Ulcerative colitis" or "UC" is a chronic, episodic, inflammatory disease of the large intestine and rectum characterized by bloody diarrhea. Ulcerative colitis is characterized by chronic inflammation in the colonic mucosa and can be categorized according to location: "proctitis" involves only the rectum; "proctosigmoiditis" affects the rectum and sigmoid colon; "left-sided colitis" encompasses the entire left side of the large intestine; and "pancolitis" inflames the entire colon.

[0035] "Crohn's disease," also called "regional enteritis," is a chronic autoimmune disease that can affect any part of the gastrointestinal tract but most commonly occurs in the ileum (the area where the small and large intestine meet). Crohn's disease, in contrast to ulcerative colitis, is characterized by chronic inflammation extending through all layers of the intestinal wall and involving the mesentery as well as regional lymph nodes. Whether or not the small bowel or colon is involved, the basic pathologic process is the same.

[0036] Ulcerative Colitis and Crohn's disease can be distinguished from each other clinically, endoscopically, pathologically, and serologically in more than 90% of cases; the remainder are considered to be indeterminate IBD (Harrison's Principles of Internal medicine, 12th edition, p. 1271 (1991)).

[0037] As used herein, the term "*treatment*" refers to clinical intervention designed to alter the natural course of the individual or cell being treated during the course of clinical pathology. Desirable effects of treatment include decreasing the rate of disease progression, ameliorating or palliating the disease state, and remission or improved prognosis. An individual is successfully "treated", for example, if one or more symptoms associated with inflammatory bowel disease (such as Crohn's disease or ulcerative colitis) are mitigated or eliminated.

[0038] As used herein, the term "*prevention*" includes providing prophylaxis with respect to occurrence or recurrence of a disease in an individual. An individual may be predisposed to, susceptible to IBD, or at risk of developing IBD, but has not yet been diagnosed with the disease.

[0039] As used herein, an individual "at risk" of developing IBD may or may not have detectable disease or symptoms of disease, and may or may not have displayed detectable disease or symptoms of disease prior to the treatment methods described herein. "At risk" denotes that an individual has one or more risk factors, which are measurable parameters that correlate with development of IBD, as known in the art. An individual having one or more of these risk factors

has a higher probability of developing IBD than an individual without one or more of these risk factors.

[0040] An "*effective amount*" refers to at least an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result. An effective amount can be provided in one or more administrations.

[0041] A "*therapeutically effective amount*" is at least the minimum concentration required to effect a measurable improvement of a particular disorder (*e.g.*, IBD). A therapeutically effective amount herein may vary according to factors such as the disease state, age, sex, and weight of the patient, and the ability of the IL-27 antagonist to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the IL-27 antagonist are outweighed by the therapeutically beneficial effects. A "*prophylactically effective amount*" refers to an amount effective, at the dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically but not necessarily, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, a prophylactically effective amount may be less than a therapeutically effective amount.

[0042] "*Chronic*" administration refers to administration of the medicament(s) in a continuous as opposed to acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time. "*Intermittent*" administration refers to treatment that is not consecutively done without interruption, but rather is cyclic in nature.

[0043] As used herein, administration "*in conjunction*" with another compound or composition includes simultaneous administration and/or administration at different times. Administration in conjunction also encompasses administration as a co-formulation or administration as separate compositions, including at different dosing frequencies or intervals, and using the same route of administration or different routes of administration.

[0044] An "*individual*" for purposes of treatment or prevention refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sport, or pet animals, such as dogs, horses, rabbits, cattle, pigs, hamsters, gerbils, mice, ferrets, rats, cats, and the like. Preferably, the individual is human.

[0045] As used herein, the term "*cytokine*" refers generically to proteins released by one cell population that act on another cell as intercellular mediators. Examples of such cytokines include lymphokines, monokines; interleukins ("ILs") such as IL-1, IL-1α, IL-2, IL-3, IL-4, IL-5,

IL-6, IL-7, IL-8, IL-9, IL-11, IL-12, IL-13, IL-15, IL-17A-F, IL-18 to IL-29, IL-31, including PROLEUKIN® rIL-2; a tumor-necrosis factor such as TNF-α or TNF-β, TGF-β1-3; and other polypeptide factors including leukemia inhibitory factor ("LIF"), ciliary neurotrophic factor ("CNTF"), CNTF-like cytokine ("CLC"), cardiotrophin ("CT"), and kit ligand ("KL").

[0046] As used herein, the term "*IL-27*" encompasses native sequence IL-27 heterodimer, native sequence IL-27 components Ebi3 and p28, naturally occurring variants of IL-27 heterodimer, and naturally occurring variants of IL-27 components Ebi3 and p28. IL-27 heterodimer and components thereof may be isolated from a variety of sources, such as from mammalian (including human) tissue types or from another source, or prepared by recombinant and/or synthetic methods.

[0047] As used herein, the term "*IL-27 receptor*" encompasses native sequence IL-27 receptor heterodimer, native sequence IL-27 receptor components IL-27Ra (also known as "WSX-1" or "TCCR") and gp130, naturally occurring variants of IL-27 receptor heterodimer, and naturally occurring variants of IL-27 receptor components IL-27Ra and gp130. IL-27 receptor heterodimer and components thereof may be isolated from a variety of sources, such as from mammalian (including human) tissue types or from another source, or prepared by recombinant and/or synthetic methods.

[0048] As used herein, the term "*IL-27 antagonist*" refers to a molecule that blocks, inhibits, reduces (including significantly), or interferes with IL-27 (mammalian, such as human IL-27) biological activity *in vitro*, *in situ*, and/or *in vivo*, including downstream pathways mediated by IL-27 signaling, such as receptor binding and/or elicitation of a cellular response to IL-27. The term "antagonist" implies no specific mechanism of biological action whatsoever, and expressly includes and encompasses all possible pharmacological, physiological, and biochemical interactions with IL-27 whether direct or indirect, and whether interacting with IL-27, its receptors, or through another mechanism, and its consequences which can be achieved by a variety of different, and chemically divergent, compositions. Exemplary IL-27 antagonists include, but are not limited to, an anti-IL-27 antibody that specifically binds to IL-27 or one or both subunits of IL-27, an anti-sense molecule directed to a nucleic acid encoding a subunit of IL-27, a short interfering RNA ("siRNA") molecule directed to a nucleic acid encoding one or both subunits of IL-27 (*i.e.*, IL-27p28 or IL-27Ebi3) or IL-27Ra, an IL-27 inhibitory compound, an RNA or DNA aptamer that binds to IL-27, one or both subunits of IL-27, or to IL-27Ra, an

IL-27 structural analog, a soluble IL-27Ra protein and fusion polypeptide thereof, and an anti-IL-27Ra antibody. In some embodiments, an IL-27 antagonist (*e.g.*, an antibody) binds (physically interacts with) IL-27, binds to an IL-27Ra, reduces (impedes and/or blocks) downstream IL-27Ra signaling, and/or inhibits (reduces) IL-27 synthesis, production or release. In other embodiments, an IL-27 antagonist binds IL-27 and prevents its binding to its receptor. In still other embodiments, an IL-27 antagonist reduces or eliminates expression (*i.e.*, transcription or translation) of IL-27, an IL-27 subunit, or IL-27Ra. Examples of types of IL-27 antagonists are provided herein.

[0049] The term "immunoglobulin" (Ig) is used interchangeably with "antibody" herein. The term "antibody" herein is used in the broadest sense and specifically covers monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g. bispecific antibodies) formed from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity.

[0050] The basic 4-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains. 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 Ed., Daniel P. Stites, Abba I. Terr and Tristram G. Parslow (eds.), Appleton & Lange, Norwalk, CT, 1994, page 71 and Chapter 6.

[0051] The L chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa ("κ") and lambda ("λ"), based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains (CH), 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 alpha ("α"), delta ("δ"), epsilon ("ε"), gamma ("γ") and mu ("μ"), respectively. The γ and α classes are further divided into subclasses (isotypes) on the basis of relatively minor differences in the CH sequence and function, *e.g.*, humans express the following subclasses: IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The subunit structures and three dimensional configurations of different classes of immunoglobulins are well known and described generally in, for example, Abbas *et al.*, Cellular and Molecular Immunology, 4th ed. (W.B. Saunders Co., 2000).

[0052] "Native antibodies" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains.

[0053] An "*isolated*" antibody is one that has been identified, separated and/or recovered from a component of its production environment (*e.g.*, naturally or recombinantly). Preferably, the isolated polypeptide is free of association with all other contaminant components from its production environment. Contaminant components from its production environment, such as those resulting from recombinant transfected cells, are materials that would typically interfere with research, diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified: (1) to greater than 95% by weight of antibody as determined by, for example, the Lowry method, and in some embodiments, to greater 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 non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody *in situ* within recombinant T-cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, an isolated polypeptide or antibody will be prepared by at least one purification step.

[0054] The "*variable region*" or "*variable domain*" of an antibody refers to the amino-terminal domains of the heavy or light chain of the antibody. The variable domains of the heavy chain and light chain may be referred to as "V_H" and "V_L", respectively. These domains are generally the most variable parts of the antibody (relative to other antibodies of the same class) and contain the antigen binding sites.

[0055] 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 defines the specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the entire span of the variable domains. Instead, it is concentrated in three segments called hypervariable regions (HVRs) both in the light-chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a beta-sheet configuration, connected by three HVRs, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The HVRs in each chain are held together in close proximity by the FR regions and, with the HVRs from the other chain, contribute to the formation of the antigen binding site of antibodies (*see* Kabat et al., *Sequences of Immunological Interest*, Fifth Edition, National Institute of Health, Bethesda, MD (1991)). The constant domains are not involved directly in the binding of antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent-cellular toxicity.

[0056] The term "*monoclonal antibody*" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations and/or post-translation modifications (*e.g.*, isomerizations, amidations) that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. In contrast to polyclonal antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, 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 and Milstein., *Nature*, 256:495-97 (1975); Hongo et al., *Hybridoma,* 14 (3):253-260 (1995), Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2d 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 (1992); 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'l Acad. Sci. USA* 101(34):12467-472 (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. Nat'l Acad. Sci. USA* 90:2551 (1993); Jakobovits *et al.*, *Nature* 362:255-258 (1993); Bruggemann *et al.*, *Year in Immunol.* 7:33 (1993); 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 Biotechnol.* 14:845-851 (1996); Neuberger, *Nature Biotechnol.* 14:826 (1996); and Lonberg and Huszar, *Intern. Rev. Immunol.* 13:65-93 (1995).

[0057] The term "*naked antibody*" refers to an antibody that is not conjugated to a cytotoxic moiety or radiolabel.

[0058] The terms "*full-length antibody*," "*intact antibody*" or "*whole antibody*" are used interchangeably to refer to an antibody in its substantially intact form, as opposed to an antibody fragment. Specifically whole antibodies include those with heavy and light chains including an Fc region. The constant domains may be native sequence constant domains (*e.g.*, human native sequence constant domains) or amino acid sequence variants thereof. In some cases, the intact antibody may have one or more effector functions.

[0059] An "*antibody fragment*" comprises a portion of an intact antibody, preferably the antigen binding and/or the 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 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.

[0060] Papain digestion of antibodies produced 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 (V_H), 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 different antigen-binding activity and is still capable of cross-linking antigen. Fab' fragments differ from Fab fragments by having a few additional 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.

[0061] 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, the region which is also recognized by Fc receptors (FcR) found on certain types of cells.

[0062] "*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 HVRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0063] "*Single-chain Fv*" also abbreviated as "*sFv*" or "*scFv*" are antibody fragments that comprise the VH and VL 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 the sFv, *see* Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

[0064] "*Functional fragments*" of the antibodies of the invention comprise a portion of an intact antibody, generally including the antigen binding or variable region of the intact antibody or the F region of an antibody which retains or has modified FcR binding capability. Examples

of antibody fragments include linear antibody, single-chain antibody molecules and multispecific antibodies formed from antibody fragments.

[0065] The term "*diabodies*" refers to small antibody fragments prepared by constructing sFv fragments (see preceding paragraph) with short linkers (about 5-10) residues) between the V_H and V_L domains such that inter-chain but not intra-chain pairing of the V domains is achieved, thereby resulting in a bivalent fragment, *i.e.*, a fragment having two antigen-binding sites. Bispecific diabodies are heterodimers of two "crossover" sFv fragments in which the V_H and V_L domains of the two antibodies are present on different polypeptide chains. Diabodies are described in greater detail in, for example, EP 404,097; WO 93/11161; Hollinger et al., *Proc. Nat'l Acad. Sci. USA* 90:6444-48 (1993).

[0066] The monoclonal antibodies herein specifically include "*chimeric*" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is 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(are) 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; Morrison et al., *Proc. Nat'l Acad. Sci. USA*, 81:6851-55 (1984)). Chimeric antibodies of interest herein include PRIMATIZED® antibodies wherein the antigen-binding region of the antibody is derived from an antibody produced by, *e.g.*, immunizing macaque monkeys with an antigen of interest. As used herein, "humanized antibody" is used a subset of "chimeric antibodies."

[0067] "*Humanized*" forms of non-human (*e.g.*, murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In one embodiment, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from an HVR of the recipient are replaced by residues from an HVR of a non-human species (donor antibody) such as mouse, rat, rabbit or non-human primate having the desired specificity, affinity, and/or capacity. In some instances, FR residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine antibody performance, such as binding affinity. In general, a 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 sequence, 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 individual FR residue substitutions that improve antibody performance, such as binding affinity, isomerization, immunogenicity, and the like. The number of these amino acid substitutions in the FR is typically no more than 6 in the H chain, and in the L chain, no more than 3. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, *see, e.g.*, 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). *See also*, for example, Vaswani and Hamilton, *Ann. Allergy, Asthma & Immunol.* 1:105-115 (1998); Harris, *Biochem. Soc. Transactions* 23:1035-1038 (1995); Hurle and Gross, *Curr. Op. Biotech.* 5:428-433 (1994); and U.S. Patent Nos. 6,982,321 and 7,087,409.

[0068] A "*human antibody*" is one that possesses an amino-acid sequence corresponding to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks et al., *J. Mol. Biol.*, 222:581 (1991). Also available for the preparation of human monoclonal antibodies are methods described in Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner et al., *J. Immunol.*, 147(1):86-95 (1991). *See also* van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5:368-74 (2001). Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, *e.g.*, immunized xenomice (*see, e.g.*, U.S. Patent Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE™ technology). *See also*, for example, Li et al., *Proc. Nat'l Acad. Sci. USA*, 103:3557-3562 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

[0069] The term "*hypervariable region*," "*HVR*," or "*HV*," when used herein refers to the regions of an antibody-variable domain that are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six HVRs; three in the VH (H1, H2,

H3), and three in the VL (L1, L2, L3). In native antibodies, H3 and L3 display the most diversity of the six HVRs, and H3 in particular is believed to play a unique role in conferring fine specificity to antibodies. *See, e.g.*, Xu et al., *Immunity* 13:37-45 (2000); Johnson and Wu in *Methods in Molecular Biology* 248:1-25 (Lo, ed., Human Press, Totowa, NJ, 2003)). Indeed, naturally occurring camelid antibodies consisting of a heavy chain only are functional and stable in the absence of light chain. See, *e.g.*, Hamers-Casterman et al., *Nature* 363:446-448 (1993) and Sheriff et al., *Nature Struct. Biol.* 3:733-736 (1996).

[0070] A number of HVR delineations are in use and are encompassed herein. The HVRs that are Kabat complementarity-determining regions (CDRs) are based on sequence variability and are the most commonly used (Kabat et al., *supra*). Chothia refers instead to the location of the structural loops (Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)). The AbM HVRs represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody-modeling software. The "contact" HVRs are based on an analysis of the available complex crystal structures. The residues from each of these HVRs are noted below.

Loop	Kabat	AbM	Chothia	Contact
L1	L24-L34	L24-L34	L26-L32	L30-L36
L2	L50-L56	L50-L56	L50-L52	L46-L55
L3	L89-L97	L89-L97	L91-L96	L89-L96
H1	H31-H35B	H26-H35B	H26-H32	H30-H35B (Kabat numbering)
H1	H31-H35	H26-H35	H26-H32	H30-H35 (Chothia numbering)
H2	H50-H65	H50-H58	H53-H55	H47-H58
H3	H95-H102	H95-H102	H96-H101	H93-H101

[0071] HVRs may comprise "extended HVRs" as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2), and 89-97 or 89-96 (L3) in the VL, and 26-35 (H1), 50-65 or 49-65 (a preferred embodiment) (H2), and 93-102, 94-102, or 95-102 (H3) in the VH. The variable-domain residues are numbered according to Kabat et al., *supra*, for each of these extended-HVR definitions.

[0072] "*Framework*" or "*FR*" residues are those variable-domain residues other than the HVR residues as herein defined.

[0073] The phrase "*variable-domain residue-numbering as in Kabat*" or "*amino-acid-position numbering as in Kabat*," and variations thereof, refers to the numbering system used for heavy-chain variable domains or light-chain variable domains of the compilation of antibodies in Kabat et al., *supra*. Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or HVR of the variable domain. For example, a heavy-chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (*e.g.* residues 82a, 82b, and 82c, *etc*. according to Kabat) after heavy-chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

[0074] The Kabat numbering system is generally used when referring to a residue in the variable domain (approximately residues 1-107 of the light chain and residues 1-113 of the heavy chain) (e.g., Kabat et al., Sequences of Immunological Interest. 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). The "EU numbering system" or "EU index" is generally used when referring to a residue in an immunoglobulin heavy chain constant region (e.g., the EU index reported in Kabat *et al., supra*). The "EU index as in Kabat" refers to the residue numbering of the human IgG1 EU antibody. Unless stated otherwise herein, references to residue numbers in the variable domain of antibodies means residue numbering by the Kabat numbering system. Unless stated otherwise herein, references to residue numbers in the constant domain of antibodies means residue numbering by the EU numbering system (*e.g., see* United States Provisional Application No. 60/640,323, Figures for EU numbering).

[0075] An "*acceptor human framework*" as used herein is a framework comprising the amino acid sequence of a VL or VH framework derived from a human immunoglobulin framework or a human consensus framework. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain pre-existing amino acid sequence changes. In some embodiments, the number of pre-existing amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. Where pre-existing amino acid changes are present in a VH, preferable those changes occur at only three, two, or one of

positions 71H, 73H and 78H; for instance, the amino acid residues at those positions may by 71A, 73T and/or 78A. In one embodiment, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

[0076] A "*human consensus framework*" is a framework that represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991). Examples include for the VL, the subgroup may be subgroup kappa I, kappa II, kappa III or kappa IV as in Kabat *et al., supra.* Additionally, for the VH, the subgroup may be subgroup I, subgroup II, or subgroup III as in Kabat *et al., supra.*

[0077] A "*VH subgroup III consensus framework*" comprises the consensus sequence obtained from the amino acid sequences in variable heavy subgroup III of Kabat et al., *supra.*

[0078] A "*VL subgroup I consensus framework*" comprises the consensus sequence obtained from the amino acid sequences in variable light kappa subgroup I of Kabat et al., *supra.*

[0079] An "*amino-acid modification*" at a specified position, *e.g.*, of the Fc region, refers to the substitution or deletion of the specified residue, or the insertion of at least one amino acid residue adjacent the specified residue. Insertion "adjacent" to a specified residue means insertion within one to two residues thereof. The insertion may be N-terminal or C-terminal to the specified residue. The preferred amino acid modification herein is a substitution.

[0080] An "*affinity-matured*" antibody is one with one or more alterations in one or more HVRs thereof that result in an improvement in the affinity of the antibody for antigen, compared to a parent antibody that does not possess those alteration(s). In one embodiment, an affinity-matured antibody has nanomolar or even picomolar affinities for the target antigen. Affinity-matured antibodies are produced by procedures known in the art. For example, Marks et al., *Bio/Technology* 10:779-783 (1992) describes affinity maturation by VH- and VL-domain shuffling. Random mutagenesis of HVR and/or framework residues is described by, for example: Barbas et al. *Proc Nat. Acad. Sci. USA* 91:3809-3813 (1994); Schier et al. *Gene*

169:147-155 (1995); Yelton et al. *J. Immunol.* 155:1994-2004 (1995); Jackson et al., *J. Immunol.* 154(7):3310-9 (1995); and Hawkins *et al, J. Mol. Biol.* 226:889-896 (1992).

[0081] As use herein, the term "*specifically binds to*" or is "*specific for*" refers to measurable and reproducible interactions such as binding between a target and an antibody, that is determinative of the presence of the target in the presence of a heterogeneous population of molecules including biological molecules. For example, an antibody that specifically binds to a target (which can be an epitope) is an antibody that binds this target with greater affinity, avidity, more readily, and/or with greater duration than it binds to other targets. In one embodiment, the extent of binding of an antibody to an unrelated target is less than about 10% of the binding of the antibody to the target as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that specifically binds to a target has a dissociation constant (Kd) of $\leq$ 1μM, $\leq$ 100 nM, $\leq$ 10 nM, $\leq$ 1 nM, or $\leq$ 0.1 nM. In certain embodiments, an antibody specifically binds to an epitope on a protein that is conserved among the protein from different species. In another embodiment, specific binding can include, but does not require exclusive binding.

[0082] A "*blocking*" antibody or an "*antagonist*" antibody is one that inhibits or reduces a biological activity of the antigen it binds. In some embodiments, blocking antibodies or antagonist antibodies substantially or completely inhibit the biological activity of the antigen.

[0083] The term "*solid phase*" describes a non-aqueous matrix to which the antibody of the present invention can adhere. Examples of solid phases encompassed herein include those formed partially or entirely of glass (*e.g.*, controlled pore glass), polysaccharides (*e.g.*, agarose), polyacrylamides, polystyrene, polyvinyl alcohol and silicones. In certain embodiments, depending on the context, the solid 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.

[0084] Antibody "*effector functions*" refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody, and vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity; Fc receptor binding; antibody-dependent-cell-

mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.*, B-cell receptors); and B-cell activation.

[0085] "*Antibody-dependent-cell-mediated cytotoxicity*" or "*ADCC*" refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors ("FcRs") present on certain cytotoxic cells (*e.g.*, natural killer ("NK") cells, neutrophils and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target-cell and subsequently kill the target-cell with cytotoxins. The antibodies "arm" the cytotoxic cells and are required for killing of the target-cell by this mechanism. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in U.S. Patent No. 5,500,362, 5,821,337 or 6,737,056 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells ("PBMC") and NK cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, *e.g.*, in an animal model such as that disclosed in Clynes et al., *Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998).

[0086] The term "*Fc region*" herein is used to define a C-terminal region of an immunoglobulin heavy chain, including native-sequence Fc regions and variant Fc regions. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy-chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during production or purification of the antibody, or by recombinantly engineering the nucleic acid encoding a heavy chain of the antibody. Accordingly, a composition of intact antibodies may comprise antibody populations with all K447 residues removed, antibody populations with no K447 residues removed, and antibody populations having a mixture of antibodies with and without the K447 residue. Suitable native-sequence Fc regions for use in the antibodies of the invention include human IgG1, IgG2, IgG3 and IgG4.

[0087] A "*functional Fc region*" possesses an "*effector function*" of a native sequence Fc region. Exemplary "effector functions" include C1q binding; CDC; Fc receptor binding; ADCC; phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor; BCR), etc. Such

effector functions generally require the Fc region to be combined with a binding domain (e.g., an antibody variable domain) and can be assessed using various assays as disclosed, for example, in definitions herein.

[0088] A "*native sequence Fc region*" comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature. Native sequence human Fc regions include a native sequence human IgG1 Fc region (non-A and A allotypes); native sequence human IgG2 Fc region; native sequence human IgG3 Fc region; and native sequence human IgG4 Fc region as well as naturally occurring variants thereof.

[0089] A "*variant Fc region*" comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification, preferably one or more amino acid substitution(s). Preferably, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, e.g. from about one to about ten amino acid substitutions, and preferably from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of the parent polypeptide. The variant Fc region herein will preferably possess at least about 80% homology with a native sequence Fc region and/or with an Fc region of a parent polypeptide, and most preferably at least about 90% homology therewith, more preferably at least about 95% homology therewith.

[0090] "*Fc receptor*" or "*FcR*" describes a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors, FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif ("ITAM") in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif ("ITIM") in its cytoplasmic domain. (*see, e.g.*, M. Daëron, *Annu. Rev. Immunol.* 15:203-234 (1997)). FcRs are reviewed in Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-92 (1991); Capel et al., *Immunomethods* 4:25-34 (1994); and de Haas et al., *J. Lab. Clin. Med.* 126: 330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein.

[0091] The term "*Fc receptor*" or "*FcR*" also includes the neonatal receptor, "*FcRn*," which is responsible for the transfer of maternal IgGs to the fetus. Guyer et al., *J. Immunol.* 117:587 (1976); and Kim et al., *J. Immunol.* 24:249 (1994). Methods of measuring binding to FcRn are known (*see, e.g.*, Ghetie and Ward, *Immunol. Today* 18: (12):592-98 (1997); Ghetie et al., *Nature Biotechnology* 15(7):637-40 (1997); Hinton et al., *J. Biol. Chem.* 279(8):6213-16 (2004); WO 2004/92219 (Hinton et al.).

[0092] Binding to FcRn *in vivo* and serum half-life of human FcRn high-affinity binding polypeptides can be assayed, *e.g.*, in transgenic mice or transfected human cell lines expressing human FcRn, or in primates to which the polypeptides having a variant Fc region are administered. WO 2004/42072 (Presta) describes antibody variants with improved or diminished binding to FcRs. *See also, e.g.*, Shields et al., *J. Biol. Chem.* 9(2):6591-6604 (2001).

[0093] "*Human effector cells*" are leukocytes which express one or more FcRs and perform effector functions. Preferably, the cells express at least FcγRIII and perform ADCC effector function. Examples of human leukocytes which mediate ADCC include PBMCs, NK cells, monocytes, cytotoxic T-cells and neutrophils, with PBMCs and MNK cells being preferred. The effector cells may be isolated from a native source, *e.g.*, blood.

[0094] "*Complement dependent cytotoxicity*" or "*CDC*" refers to the lysis of a target-cell in the presence of complement. Activation of the classical complement pathway is initiated by the binding of the first component of the complement system (C1q) to antibodies (of the appropriate subclass) which are bound to their cognate antigen. To assess complement activation, a CDC assay, *e.g.*, as described in Gazzano-Santoro *et al., J. Immunol. Methods* 202: 163 (1996), may be performed.

[0095] Polypeptide variants with altered Fc region amino acid sequences and increased or decreased C1q binding capability are described in US patent No. 6,194,551B1 and WO99/51642. The contents of those patent publications are specifically incorporated herein by reference. See, also, Idusogie *et al. J. Immunol.* 164: 4178-4184 (2000).

[0096] "*Binding affinity*" generally refers to the strength of the sum total of non-covalent interactions between a single binding site of a molecule (*e.g.*, an antibody) and its binding partner (*e.g.*, an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity that reflects a 1:1 interaction between members of a binding pair (*e.g.*, antibody and antigen). The affinity of a molecule X for its partner Y can generally be

represented by the dissociation constant ("Kd," see below). Affinity can be measured by common methods known in the art, including those described herein. Low-affinity antibodies generally bind antigen slowly and tend to dissociate readily, whereas high-affinity antibodies generally bind antigen faster and tend to remain bound longer. A variety of methods of measuring binding affinity are known in the art, any of which can be used for purposes of the present invention. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

[0097] In one embodiment, the "*Kd*" or "*Kd value*" according to this invention is measured by a radiolabeled antigen-binding assay (RIA) performed with the Fab version of an antibody of interest and its antigen as described by the following assay. Solution-binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (*see, e.g.*, Chen *et al.*, *J. Mol. Biol.* 293:865-881 (1999)). To establish conditions for the assay, microtiter plates (DYNEX Technologies, Inc., Chantilly, VA) are coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs, Cochranville, PA) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620, Nalge Nunc International , Rochester, NY), 100 pM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest (*e.g.*, consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta *et al.*, *Cancer Res.* 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (*e.g.*, about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (*e.g.*, for one hour). The solution is then removed and the plate washed eight times with 0.1% TWEEN-20™ surfactant in PBS. When the plates have dried, 150 μl/well of scintillant (MICROSCINT-20™; Packard) is added, and the plates are counted on a TOPCOUNT™ gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

[0098] According to another embodiment, the *Kd* is measured by using surface-plasmon resonance assays using a BIACORE®-2000 or a BIACORE®-3000 instrument (BIAcore, Inc., Piscataway, NJ) at 25°C with immobilized antigen CM5 chips at ~10 response units (RU).

Briefly, carboxymethylated dextran biosensor chips (CM5, BIAcore Inc.) are activated with N-ethyl-N'- (3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 μg/ml (~0.2 μM) before injection at a flow rate of 5 μl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% TWEEN 20™ surfactant (PBST) at 25°C at a flow rate of approximately 25 μl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIAcore® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (Kd) is calculated as the ratio k_{off}/k_{on}. See, *e.g.*, Chen *et al.*, *J. Mol. Biol.* 293:865-881 (1999). If the on-rate exceeds 10^6 M^{-1} s^{-1} by the surface-plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence-emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25°C of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow-equipped spectrophotometer (Aviv Instruments) or a 8000-series SLM-AMINCO™ spectrophotometer (ThermoSpectronic, Madison, WI) with a stirred cuvette.

[0099] An "*on-rate*," "*rate of association*," "*association rate*," or "k_{on}" according to this invention can also be determined as described above using a BIACORE®-2000 or a BIACORE®-3000 system (BIAcore, Inc., Piscataway, NJ).

[0100] The phrase "*substantially reduced*," or "*substantially different*," as used herein, denotes a sufficiently high degree of difference between two numeric values (generally one associated with a molecule and the other associated with a reference/comparator molecule) such that one of skill in the art would consider the difference between the two values to be of statistical significance within the context of the biological characteristic measured by said values (*e.g.*, Kd values). The difference between said two values is, for example, greater than about 10%, greater than about 20%, greater than about 30%, greater than about 40%, and/or greater than about 50% as a function of the value for the reference/comparator molecule.

[0101] The term "*substantially similar*" or "*substantially the same*," as used herein, denotes a sufficiently high degree of similarity between two numeric values (for example, one associated with an antibody of the invention and the other associated with a reference/comparator antibody), such that one of skill in the art would consider the difference between the two values to be of little or no biological and/or statistical significance within the context of the biological characteristic measured by said values (*e.g.*, Kd values). The difference between said two values is, for example, less than about 50%, less than about 40%, less than about 30%, less than about 20%, and/or less than about 10% as a function of the reference/comparator value.

[0102] As used herein, "*percent (%) amino acid sequence identity*" and "*homology*" with respect to a peptide, polypeptide or antibody sequence refers to the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific peptide or 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, authored by Genentech, Inc. The source code of ALIGN-2 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. 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.

[0103] In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A.

[0104] Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

[0105] An "*isolated*" nucleic acid molecule encoding the antibodies herein 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 environment in which it was produced. Preferably, the isolated nucleic acid is free of association with all components associated with the production environment. The isolated nucleic acid molecules encoding the polypeptides and antibodies herein is in a form other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from nucleic acid encoding the polypeptides and antibodies herein existing naturally in cells.

[0106] The term "*vector*," as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid," which refers to a circular double stranded DNA into which additional DNA segments may be ligated. Another type of vector is a phage vector. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors," or simply, "expression vectors." In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification,

"plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector.

[0107] "*Polynucleotide*," or "*nucleic acid*," as used interchangeably herein, refer to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase or by a synthetic reaction. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. If present, modification to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may comprise modification(s) made after synthesis, such as conjugation to a label. Other types of modifications include, for example, "caps," substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example, proteins (e.g., nucleases, toxins, antibodies, signal peptides, ply-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotides(s). Further, any of the hydroxyl groups ordinarily present in the sugars may be replaced, for example, by phosphonate groups, phosphate groups, protected by standard protecting groups, or activated to prepare additional linkages to additional nucleotides, or may be conjugated to solid or semi-solid supports. The 5' and 3' terminal OH can be phosphorylated or substituted with amines or organic capping group moieties of from 1 to 20 carbon atoms. Other hydroxyls may also be derivatized to standard protecting groups. Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including, for example, 2'-O-methyl-, 2'-O-allyl-, 2'-fluoro- or 2'-azido-ribose, carbocyclic sugar analogs, α-anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs, and basic nucleoside analogs such as methyl riboside. One or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups include, but are not limited to, embodiments

wherein phosphate is replaced by P(O)S ("thioate"), P(S)S ("dithioate"), (O)NR2 ("amidate"), P(O)R, P(O)OR', CO, or CH2 ("formacetal"), in which each R or R' is independently H or substituted or unsubstituted alkyl (1-20 C) optionally containing an ether (-O-) linkage, aryl, alkenyl, cycloalkyl, cycloalkenyl or araldyl. Not all linkages in a polynucleotide need be identical. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

[0108] "*Oligonucleotide*," as used herein, generally refers to short, generally single-stranded, generally synthetic polynucleotides that are generally, but not necessarily, less than about 200 nucleotides in length. The terms "oligonucleotide" and "polynucleotide" are not mutually exclusive. The description above for polynucleotides is equally and fully applicable to oligonucleotides.

[0109] The term "*epitope tagged*" when used herein refers to a chimeric polypeptide comprising a polypeptide or antibody described herein 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 activity of the polypeptide to which it is fused. The tag polypeptide preferably also is fairly unique so that the 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).

[0110] 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. The Ig fusions preferably include the substitution of a domain of a polypeptide or antibody described herein in the place of at least one variable region within an Ig molecule. In a particularly preferred embodiment, the immunoglobulin

fusion includes the hinge, C_H2 and C_H3, or the hinge, C_H1, C_H2 and C_H3 regions of an IgG1 molecule. For the production of immunoglobulin fusions see also US Patent No. 5,428,130 issued June 27, 1995. For example, useful immunoadhesins as medicaments include polypeptides that comprise a ligand binding subunit of IL-27 receptor or a receptor binding subunit of IL-27 is fused to a constant domain of an immunoglobulin sequence.

[0111] A "*fusion protein*" and a "*fusion polypeptide*" refer to a polypeptide having two portions covalently linked together, where each of the portions is a polypeptide having a different properly. The property may be a biological property, such as activity *in vitro* or *in vivo*. The property may also be simple chemical or physical property, such as binding to a target molecule, catalysis of a reaction, *etc*. The two portions may be linked directly by a single peptide bond or through a peptide linker will be in reading frame with each other.

[0112] As used herein, the term "*RNA interference*" or "*RNAi*" refers generally to a process in which a double-stranded RNA molecule or a short hairpin RNA molecule reducing or inhibiting the expression of a nucleic acid sequence with which the double-stranded or short hairpin RNA molecule shares substantial or total homology. The term "*short interfering RNA*" or "*siRNA*" or "*RNAi agent*" refers to an RNA sequence that elicits RNA interference. *See* Kreutzer et al., WO 00/44895; Zernicka-Goetz et al., WO 01/36646; Fire, WO 99/32619; Mello and Fire, WO 01/29058. As used herein, siRNA molecules include RNA molecules encompassing chemically modified nucleotides and non-nucleotides. The term "*ddRNAi agent*" refers to a DNA-directed RNAi agent that is transcribed from an exogenous vector. The terms "*short hairpin RNA*" or "*shRNA*" refer to an RNA structure having a duplex region and a loop region. In certain embodiments, ddRNAi agents are expressed initially as shRNAs.

[0113] As used herein, the term "*aptamer*" refers to a heterologous oligonucleotide capable of binding tightly and specifically to a desired molecular target, such as, for example, common metabolic cofactors (*e.g.*, Coenzyme A, S-adenosyl methionine, and the like), proteins (*e.g.*, complement protein C5, antibodies, and the like), or conserved structural elements in nucleic acid molecules (*e.g.*, structures important for binding of transcription factors and the like). Aptamers typically comprise DNA or RNA nucleotide sequences ranging from about 10 to about 100 nucleotides in length, from about 10 to about 75 nucleotides in length, from about 10 to about 50 nucleotides in length, from about 10 to about 35 nucleotides in length, and from about 10 to about 25 nucleotides in length. Synthetic DNA or RNA oligonucleotides can be made

using standard solid phase phosphoramidite methods and equipment, such as by using a 3900 High Throughput DNA Synthesizer™, available from Applied Biosystems (Foster City, CA). Aptamers frequently incorporate derivatives or analogs of the commonly occurring nucleotides found in DNA and RNA (*e.g.*, A, G, C, and T/U), including backbone or linkage modifications (*e.g.*, peptide nucleic acid (PNA) or phosphothioate linkages) to increase resistance to nucleases, binding avidity, or to otherwise alter their pharmacokinetic properties. Exemplary modifications are set forth in U.S. Patent Nos. 6,455,308; 4,469,863; 5,536,821; 5,541,306; 5,637,683; 5,637,684; 5,700,922; 5,717,083; 5,719,262; 5,739,308; 5,773,601; 5,886,165; 5,929,226; 5,977,296; 6,140,482; and in WIPO publications WO 00/56746 and WO 01/14398. Methods for synthesizing oligonucleotides comprising such analogs or derivatives are disclosed, for example, in the patent publications cited above, and in U.S. Patent Nos. 6,455,308; 5,614,622; 5,739,314; 5,955,599; 5,962,674; 6,117,992; and in WO 00/75372.

[0114] A "*stable*" formulation is one in which the protein therein essentially retains its physical and chemical stability and integrity upon storage. Various analytical techniques for measuring protein stability are available in the art and are reviewed in *Peptide and Protein Drug Delivery,* pp. 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, New York, Pubs. (1991) and Jones, A. *Adv. Drug Delivery Rev.* 10:29-90 (1993). Stability can be measured at a selected temperature for a selected time period. For rapid screening, the formulation may be kept at 40°C for 2 weeks to 1 month, at which time stability is measured. Where the formulation is to be stored at 2-8°C, generally the formulation should be stable at 30°C or 40°C for at least 1 month and/or stable at 2-8°C for at least 2 years. Where the formulation is to be stored at 30°C, generally the formulation should be stable for at least 2 years at 30°C and/or stable at 40°C for at least 6 months. For example, the extent of aggregation during storage can be used as an indicator of protein stability. Thus, a "stable" formulation may be one wherein less than about 10% and preferably less than about 5% of the protein are present as an aggregate in the formulation. In other embodiments, any increase in aggregate formation during storage of the formulation can be determined.

[0115] A "*reconstituted*" formulation is one which has been prepared by dissolving a lyophilized protein or antibody formulation in a diluent such that the protein is dispersed throughout. The reconstituted formulation is suitable for administration (e.g. parenteral

administration) to a patient to be treated with the protein of interest and, in certain embodiments of the invention, may be one which is suitable for subcutaneous administration.

[0116] An "*isotonic*" formulation is one which has essentially the same osmotic pressure as human blood. Isotonic formulations will generally have an osmotic pressure from about 250 to 350 mOsm. The term "hypotonic" describes a formulation with an osmotic pressure below that of human blood. Correspondingly, the term "hypertonic" is used to describe a formulation with an osmotic pressure above that of human blood. Isotonicity can be measured using a vapor pressure or ice-freezing type osmometer, for example. The formulations of the present invention are hypertonic as a result of the addition of salt and/or buffer.

[0117] "*Carriers*" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers that 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 TWEEN™, polyethylene glycol (PEG), and PLURONICS™.

[0118] A "*package insert*" refers to instructions customarily included in commercial packages of medicaments that contain information about the indications customarily included in commercial packages of medicaments that contain information about the indications, usage, dosage, administration, contraindications, other medicaments to be combined with the packaged product, and/or warnings concerning the use of such medicaments, *etc.*

[0119] A "*pharmaceutically acceptable acid*" includes inorganic and organic acids which are non toxic at the concentration and manner in which they are formulated. For example, suitable inorganic acids include hydrochloric, perchloric, hydrobromic, hydroiodic, nitric, sulfuric, sulfonic, sulfinic, sulfanilic, phosphoric, carbonic, *etc.* Suitable organic acids include straight and branched-chain alkyl, aromatic, cyclic, cycloaliphatic, arylaliphatic, heterocyclic, saturated, unsaturated, mono, di- and tri-carboxylic, including for example, formic, acetic, 2-hydroxyacetic,

trifluoroacetic, phenylacetic, trimethylacetic, *t*-butyl acetic, anthranilic, propanoic, 2-hydroxypropanoic, 2-oxopropanoic, propandioic, cyclopentanepropionic, cyclopentane propionic, 3-phenylpropionic, butanoic, butandioic, benzoic, 3-(4-hydroxybenzoyl)benzoic, 2-acetoxy-benzoic, ascorbic, cinnamic, lauryl sulfuric, stearic, muconic, mandelic, succinic, embonic, fumaric, malic, maleic, hydroxymaleic, malonic, lactic, citric, tartaric, glycolic, glyconic, gluconic, pyruvic, glyoxalic, oxalic, mesylic, succinic, salicylic, phthalic, palmoic, palmeic, thiocyanic, methanesulphonic, ethanesulphonic, 1,2-ethanedisulfonic, 2-hydroxyethanesulfonic, benzenesulphonic, 4-chorobenzenesulfonic, napthalene-2-sulphonic, *p*-toluenesulphonic, camphorsulphonic, 4-methylbicyclo[2.2.2]-oct-2-ene-1-carboxylic, glucoheptonic, 4,4'-methylenebis-3-(hydroxy-2-ene-1-carboxylic acid), hydroxynapthoic.

[0120] "*Pharmaceutically-acceptable bases*" include inorganic and organic bases which are non-toxic at the concentration and manner in which they are formulated. For example, suitable bases include those formed from inorganic base forming metals such as lithium, sodium, potassium, magnesium, calcium, ammonium, iron, zinc, copper, manganese, aluminum, N-methylglucamine, morpholine, piperidine and organic nontoxic bases including, primary, secondary and tertiary amines, substituted amines, cyclic amines and basic ion exchange resins, [*e.g.*, $N(R')_4^+$ (where R' is independently H or C_{1-4} alkyl, *e.g.*, ammonium, Tris)], for example, isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, ethanolamine, 2-diethylaminoethanol, trimethamine, dicyclohexylamine, lysine, arginine, histidine, caffeine, procaine, hydrabamine, choline, betaine, ethylenediamine, glucosamine, methylglucamine, theobromine, purines, piperazine, piperidine, N-ethylpiperidine, polyamine resins and the like. Particularly preferred organic non-toxic bases are isopropylamine, diethylamine, ethanolamine, trimethamine, dicyclohexylamine, choline, and caffeine.

[0121] Additional pharmaceutically acceptable acids and bases useable with the present invention include those which are derived from the amino acids, for example, histidine, glycine, phenylalanine, aspartic acid, glutamic acid, lysine and asparagine.

[0122] "*Pharmaceutically acceptable*" buffers and salts include those derived from both acid and base addition salts of the above indicated acids and bases. Specific buffers and/ or salts include histidine, succinate and acetate.

[0123] A "*pharmaceutically acceptable sugar*" is a molecule which, when combined with a protein of interest, significantly prevents or reduces chemical and/or physical instability of the

protein upon storage. When the formulation is intended to be lyophilized and then reconstituted, "pharmaceutically acceptable sugars" may also be known as a "lyoprotectant". Exemplary sugars and their corresponding sugar alcohols include: an amino acid such as monosodium glutamate or histidine; a methylamine such as betaine; a lyotropic salt such as magnesium sulfate; a polyol such as trihydric or higher molecular weight sugar alcohols, *e.g.* glycerin, dextran, erythritol, glycerol, arabitol, xylitol, sorbitol, and mannitol; propylene glycol; polyethylene glycol; PLURONICS®; and combinations thereof. Additional exemplary lyoprotectants include glycerin and gelatin, and the sugars mellibiose, melezitose, raffinose, mannotriose and stachyose. Examples of reducing sugars include glucose, maltose, lactose, maltulose, iso-maltulose and lactulose. Examples of non-reducing sugars include non-reducing glycosides of polyhydroxy compounds selected from sugar alcohols and other straight chain polyalcohols. Preferred sugar alcohols are monoglycosides, especially those compounds obtained by reduction of disaccharides such as lactose, maltose, lactulose and maltulose. The glycosidic side group can be either glucosidic or galactosidic. Additional examples of sugar alcohols are glucitol, maltitol, lactitol and iso-maltulose. The preferred pharmaceutically-acceptable sugars are the non-reducing sugars trehalose or sucrose. Pharmaceutically acceptable sugars are added to the formulation in a "protecting amount" (*e.g.* pre-lyophilization) which means that the protein essentially retains its physical and chemical stability and integrity during storage (*e.g.*, after reconstitution and storage).

[0124] The "*diluent*" of interest herein is one which is pharmaceutically acceptable (safe and non-toxic for administration to a human) and is useful for the preparation of a liquid formulation, such as a formulation reconstituted after lyophilization. Exemplary diluents include sterile water, bacteriostatic water for injection (BWFI), a pH buffered solution (*e.g.* phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution. In an alternative embodiment, diluents can include aqueous solutions of salts and/or buffers.

[0125] A "*preservative*" is a compound which can be added to the formulations herein to reduce bacterial activity. The addition of a preservative may, for example, facilitate the production of a multi-use (multiple-dose) formulation. Examples of potential preservatives include octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride (a mixture of alkylbenzyldimethylammonium chlorides in which the alkyl groups are long-chain compounds), and benzethonium chloride. Other types of preservatives include

aromatic alcohols such as phenol, butyl and benzyl alcohol, alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, 3-pentanol, and *m*-cresol. The most preferred preservative herein is benzyl alcohol.

[0126] The term "*pharmaceutical formulation*" refers to a preparation that is in such form as to permit the biological activity of the active ingredient to be effective, and that contains no additional components that are unacceptably toxic to a subject to which the formulation would be administered. Such formulations are sterile.

[0127] A "*sterile*" formulation is aseptic or free from all living microorganisms and their spores.

[0128] As used herein, the term "sample" refers to a composition that is obtained or derived from an individual of interest that contains a cellular and/or other molecular entity that is to be characterized and/or identified, for example based on physical, biochemical, chemical and/or physiological characteristics. For example, the phrase "disease sample" and variations thereof refers to any sample obtained from an individual of interest that would be expected or is known to contain the cellular and/or molecular entity that is to be characterized.

[0129] By "tissue or cell sample" is meant a collection of similar cells obtained from a tissue of an individual or a patient. The source of the tissue or cell sample may be solid tissue as from a fresh, frozen and/or preserved organ or tissue sample or biopsy or aspirate; blood or any blood constituents; bodily fluids such as cerebral spinal fluid, amniotic fluid, peritoneal fluid, or interstitial fluid; cells from any time in gestation or development of the individual. The tissue sample may also be primary or cultured cells or cell lines. Optionally, the tissue or cell sample is obtained from a disease tissue/organ. The tissue sample may contain compounds which are not naturally intermixed with the tissue in nature such as preservatives, anticoagulants, buffers, fixatives, nutrients, antibiotics, or the like. A "reference sample", "reference cell", or "reference tissue", as used herein, refers to a sample, cell or tissue obtained from a source known, or believed, not to be afflicted with the disease or condition for which a method or composition of the invention is being used to identify. In one embodiment, a reference sample, reference cell or reference tissue is obtained from a healthy part of the body of the same individual or patient in whom a disease or condition is being identified using a composition or method of the invention. In one embodiment, a reference sample, reference cell or reference tissue is obtained from a

healthy part of the body of an individual who is not the individual or patient in whom a disease or condition is being identified using a composition or method of the invention.

[0130] For the purposes herein a "section" of a tissue sample is meant a single part or piece of a tissue sample, *e.g.* a thin slice of tissue or cells cut from a tissue sample. It is understood that multiple sections of tissue samples may be taken and subjected to analysis according to the present invention, provided that it is understood that the present invention comprises a method whereby the same section of tissue sample is analyzed at both morphological and molecular levels, or is analyzed with respect to both protein and nucleic acid.

[0131] The term "*about*" as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to "*about*" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se.

[0132] As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly indicates otherwise. For example, reference to an "antibody" is a reference to from one to many antibodies, such as molar amounts, and includes equivalents thereof known to those skilled in the art, and so forth.

[0133] It is understood that aspect and embodiments of the invention described herein include "comprising," "consisting," and "consisting essentially of" aspects and embodiments.

III. Modes for Carrying out the Invention

[0134] The invention provides methods for treating or preventing inflammatory bowel disease in an individual comprising administering to the individual an effective amount of an IL-27 antagonist. In some embodiments, an effective amount of an IL-27 antagonist is administered to an individual for treating or preventing ulcerative colitis in the individual. In some embodiments, an effective amount of an IL-27 antagonist is administered to an individual for treating or preventing Crohn's disease in the individual.

[0135] With respect to all methods described herein, reference to an IL-27 antagonist also includes compositions comprising one or more of those agents. Such compositions may further comprise suitable excipients, such as pharmaceutically acceptable excipients (carriers) including buffers, acids, bases, sugars, diluents, preservatives, and the like, which are well known in the art

and are described herein. The present methods can be used alone or in combination with other conventional methods of treatment.

A. IL-27 Antagonists

[0136] The methods of the invention use IL-27 antagonists, which term refers to any molecule that blocks, inhibits, reduces (including significantly), or interferes with IL-27 biological activity *in vitro*, *in situ*, and/or *in vivo*, including downstream pathways mediated by IL-27 signaling, such as receptor binding and/or elicitation of a cellular response to IL-27. An IL-27 antagonist should exhibit one or more of the following characteristics: (1) the ability to inhibit IL-27 biological activity and/or activity of downstream pathways mediated by IL-27 signaling; (2) the ability to block or reduce IL-27 receptor activation; (3) the ability to increase clearance of IL-27; (4) the ability to inhibit or reduce IL-27 synthesis, production or release; and (5) ability to treat or prevent any aspect of inflammatory bowel disease (such as Crohn's disease or ulcerative colitis).

[0137] In some embodiments, the IL-27 antagonist blocks, inhibits, reduces, or interferes the biological activity of a human IL-27 and/or a human IL-27 receptor. In some embodiments, the antagonist (such as an antibody) targets a human Ebi3 and/or p28. In some embodiments, the human Ebi3 comprises the amino acid sequence of amino acid residues 21-229 shown in SEQ ID NO:1. In some embodiments, the human p28 comprises the amino acid sequence of amino acid residues 29-243 shown in SEQ ID NO:2. In some embodiments, the IL-27 antagonists (such as an antibody) targets IL-27Ra. In some embodiments, the human IL-27Ra comprises the amino acid sequence shown in SEQ ID NO:3 with the signal peptide deleted.

Human Ebi3 amino acid sequence (GenBank Accession Number: NP_005746)

MTPQLLLALV LWASCPPCSG RKGPPAALTL PRVQCRASRY PIAVDCSWTL
PPAPNSTSPV SFIATYRLGM AARGHSWPCL QQTPTSTSCT ITDVQLFSMA
PYVLNVTAVH PWGSSSSFVP FITEHIIKPD PPEGVRLSPL AERQLQVQWE
PPGSWPFPEI FSLKYWIRYK RQGAARFHRV GPIEATSFIL RAVRPRARYY
VQVAAQDLTD YGELSDWSLP ATATMSLGK (SEQ ID NO:1)

Human p28 amino acid sequence (GenBank Accession Number: NP_663634)

MGQTAGDLGW RLSLLLLPLL LVQAGVWGFP RPPGRPQLSL QELRREFTVS
LHLARKLLSE VRGQAHRFAE SHLPGVNLYL LPLGEQLPDV SLTFQAWRRL
SDPERLCFIS TTLQPFHALL GGLGTQGRWT NMERMQLWAM RLDLRDLQRH

LRFQVLAAGF NLPEEEEEEE EEEEEERKGL LPGALGSALQ GPAQVSWPQL
LSTYRLLHSL ELVLSRAVRE LLLLSKAGHS VWPLGFPTLS PQP (SEQ ID NO:2)

Human IL27Ra amino acid sequence (GenBank Accession Number: NP_004834)
MRGGRGAPFW LWPLPKLALL PLLWVLFQRT RPQGSAGPLQ CYGVGPLGDL
NCSWEPLGDL GAPSELHLQS QKYRSNKTQT VAVAAGRSWV AIPREQLTMS
DKLLVWGTKA GQPLWPPVFV NLETQMKPNA PRLGPDVDFS EDDPLEATVH
WAPPTWPSHK VLICQFHYRR CQEAAWTLLE PELKTIPLTP VEIQDLELAT
GYKVYGRCRM EKEEDLWGEW SPILSFQTPP SAPKDVWVSG NLCGTPGGEE
PLLLWKAPGP CVQVSYKVWF WVGGRELSPE GITCCCSLIP SGAEWARVSA
VNATSWEPLT NLSLVCLDSA SAPRSVAVSS IAGSTELLVT WQPGPGEPLE
HVVDWARDGD PLEKLNWVRL PPGNLSALLP GNFTVGVPYR ITVTAVSASG
LASASSVWGF REELAPLVGP TLWRLQDAPP GTPAIAWGEV PRHQLRGHLT
HYTLCAQSGT SPSVCMNVSG NTQSVTLPDL PWGPCELWVT ASTIAGQGPP
GPILRLHLPD NTLRWKVLPG ILFLWGLFLL GCGLSLATSG RCYHLRHKVL
PRWVWEKVPD PANSSSGQPH MEQVPEAQPL GDLPILEVEE MEPPPVMESS
QPAQATAPLD SGYEKHFLPT PEELGLLGPP RPQVLA (SEQ ID NO:3)

[0138] Exemplary IL-27 antagonists include, but are not limited to, anti-IL-27 antibodies that specifically bind to a subunit of IL-27 (IL-27p28 or IL-27Ebi3), or heterodimeric IL-27, anti-IL-27 receptor antibodies that specifically bind to a component of IL-27 receptor (such as IL-27Ra) or the heterodimeric IL-27 receptor, antisense molecules directed to a subunit of IL-27 (*i.e.*, IL-27p28 or IL-27Ebi3) or IL-27Ra, a short interfering RNA ("siRNA") molecule directed to a nucleic acid a subunit of IL-27 (*i.e.*, IL-27p28 or IL-27Ebi3) or IL-27Ra, an IL-27 inhibitory compound, an RNA or DNA aptamer that binds to IL-27, IL-27p28, IL-27 Ebi3, the heterodimeric IL-27 receptor, or IL-27Ra, an IL-27 structural analog, an IL-27Ra structural analog, a soluble receptor IL-27Ra and fusion polypeptide thereof, a subunit of IL-27 that binds to IL-27 receptor and a fusion polypeptide thereof, an IL-27 binding polypeptide, compounds that specifically inhibit IL-27 synthesis and/or release, and compounds that specifically inhibit IL-27Ra signal transduction.

[0139] In certain embodiments, the IL-27 antagonist inhibits IL-27 signal transduction. In certain embodiments, the IL-27 antagonist inhibits the production of IL-10 (for example, IL-27-induced IL-10 production). In certain embodiments, the IL-27 antagonist inhibits the production

of IL-21 (for example, IL-27-induced IL-21 production). In certain embodiments, the IL-27 antagonist reduces the number of T_{FH} cells. In certain embodiments, the IL-27 antagonist reduces the amount of high affinity antibodies.

[0140] In certain embodiments, the IL-27 antagonist is an antibody that binds or physically interacts with a subunit of IL-27 (IL-27p28 or IL-27Ebi3). In certain embodiments, the antibody binds to IL-27p28 or IL-27Ebi3 and blocks and/or prevents formation of the heterodimeric IL-27. In certain embodiments, the antibody binds to IL-27p28 and blocks and/or prevents formation of the heterodimeric IL-27. In certain embodiments, the antibody binds to IL-27Ebi3 and blocks and/or prevents formation of the heterodimeric IL-27. In certain embodiments, the antibody is an anti-IL-27p28 antibody.

[0141] In certain embodiments, the IL-27 antagonist is an antibody that binds or physically interacts with heterodimeric IL-27, and blocks interactions between IL-27 and its receptor. In certain embodiments, the antibody binds to an epitope on the p28 subunit of IL-27. In certain embodiments, the antibody binds to an epitope on the Ebi3 subunit of IL-27. In certain embodiments, the antibody binds to both subunits of IL-27.

[0142] In certain embodiments, the IL-27 antagonist is an antibody that binds or physically interacts with IL-27Ra. In certain embodiments, the antibody binds IL-27Ra and inhibits and/or prevents formation of heterodimeric IL-27 receptor. In certain embodiments, the antibody binds IL-27Ra and inhibits and/or prevents binding between IL-27 and IL-27Ra.

[0143] In certain embodiments, the IL-27 antagonist is an antibody that binds or physically interacts with the heterodimeric IL-27 receptor, and reduces, impedes, or blocks downstream IL-27 signaling.

[0144] The antibody may have nanomolar or even picomolar affinities for the target antigen (e.g., IL-27, IL-27p28, IL-27Ebi3, IL-27 receptor, or IL-27Ra). In certain embodiments, the Kd of the antibody is about 0.05 to about 100 nM. For example, Kd of the antibody is any of about 100 nM, about 50 nM, about 10 nM, about 1 nM, about 500 pM, about 100 pM, or about 50 pM to any of about 2 pM, about 5 pM, about 10 pM, about 15 pM, about 20 pM, or about 40 pM.

[0145] In certain embodiments, the IL-27 antagonist is a small molecule antagonist, including, but is not limited to, small peptides or peptide-like molecules, soluble peptides, and synthetic non-peptidyl organic or inorganic compounds. A small molecule antagonist may have a molecular weight of any of about 100 to about 20,000 daltons (Da), about 500 to about 15,000

Da, about 1000 to about 10,000 Da. In certain embodiments, an IL-27 antagonist comprises a small molecule that binds IL-27. Exemplary sites of small molecule binding include, but are not limited to, the portion of IL-27 that binds to the IL-27 receptor, to IL-27Ra or to the portions of IL-27 adjacent to the IL-27 receptor binding region and which are responsible in whole or in part for establishing and/or maintaining the correct three-dimensional conformation of the receptor binding portion of IL-27. In certain other embodiments, an IL-27 antagonist comprises a small molecule that binds to the IL-27 receptor or to IL-27Ra and inhibits an IL-27 biological activity. Exemplary sites of small molecule binding include, but are not limited to, those portions of the IL-27 receptor and/or IL-27Ra that bind to IL-27.

[0146] In certain embodiments, the IL-27 antagonist is an RNA or DNA aptamer that binds or physically interacts with IL-27, and blocks interactions between IL-27 and its receptor. In certain embodiments, the aptamer comprises at least one RNA or DNA aptamer that binds to the p28 subunit of IL-27. In certain embodiments, the aptamer comprises at least one RNA or DNA aptamer that binds to the Ebi3 subunit of IL-27. In certain embodiments, the IL-27 antagonist comprises at least one RNA or DNA aptamer that binds to both subunits of IL-27.

[0147] In certain embodiments, the IL-27 antagonist is an RNA or DNA aptamer that binds or physically interacts with the heterodimeric IL-27 receptor or the IL-27Ra subunit, and reduces, impedes, or blocks downstream IL-27 signaling.

[0148] In certain embodiments, the IL-27 antagonist comprises at least one IL-27 or IL-27 receptor structural analog. The terms IL-27 structural analogs and IL-27 receptor structural analogs refer to compounds that have a similar three dimensional structure as part of that of IL-27 or IL-27 receptor, or IL-27Ra and which bind to IL-27 (*e.g.*, IL-27 receptor or IL-27Ra structural analogs) or to IL-27 receptor (*e.g.*, IL-27, IL-27p28, and IL-27Ebi3 structural analogs) under physiological conditions *in vitro* or *in vivo*, wherein the binding at least partially inhibits an IL-27 biological activity or an IL-27 receptor biological activity. Suitable IL-27 structural analogs and IL-27 receptor structural analogs can be designed and synthesized through molecular modeling of IL-27 receptor binding. The IL-27 structural analogs and IL-27 receptor structural analogs can be monomers, dimers, or higher order multimers in any desired combination of the same or different structures to obtain improved affinities and biological effects.

[0149] In certain embodiments, an IL-27 antagonist comprising at least one soluble IL-27 receptor (*e.g.*, IL-27Ra) or fusion polypeptide thereof is provided. In certain embodiments, the soluble IL-27Ra is fused to an immunoglobulin constant domain, such as an Fc domain.

[0150] In certain embodiments, the IL-27 antagonist comprises at least one antisense molecule capable of blocking or decreasing the expression of functional IL-27 or IL-27 receptor by targeting nucleic acids encoding a subunit of IL-27 (i.e., IL-27p28 or IL-27Ebi3), or IL-27Ra. Nucleotide sequences of IL-27 and IL-27 receptor are known. *See, e.g.*, GenBank Accession Nos. NM 005755 (human IL-27Ebi3 mRNA); NM 145659 (human IL-27p28 mRNA); and NM 004843 (human IL-27Ra mRNA). Methods are known for the preparation of antisense oligonucleotide molecules that will specifically bind one or more of IL-27p28, IL-27Ebi3, and IL-27Ra mRNA without cross-reacting with other polynucleotides. Exemplary sites of targeting include, but are not limited to, the initiation codon, the 5' regulatory regions, including promoters or enhancers, the coding sequence, including any conserved consensus regions, and the 3' untranslated region. In certain embodiments, the antisense oligonucleotides are about 10 to about 100 nucleotides in length, about 15 to about 50 nucleotides in length, about 18 to about 25 nucleotides in length, or more. In certain embodiments, the oligonucleotides further comprise chemical modifications to increase nuclease resistance and the like, such as, for example, phosphorothioate linkages and 2'-O-sugar modifications known to those of ordinary skill in the art.

[0151] In certain embodiments, the IL-27 antagonist comprises at least one siRNA molecule capable of blocking or decreasing the expression of functional IL-27 or IL-27 receptor by targeting nucleic acids encoding IL-27, a subunit of IL-27 (*i.e.*, IL-27p28 or IL-27Ebi3), or IL-27Ra. It is routine to prepare siRNA molecules that will specifically target one or more of IL-27p28, IL-27Ebi3, and IL-27Ra mRNA without cross-reacting with other polynucleotides.

[0152] siRNA molecules may be generated by methods known in the art such as by typical solid phase oligonucleotide synthesis, and often will incorporate chemical modifications to increase half life and/or efficacy of the siRNA agent, and/or to allow for a more robust delivery formulation. Alternatively, siRNA molecules are delivered using a vector encoding an expression cassette for intracellular transcription of siRNA.

[0153] IL-27 antagonists can be identified or characterized using methods known in the art, such as protein-protein binding assays, biochemical screening assays, immunoassays, and cell-based assays, which are well known in the art.

[0154] To identify a molecule that inhibits interaction between IL-27 and its receptor, binding assays may be used. For example, IL-27 or receptor polypeptide is immobilized on a microtiter plate by covalent or non-covalent attachment. The assay is performed by adding the non-immobilized component (ligand or receptor polypeptide), which may be labeled by a detectable label, to the immobilized component, in the presence or absence of the testing molecule. When the reaction is complete, the non-reacted components are removed and binding complexes are detected. If formation of binding complexes is inhibited by the presence of the testing molecule, the testing molecule may be a candidate antagonist that inhibits binding between IL-27 and its receptor.

[0155] A cell-based assay may also be used to identify IL-27 antagonists. For example, IL-27 may be added to a cell along with the testing molecule to be screened for a particular activity (*e.g.*, expression of IL-10 or IL-21), and the ability of the testing molecule to inhibit the activity of interest indicates that the testing molecule is an IL-27 antagonist.

[0156] By detecting and/or measuring levels of IL-27 gene expression, antagonist molecules that inhibit IL-27 gene expression may be tested. IL-27 gene expression can be detected and/or measured by a variety of methods, such as real time RT-PCR, enzyme-linked immunosorbent assay ("ELISA"), Northern blotting, or flow cytometry.

B. Recombinant Preparation of IL-27 Antagonists

[0157] The invention also provides methods of producing IL-27 polypeptide antagonists (such as antibodies) using recombinant techniques. For example, polypeptides can be prepared using isolated nucleic acids encoding such polypeptides (for example, anti-IL-27, anti-IL-27p28, anti-IL-27Ebi3, anti-IL-27 receptor and anti-IL-27Ra antibodies) or fragments thereof, vectors and host-cells comprising such nucleic acids. Although the methods described under Section B generally refer to production of antibodies, these methods may also be used to produce any polypeptides described herein.

[0158] For recombinant production of antibodies or fragments thereof, nucleic acids encoding the desired antibodies or antibody fragments are isolated and inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. DNA encoding the polyclonal or

monoclonal antibodies is readily isolated (*e.g.*, with oligonucleotide probes that specifically bind to genes encoding the heavy and light chains of the antibody) and sequenced using conventional procedures. Many cloning and/or expression vectors are commercially available. Vector components generally include, but are not limited to, one or more of the following, a signal sequence, an origin of replication, one or more marker genes, a multiple cloning site containing recognition sequences for numerous restriction endonucleases, an enhancer element, a promoter, and a transcription termination sequence.

(1) Signal sequence component

[0159] The antibodies or fragments thereof may be produced recombinantly not only directly, but also as a fusion protein, where the antibody is fused to a heterologous polypeptide, preferably a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. The heterologous signal sequence selected preferably is one that is recognized and processed (*i.e.*, cleaved by a signal peptidase) by eukaryotic host-cells. For prokaryotic host-cells that do not recognize and process native mammalian signal sequences, the eukaryotic (*i.e.*, mammalian) signal sequence is replaced by a prokaryotic signal sequence selected, for example, from the group consisting of leader sequences from alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II genes. For yeast secretion the native signal sequence may be substituted by, *e.g.,* the yeast invertase leader, factor leader (including *Saccharomyces* and *Kluyveromyces* -factor leaders), or acid phosphatase leader, the *C. albicans* glucoamylase leader, or the signal described in WO 90/13646. In mammalian cell expression, mammalian signal sequences as well as viral secretory leaders, for example, the herpes simplex virus gD signal, are available.

[0160] The DNA for such precursor region is ligated in reading frame to the DNA encoding the antibodies or fragments thereof.

(2) Origin of replication

[0161] Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host-cells. Generally, in cloning vectors this sequence is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. 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, vesicular stomatitis virus ("VSV") or bovine papilloma virus ("BPV") are useful for cloning vectors in mammalian cells. Generally, the origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

(3) Selection gene component

[0162] Expression and cloning vectors may also contain a selection gene, known as 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.*

[0163] One example of a selection scheme utilizes a drug to arrest growth of a host-cell. Those cells that are successfully transformed with a heterologous gene produce a protein conferring drug resistance and thus survive the selection regimen. Examples of such dominant selection strategies use the drugs neomycin, mycophenolic acid and hygromycin.

[0164] Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the antibody- or antibody fragment-encoding nucleic acids, such as dihydrofolate reductase ("DHFR"), thymidine kinase, metallothionein-I and -II, preferably primate metallothionein genes, adenosine deaminase, ornithine decarboxylase, and the like.

[0165] For example, cells transformed with the DHFR selection gene are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. An exemplary host-cell strain for use with wild-type DHFR is the Chinese hamster ovary ("CHO") cell line lacking DHFR activity (*e.g.*, ATCC CRL-9096).

[0166] Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent to take up the antibody- or antibody fragment-encoding nucleic acids, such as dihydrofolate reductase ("DHFR"), glutamine synthetase (GS), thymidine kinase, metallothionein-I and -II, preferably primate metallothionein genes, adenosine deaminase, ornithine decarboxylase, and the like.

[0167] Alternatively, cells transformed with the GS (glutamine synthetase) gene are identified by culturing the transformants in a culture medium containing L-methionine sulfoximine (Msx), an inhibitor of GS. Under these conditions, the GS gene is amplified along with any other co-

transformed nucleic acid. The GS selection/amplification system may be used in combination with the DHFR selection/amplification system described above.

[0168] Alternatively, host-cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding antibodies (*e.g.*, antibodies directed to IL-27, IL-27p28, IL-27Ebi3, IL-27 receptor or IL-27Ra) or fragments thereof, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3'-phosphotransferase ("APH") can be selected by cell growth in medium containing a selection agent for the appropriate selectable marker, such as an aminoglycosidic antibiotic, *e.g.*, kanamycin, neomycin, or G418. See U.S. Patent No. 4,965,199.

[0169] A suitable selection gene for use in yeast is the *trp1* gene present in the yeast plasmid YRp7 (Stinchcomb et al., *Nature*, 282:39 (1979)). The *trp1* gene provides a selection marker for a mutant strain of yeast lacking the ability to grow medium containing tryptophan (*e.g.*, ATCC No. 44076 or PEP4-1). Jones, *Genetics*, 85:12 (1977). The presence of the *trp1* lesion in the yeast host-cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan. Similarly, *Leu2*-deficient yeast strains (*e.g.*, ATCC 20,622 or 38,626) can be complemented by known plasmids bearing the *Leu2* gene.

[0170] In addition, vectors derived from the 1.6 μm circular plasmid pKD1 can be used for transformation of *Kluyveromyces* yeasts. Alternatively, an expression system for large-scale production of recombinant calf chymosin was reported for *K. lactis*. Van den Berg, *Bio/Technology*, 8:135 (1990). Stable multi-copy expression vectors for secretion of mature recombinant human serum albumin by industrial strains of *Kluyveromyces* have also been disclosed. Fleer et al., *Bio/Technology*, 9:968-975 (1991).

(4) Promoter component

[0171] Expression and cloning vectors usually contain a promoter that is recognized by the host organism and is operably linked to the nucleic acid encoding the antibodies (*e.g.*, antibodies directed to IL-27, IL-27p28, IL-27Ebi3, IL-27 receptor and IL-27Ra) or fragments thereof. Promoters suitable for use with prokaryotic hosts include the *phoA* promoter, lactamase and lactose promoter systems, alkaline phosphatase promoter, a tryptophan promoter system, and hybrid promoters such as the tac promoter, although other known bacterial promoters are also suitable. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding the antibodies and antibody fragments.

[0172] Promoter sequences are known for eukaryotes. Virtually all eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CNCAAT region where N may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA sequence that may be the signal for addition of the polyA tail to the 3' end of the coding sequence. All of these sequences may be inserted into eukaryotic expression vectors.

[0173] Examples of suitable promoter sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase or other glycolytic enzymes, such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phospho-fructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

[0174] Inducible promoters in yeast have the additional advantage of permitting transcription controlled by growth conditions. Exemplary inducible promoters include 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. Yeast enhancers also are advantageously used with yeast promoters.

[0175] Transcription of nucleic acids encoding antibodies or fragments thereof from vectors in mammalian host-cells can be controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus, adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), by heterologous mammalian promoters, *e.g.,* the actin promoter or an immunoglobulin promoter, and by heat-shock gene promoters, provided such promoters are compatible with the desired host-cell systems.

[0176] The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment. A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. Patent No. 4,419,446. A modification of this system is described

in U.S. Patent No. 4,601,978. *See also* Reyes et al., *Nature* 297:598-601 (1982), regarding methods for expression of human interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus. Alternatively, the Rous Sarcoma Virus long terminal repeat can be used as the promoter.

(5) Enhancer element component

[0177] Transcription of a DNA encoding the antibodies or fragments thereof by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α-fetoprotein, and insulin). Typically, however, one of ordinary skill in the art will use an enhancer from a eukaryotic 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. *See also* Yaniv, *Nature* 297:17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the antibody- or antibody-fragment encoding sequences, but is preferably located at a site 5' of the promoter.

(6) Transcription termination component

[0178] 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 antibodies or fragments thereof. One useful transcription termination component is the bovine growth hormone polyadenylation region. *See* WO94/11026 and the expression vector disclosed therein.

(7) Selection and transformation of host-cells

[0179] Suitable host-cells for cloning or expressing the DNA encoding antibodies (*e.g.*, antibodies directed to IL-27, IL-27p28, IL-27Ebi3, IL-27 receptor and IL-27Ra) or fragments thereof in the vectors described herein include the prokaryotic, yeast, or higher eukaryotic cells described above. Suitable prokaryotes for this purpose include eubacteria, such as Gram-negative or Gram-positive organisms, for example, 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*. One preferred *E. coli* cloning host is *E. coli* 294 (ATCC 31,446), although other strains such as *E. coli* B, *E. coli* X1776 (ATCC 31,537), and *E. coli* W3110 (ATCC 27,325) are also suitable. These examples are illustrative rather than limiting.

[0180] Full length antibodies, 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). 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, antibodies or antibody fragments are 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 by the same process used to purify antibodies or antibody fragments expressed, *e.g.*, in CHO cells.

[0181] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are also suitable cloning or expression hosts for antibody- or antibody-fragment encoding vectors. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe*; *Kluyveromyces spp.*, such as *K. lactis*, *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), *K. thermotolerans*, and *K. marxianus; yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070); *Candida; Trichoderma reesia* (EP 244,234); *Neurospora crassa*; *Schwanniomyces* such as *Schwanniomyces occidentalis*; and filamentous fungi such as, *e.g.*, *Neurospora, Penicillium, Tolypocladium*, and *Aspergillus* hosts such as *A. nidulans* and *A. niger*. For a review discussing the use of yeasts and filamentous fungi for the production of therapeutic proteins, see, e.g., Gerngross, *Nat. Biotech.* 22: 1409-1414 (2004).

[0182] Certain fungi and yeast strains may be selected in which glycosylation pathways have been "humanized," resulting in the production of an antibody with a partially or fully human glycosylation pattern. *See, e.g.*, Li et al., *Nat. Biotech.* 24:210-215 (2006) (describing humanization of the glycosylation pathway in *Pichia pastoris*); and Gerngross et al., *supra.*

[0183] Suitable host-cells for the expression of glycosylated antibodies or antibody fragments are derived from multicellular organisms. Examples of invertebrate cells include plant and insect-cells. 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* (moth) 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. Such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells.

[0184] Plant-cell cultures of cotton, corn, potato, soybean, petunia, tomato, and tobacco can also be utilized as hosts.

[0185] 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. Nat'l 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). Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including $DHFR^-$ CHO cells (Urlaub et al., Proc. Natl. Acad. Sci. USA 77:4216 (1980)); and myeloma cell lines such as NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, e.g., Yazaki and Wu,

Methods in Molecular Biology, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ, 2003), pp. 255-268.

[0186] Host-cells are transformed with the above-described expression or cloning vectors for antibody or antibody fragment production and cultured in conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

(8) Culturing the host-cells

[0187] The host-cells used to produce the antibodies (*e.g.*, antibodies directed to IL-27, IL-27p28, IL-27Ebi3, IL-27 receptor and IL-27Ra) or antibody fragments described herein 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. Patent Nos. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WIPO Publication Nos. 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 GENTAMYCIN™ 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.

(9) Purification of antibody

[0188] When using recombinant techniques, the antibodies (*e.g.*, antibodies directed to IL-27, IL-27p28, IL-27Ebi3, IL-27 receptor or IL-27Ra) or antibody fragments can be produced intracellularly, in the periplasmic space, or secreted directly into the medium. If the antibodies are produced intracellularly, as a first step, the particulate debris from either host-cells or lysed fragments is 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 minutes. 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.

[0189] The antibody or antibody fragment compositions prepared from such cells can be purified 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 or antibody fragments that are based on human 1, 2, or 4 heavy chains (Lindmark *et al., J. Immunol. Meth.* 62:1-13 (1983)). Protein G is recommended for all mouse isotypes and for human 3 heavy chain antibodies or antibody fragments (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(styrene-divinyl)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibodies or antibody fragments comprise a C_H3 domain, the Bakerbond ABX™resin (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, heparin, SEPHAROSE™, or anion or cation exchange resins (such as a polyaspartic acid column), as well as chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody or antibody fragment to be recovered.

[0190] Following any preliminary purification step or steps, the mixture comprising the antibody or antibody fragment 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 0-0.25 M salt).

[0191] In general, various methodologies for preparing antibodies for use in research, testing, and clinical applications are well-established in the art, consistent with the above-described methodologies and/or as deemed appropriate by one skilled in the art for a particular antibody of interest.

C. Antibody Preparation

[0192] The antibodies useful in the present invention can encompass monoclonal antibodies, polyclonal antibodies, antibody fragments (*e.g.*, Fab, Fab'-SH, Fv, scFv, and $F(ab')_2$), chimeric antibodies, bispecific antibodies, multivalent antibodies, heteroconjugate antibodies, fusion proteins comprising an antibody portion, humanized antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity (*e.g.*, for IL-27, IL-27p28, IL-27Ebi3, IL-27 receptor, or IL-27Ra), including glycosylation variants of antibodies, amino acid sequence variants of antibodies, and covalently modified antibodies. The antibodies may be murine, rat, human, or of any other origin (including chimeric or humanized antibodies).

(1) Polyclonal antibodies

[0193] Polyclonal antibodies are generally 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 (*e.g.*, purified or recombinant IL-27, IL-27p28, IL-27Ebi3, IL-27 receptor, or IL-27Ra) to a protein that is immunogenic in the species to be immunized, *e.g.*, 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 independently lower alkyl groups. Examples of adjuvants which may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

[0194] The animals are immunized against the desired antigen, immunogenic conjugates, or derivatives by combining, *e.g.*, 100 μg (for rabbits) or 5 μg (for mice) of the protein or conjugate 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 fourteen 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 suitable to enhance the immune response.

(2) Monoclonal antibodies

[0195] Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations and/or post-translational modifications (*e.g.*, isomerizations, amidations) that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies.

[0196] For example, the 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).

[0197] In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization (*e.g.*, purified or recombinant IL-27, IL-27p28, IL-27Ebi3, IL-27 receptor, or IL-27Ra). Alternatively, lymphocytes may be immunized *in vitro*. Lymphocytes then are fused with myeloma cells 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)).

[0198] The immunizing agent will typically include the antigenic protein (*e.g.*, purified or recombinant IL-27, IL-27p28, IL-27Ebi3, IL-27 receptor, or IL-27Ra) or a fusion variant thereof. Generally peripheral blood lymphocytes ("PBLs") are used if cells of human origin are desired, while spleen or lymph node cells are used if non-human mammalian sources are desired. The lymphoctyes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell. Goding, *Monoclonal Antibodies: Principles and Practice*, Academic Press (1986), pp. 59-103.

[0199] Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine or human origin. Usually, rat or mouse myeloma cell lines are employed.

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

[0200] Preferred immortalized 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 medium such as HAT medium. Among these, preferred 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), as well as SP-2 cells and derivatives thereof (*e.g.*, X63-Ag8-653) (available from the American Type Culture Collection, Manassas, Virginia USA). Human myeloma and mouse-human heteromyeloma cell lines have also been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

[0201] Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen (*e.g.*, IL-27, IL-27p28, IL-27Ebi3, IL-27 receptor, or IL-27Ra). 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).

[0202] The culture medium in which the hybridoma cells are cultured can be assayed for the presence of monoclonal antibodies directed against the desired antigen (*e.g.*, IL-27, IL-27p28, IL-27Ebi3, IL-27 receptor, or IL-27Ra). Preferably, the binding affinity and specificity of the monoclonal antibody can be determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked assay (ELISA). Such techniques and assays are known in the in art. For example, binding affinity may be determined by the Scatchard analysis of Munson et al., *Anal. Biochem.*, 107:220 (1980).

[0203] After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *supra*). 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 tumors in a mammal.

[0204] The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose chromatography, hydroxylapatite chromatography, gel electrophoresis, dialysis, affinity chromatography, and other methods as described above.

[0205] Monoclonal antibodies may also be made by recombinant DNA methods, such as those disclosed in U.S. Patent No. 4,816,567, and as described above. DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that specifically bind 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 immunoglobulin protein, in order to synthesize monoclonal antibodies in such recombinant host-cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra et al., *Curr. Opin. Immunol.*, 5:256-262 (1993) and Plückthun, *Immunol. Rev.* 130:151-188 (1992).

[0206] In certain embodiments, antibodies 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) described the isolation of murine and human antibodies, respectively, from phage libraries. Subsequent publications describe the production of high affinity (nanomolar ("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., *Nucl. Acids Res.*, 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies of desired specificity (*e.g.*, those that bind IL-27, IL-27p28, IL-27Ebi3, IL-27 receptor, or IL-27Ra).

[0207] The DNA encoding antibodies or fragments thereof may also be modified, for example, by substituting the coding sequence for human heavy- and light-chain constant domains in place of the homologous murine sequences (U.S. Patent No. 4,816,567; Morrison, et al., *Proc. Natl*

Acad. Sci. USA, 81:6851 (1984)), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Typically such non-immunoglobulin polypeptides are substituted 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.

[0208] The monoclonal antibodies described herein (*e.g.*, IL-27, IL-27p28, IL-27Ebi3, IL-27 receptor, or IL-27Ra antibodies or fragments thereof) may by monovalent, the preparation of which is well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and a modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues may be substituted with another amino acid residue or are deleted so as to prevent crosslinking. *In vitro* methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly Fab fragments, can be accomplished using routine techniques known in the art.

[0209] Chimeric or hybrid antibodies also may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide-exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate.

(3) Humanized antibodies.

[0210] The antibodies (such as IL-27, IL-27p28, IL-27Ebi3, IL-27 receptor, or IL-27Ra antibodies) or antibody fragments of the invention may further comprise humanized or human antibodies. Humanized forms of non-human (*e.g.*, murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fab, Fab'-SH, Fv, scFv, $F(ab')_2$ 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 complementarity 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 will also 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. Opin. Struct. Biol.* 2: 593-596 (1992).

[0211] 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), or through 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.

[0212] The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. 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 sequence which is closest to that of the rodent is then accepted as the human framework (FR) 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 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. Nat'l Acad. Sci. USA* 89:4285 (1992); Presta et al., *J. Immunol.* 151:2623 (1993).

[0213] Furthermore, it is important that antibodies be humanized with retention of high 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 analyzing 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 or antigens (e.g., IL-27, IL-27p28, IL-27Ebi3, IL-27 receptor, or IL-27Ra), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

[0214] Various forms of the humanized antibody are contemplated. For example, the humanized antibody may be an antibody fragment, such as an 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.

(4) Human antibodies

[0215] Alternatively, 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. 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 in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. *See, e.g.*, Jakobovits et al., *Proc. Nat'l Acad. Sci. USA*, 90:2551 (1993); Jakobovits et al., *Nature*, 362:255-258 (1993); Bruggermann et al., *Year in Immunol.*, 7:33 (1993); U.S. Patent Nos. 5,591,669 and WO 97/17852.

[0216] Alternatively, phage display technology can be used to produce human antibodies and antibody fragments in vitro, from immunoglobulin variable (V) domain gene repertoires from

unimmunized donors. McCafferty et al., *Nature* 348:552-553 (1990); Hoogenboom and Winter, *J. Mol. Biol.* 227: 381 (1991). 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., *Curr. Opin Struct. Biol.* 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.

[0217] The techniques of Cole et al., and Boerner et al., are also available for the preparation of human monoclonal antibodies (Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985) and Boerner et al., *J. Immunol.* 147(1): 86-95 (1991). Similarly, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, *e.g.*, mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806, 5,569,825, 5,625,126, 5,633,425, 5,661,016 and in the following scientific publications: Marks et al., *Bio/Technology* 10: 779-783 (1992); Lonberg et al., *Nature* 368: 856-859 (1994); Morrison, *Nature* 368: 812-13 (1994), Fishwild et al., *Nature Biotechnology* 14: 845-51 (1996), Neuberger, *Nature Biotechnology* 14: 826 (1996) and Lonberg and Huszar, *Intern. Rev. Immunol.* 13: 65-93 (1995).

[0218] Finally, human antibodies may also be generated in vitro by activated B-cells (see U.S. Patent Nos 5,567,610 and 5,229,275).

(5) Antibody Fragments

[0219] In certain circumstances there are advantages to using antibody fragments, rather than whole antibodies. Smaller fragment sizes allow for rapid clearance, and may lead to improved access to solid tumors.

[0220] 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., *J. Biochem. Biophys. Method.* 24:107-117 (1992); and Brennan et al., *Science* 229:81 (1985)). However, these fragments can now be produced directly by recombinant host-cells, for example, using nucleic acids encoding antibodies to IL-27, IL-27p28, IL-27Ebi3, IL-27 receptor, or IL-27Ra as discussed above. Fab, Fv and scFv antibody fragments can all be expressed in and secreted from *E. coli*, thus allowing the straightforward production of large amounts of these fragments. Antibody fragments can also be isolated from the antibody phage libraries as 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. Production of Fab and $F(ab')_2$ antibody fragments with increased *in vivo* half-lives are described in U.S. Patent No. 5,869,046. 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. The antibody fragment may also be a "linear antibody," *e.g.*, as described in U.S. Patent 5,641,870. Such linear antibody fragments may be monospecific or bispecific.

(6) Bispecific and polyspecific antibodies

[0221] Bispecific antibodies (BsAbs) are antibodies that have binding specificities for at least two different epitopes, including those on the same or another protein (*e.g.*, IL-27, IL-27p28, IL-27Ebi3, IL-27 receptor, or IL-27Ra). Alternatively, one part of a BsAb can be armed to bind to the target antigen, and another can be combined with an arm that binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (*e.g.*, CD3), or Fc receptors for IgG (FcγR) such as FcγR1 (CD64), FcγRII (CD32) and FcγRIII (CD16), in order to focus and localize cellular defense mechanisms to the target antigen-expressing cell. Such antibodies can be derived from full length antibodies or antibody fragments (*e.g.*, $F(ab')_2$ bispecific antibodies).

[0222] Bispecific antibodies may also be used to localize cytotoxic agents to cells which express the target antigen. Such antibodies possess one arm that binds the desired antigen and another arm that binds the cytotoxic agent (*e.g.*, saporin, anti-interferon-a, vinca alkoloid, ricin A chain, methotrexate or radioactive isotope hapten). Examples of known bispecific antibodies include anti-ErbB2/anti-FcγRIII (WO 96/16673), anti-ErbB2/anti-FcgRI (U.S.P. 5,837,234), anti-ErbB2/anti-CD3 (U.S.P. 5,821,337).

[0223] Methods for making bispecific antibodies are known in the art. Traditional production of full length bispecific antibodies is based on the coexpression 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).

[0224] According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin 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 binding, 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 organism. This provides for great 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 yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

[0225] 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 half of the bispecific molecules provides for an easy 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).

[0226] According to another approach described in WO 96/27011 or 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 region of an antibody constant 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 chains(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.

[0227] Techniques for generating bispecific antibodies from antibody fragments have 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'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

[0228] Fab' fragments may be directly recovered from *E. coli* and chemically coupled to form bispecific antibodies. Shalaby et al., *J. Exp. Med.* 175: 217-225 (1992) describes the production of fully humanized bispecific antibody $F(ab')_2$ molecules. 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.

[0229] Various techniques for making and isolating bivalent antibody fragments directly from recombinant-cell culture have also been described. For example, bivalent heterodimers 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 re-oxidized to form the antibody heterodimers. The "diabody" technology described by Hollinger et al., *Proc. Nat'l Acad. Sci. USA*, 90: 6444-6448 (1993) has provided an alternative mechanism for making bispecific/bivalent antibody fragments. The fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (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/bivalent antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. *See* Gruber et al., *J. Immunol.*, 152:5368 (1994).

[0230] Antibodies with more than two valencies are also contemplated. For example, trispecific antibodies can be prepared. Tutt et al., *J. Immunol.* 147:60 (1991).

[0231] Exemplary bispecific antibodies may bind to two different epitopes on a given molecule (*e.g.*, IL-27, IL-27p28, IL-27Ebi3, IL-27 receptor, or IL-27Ra). Alternatively, an arm targeting an IL-27 signaling component may be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (*e.g.*, CD2, CD3, CD28 or B7), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the cell expressing the particular protein. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express a particular protein. Such antibodies possess a protein-binding arm and an arm which binds a cytotoxic agent or a radionuclide chelator, such as EOTUBE, DPTA, DOTA or TETA. Another bispecific antibody of interest binds the protein of interest and further binds tissue factor (TF).

(7) Multivalent Antibodies

[0232] 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 (*e.g.*, IL-27, IL-27p28, IL-27Ebi3, IL-27 receptor, or IL-27Ra antibodies) or antibody fragments 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 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 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 or chains comprise two or more variable domains. For instance, the polypeptide chain or chains 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. Similarly, the polypeptide chain or chains may comprise V_H-C_H1-flexible linker-V_H-C_H1-Fc region chain; or V_H-C_H1-V_H-C_H1-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.

(8) Heteroconjugate Antibodies

[0233] Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies (*e.g.*, IL-27, IL-27p28, IL-27Ebi3, IL-27 receptor, or IL-27Ra antibodies or antibody fragments). 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 have been used to treat HIV infection. International Publication Nos. WO 91/00360, WO 92/200373 and EP 0308936. It is contemplated that the antibodies may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for

example, in U.S. Patent No. 4,676,980. 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.

(9) Effector Function Engineering

[0234] It may be desirable to modify the antibody of the invention to modify effector function and/or to increase serum half life of the antibody. For example, the Fc receptor binding site on the constant region may be modified or mutated to remove or reduce binding affinity to certain Fc receptors, such as FcγRI, FcγRII, and/or FcγRIII. In some embodiments, the effector function is impaired by removing N-glycosylation of the Fc region (e.g., in the CH 2 domain of IgG) of the antibody. In some embodiments, the effector function is impaired by modifying regions such as 233-236, 297, and/or 327-331 of human IgG as described in PCT WO 99/58572 and Armour et al., *Molecular Immunology* 40: 585-593 (2003); Reddy et al., *J. Immunology* 164:1925-1933 (2000).

[0235] 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.

(10) Other Amino Acid Sequence Modifications

[0236] Amino acid sequence modifications of the antibodies described herein (*e.g.*, IL-27, IL-27p28, IL-27Ebi3, IL-27 receptor, or IL-27Ra antibodies or antibody fragments) are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibodies or antibody fragments. Amino acid sequence variants of the antibodies or antibody fragments are prepared by introducing appropriate nucleotide changes into the nucleic acid encoding the antibodies or antibody fragments, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics (*i.e.*, the ability to bind or physically interact with IL-27, IL-27p28, IL-27Ebi3, IL-27 receptor, or IL-27Ra). The amino acid changes also may alter

post-translational processes of the antibody, such as changing the number or position of glycosylation sites.

[0237] A useful method for identification of certain residues or regions of the antibody that are preferred locations for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells in *Science*, 244:1081-1085 (1989). Here, a residue or group of target residues are identified (*e.g.*, charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with the target antigen. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation *per se* need not be predetermined. For example, to analyze the performance of a mutation at a given site, ala scanning or random mutagenesis is conducted at the target codon or region and the expressed antibody variants are screened for the desired activity.

[0238] Amino acid sequence insertions include amino- ("N") and/or carboxy- ("C") terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue or the antibody fused to a cytotoxic polypeptide. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme or a polypeptide which increases the serum half-life of the antibody.

[0239] Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue in the antibody molecule replaced by a different residue. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in the Table A below 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 A, or as further described below in reference to amino acid classes, may be introduced and the products screened.

TABLE A

Amino Acid Substitutions

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)	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)	leu; val; ile; ala; tyr	tyr
Pro (P)	Ala	ala
Ser (S)	Thr	thr
Thr (T)	Ser	ser
Trp (W)	tyr; phe	tyr
Tyr (Y)	trp; phe; thr; ser	phe
Val (V)	ile; leu; met; phe; ala; norleucine	leu

[0240] Substantial modifications in the biological properties of the antibody 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) hydrophobic: norleucine, met, ala, val, leu, ile;

(2) neutral hydrophilic: cys, ser, thr;

(3) acidic: asp, glu;

(4) basic: asn, gln, his, lys, arg;

(5) residues that influence chain orientation: gly, pro; and

(6) aromatic: trp, tyr, phe.

[0241] Non-conservative substitutions entail exchanging a member of one of these classes for another class.

[0242] Any cysteine residue not involved in maintaining the proper conformation of the antibody 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 the antibody to improve its stability (particularly where the antibody is an antibody fragment, such as an Fv fragment).

[0243] 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 the antigen (*e.g.,* IL-27, IL-27p28, IL-27Ebi3, IL-27 receptor, or IL-27Ra). 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.

[0244] Another type of amino acid variant of the antibody alters the original glycosylation pattern of the antibody. By altering is meant deleting one or more carbohydrate moieties found in the antibody, and/or adding one or more glycosylation sites that are not present in the antibody.

[0245] Glycosylation of antibodies 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.

[0246] Addition of glycosylation sites to the antibody is conveniently 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 antibody (for O-linked glycosylation sites).

[0247] Nucleic acid molecules encoding amino acid sequence variants of the anti-IgE antibody 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 an earlier prepared variant or a non-variant version of the antibodies (*e.g.*, IL-27, IL-27p28, IL-27Ebi3, IL-27 receptor, or IL-27Ra antibodies) or antibody fragments.

(10) Other Antibody Modifications

[0248] The antibodies (*e.g.*, IL-27, IL-27p28, IL-27Ebi3, IL-27 receptor, or IL-27Ra antibodies) or antibody fragments of the present invention can be further modified to contain

additional nonproteinaceous moieties that are known in the art and readily available. Preferably, the moieties suitable for derivatization of the antibody are water-soluble polymers. Non-limiting examples of water-soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, polypropylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer is attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, *etc.* Such techniques and other suitable formulations are disclosed in *Remington: The Science and Practice of Pharmacy*, 20th Ed., Alfonso Gennaro, Ed., Philadelphia College of Pharmacy and Science (2000).

D. Pharmaceutical Formulations

[0249] Therapeutic formulations of IL-27 antagonists are prepared for storage by mixing the active ingredient having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (*Remington: The Science and Practice of Pharmacy*, 20th Ed., (Gennaro, A.R., ed., Lippincott Williams & Wilkins, Publishers, Philadelphia, PA 2000). Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers, antioxidants including ascorbic acid, methionine, Vitamin E, sodium metabisulfite; preservatives, isotonicifiers, stabilizers, metal complexes (*e.g.*, Zn-protein complexes), chelating agents such as EDTA and/or non-ionic surfactants, and the like.

[0250] When the therapeutic agent is an antibody fragment, the smallest inhibitory fragment which specifically binds to the binding domain of the target protein (*e.g.*, IL-27, IL-27p28, IL-27, Ebi3, IL-27 receptor, or IL-27Ra) is preferred. For example, based upon the variable region

sequences of an antibody, antibody fragments or even peptide molecules can be designed which retain the ability to bind the target protein sequence. Such peptides can be synthesized chemically and/or produced by recombinant DNA technology (see, *e.g.*, Marasco et al., *Proc. Nat'l Acad. Sci. USA* 90: 7889-7893 (1993)).

[0251] Buffers are used to control the pH in a range which optimizes the therapeutic effectiveness, especially if stability is pH dependent. Buffers are preferably present at concentrations ranging from about 50 mM to about 250 mM. Suitable buffering agents for use with the present invention include both organic and inorganic acids and salts thereof, such as citrate, phosphate, succinate, tartrate, fumarate, gluconate, oxalate, lactate, acetate. Additionally, buffers may comprise histidine and trimethylamine salts such as Tris.

[0252] Preservatives are added to retard microbial growth, and are typically present in a range from 0.2% - 1.0% (w/v). Suitable preservatives for use with the present invention include octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium halides (*e.g.*, chloride, bromide, iodide), benzethonium chloride; thimerosal, phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol, 3-pentanol, and *m*-cresol.

[0253] Tonicity agents, sometimes known as "stabilizers" are present to adjust or maintain the tonicity of liquid in a composition. When used with large, charged biomolecules such as proteins and antibodies, they are often termed "stabilizers" because they can interact with the charged groups of the amino acid side chains, thereby lessening the potential for inter- and intra-molecular interactions. Tonicity agents can be present in any amount between 0.1% to 25% by weight, or more preferably between 1% to 5% by weight, taking into account the relative amounts of the other ingredients. Preferred tonicity agents include polyhydric sugar alcohols, preferably trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol.

[0254] Additional excipients include agents which can serve as one or more of the following: (1) bulking agents, (2) solubility enhancers, (3) stabilizers and (4) and agents preventing denaturation or adherence to the container wall. Such excipients include: polyhydric sugar alcohols (listed above); amino acids such as alanine, glycine, glutamine, asparagine, histidine, arginine, lysine, ornithine, leucine, 2-phenylalanine, glutamic acid, threonine, and the like; organic sugars or sugar alcohols such as sucrose, lactose, lactitol, trehalose, stachyose, mannose,

sorbose, xylose, ribose, ribitol, myoinisitose, myoinisitol, galactose, galactitol, glycerol, cyclitols (*e.g.*, inositol), polyethylene glycol; sulfur containing reducing agents, such as urea, glutathione, thioctic acid, sodium thioglycolate, thioglycerol, α-monothioglycerol and sodium thio sulfate; low molecular weight proteins such as human serum albumin, bovine serum albumin, gelatin or other immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; monosaccharides (*e.g.*, xylose, mannose, fructose, glucose; disaccharides (*e.g.*, lactose, maltose, sucrose); trisaccharides such as raffinose; and polysaccharides such as dextrin or dextran.

[0255] Non-ionic surfactants or detergents (also known as "wetting agents") are present to help solubilize the therapeutic agent as well as to protect the therapeutic protein against agitation-induced aggregation, which also permits the formulation to be exposed to shear surface stress without causing denaturation of the active therapeutic protein or antibody. Non-ionic surfactants are present in a range of about 0.05 mg/ml to about 1.0 mg/ml, preferably about 0.07 mg/ml to about 0.2 mg/ml.

[0256] Suitable non-ionic surfactants include polysorbates (20, 40, 60, 65, 80, *etc.*), polyoxamers (184, 188, *etc.*), PLURONIC® polyols, TRITON®, polyoxyethylene sorbitan monoethers (TWEEN®-20, TWEEN®-80, *etc.*), lauromacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, sucrose fatty acid ester, methyl cellulose and carboxymethyl cellulose. Anionic detergents that can be used include sodium lauryl sulfate, dioctyle sodium sulfosuccinate and dioctyl sodium sulfonate. Cationic detergents include benzalkonium chloride or benzethonium chloride.

[0257] In order for pharmaceutical formulations comprising IL-27 antagonists to be used for *in vivo* administration, they must be sterile. The formulation may be rendered sterile by filtration through sterile filtration membranes. The therapeutic compositions herein generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[0258] The route of administration is in accordance with known and accepted methods, such as by single or multiple bolus or infusion over a long period of time in a suitable manner, *e.g.*, injection or infusion by subcutaneous, intravenous, intraperitoneal, intramuscular, intraarterial, intralesional or intraarticular routes, topical administration, inhalation or by sustained release or extended-release means.

[0259] The IL-27 antagonist 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. Alternatively, or in addition, the composition may comprise a cytotoxic agent, cytokine or growth inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[0260] The active ingredients may also be entrapped in microcapsules prepared, for example, by coascervation 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* 20th Edition, *supra*.

[0261] Stability of the proteins and antibodies described herein may be enhanced through the use of non-toxic "water-soluble polyvalent metal salts". Examples include Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{2+}, Fe^{3+}, Cu^{2+}, Sn^{2+}, Sn^{4+}, Al^{2+} and Al^{3+}. Exemplary anions that can form water soluble salts with the above polyvalent metal cations include those formed from inorganic acids and/or organic acids. Such water-soluble salts have are soluble in water (at 20°C) to at least about 20 mg/ml, alternatively at least about 100 mg/ml, alternatively at least about 200 mg/ml.

[0262] Suitable inorganic acids that can be used to form the "water soluble polyvalent metal salts" include hydrochloric, acetic, sulfuric, nitric, thiocyanic and phosphoric acid. Suitable organic acids that can be used include aliphatic carboxylic acid and aromatic acids. Aliphatic acids within this definition may be defined as saturated or unsaturated C_{2-9} carboxylic acids (*e.g.*, aliphatic mono-, di- and tri-carboxylic acids). For example, exemplary monocarboxylic acids within this definition include the saturated C_{2-9} monocarboxylic acids acetic, proprionic, butyric, valeric, caproic, enanthic, caprylic pelargonic and capryonic, and the unsaturated C_{2-9} monocarboxylic acids acrylic, propriolic methacrylic, crotonic and isocrotonic acids. Exemplary dicarboxylic acids include the saturated C_{2-9} dicarboxylic acids malonic, succinic, glutaric, adipic and pimelic, while unsaturated C_{2-9} dicarboxylic acids include maleic, fumaric, citraconic and mesaconic acids. Exemplary tricarboxylic acids include the saturated C_{2-9} tricarboxylic acids tricarballylic and 1,2,3-butanetricarboxylic acid. Additionally, the carboxylic acids of this definition may also contain one or two hydroxyl groups to form hydroxy carboxylic acids.

Exemplary hydroxy carboxylic acids include glycolic, lactic, glyceric, tartronic, malic, tartaric and citric acid. Aromatic acids within this definition include benzoic and salicylic acid.

[0263] Commonly employed water soluble polyvalent metal salts which may be used to help stabilize the encapsulated polypeptides of this invention include, for example: (1) the inorganic acid metal salts of halides (*e.g.*, zinc chloride, calcium chloride), sulfates, nitrates, phosphates and thiocyanates; (2) the aliphatic carboxylic acid metal salts (e.g., calcium acetate, zinc acetate, calcium proprionate, zinc glycolate, calcium lactate, zinc lactate and zinc tartrate); and (3) the aromatic carboxylic acid metal salts of benzoates (*e.g.*, zinc benzoate) and salicylates.

[0264] Pharmaceutical formulations of IL-27 antagonists, such as those comprising small molecules, aptamers or polypeptides other than antibodies or antibody fragments, can be designed to immediately release an IL-27 antagonist ("immediate-release" formulations), to gradually release the IL-27 antagonist over an extended period of time ("sustained-release," "controlled-release," or "extended-release" formulations), or with alternative release profiles. The additional materials used to prepare a pharmaceutical formulation can vary depending on the therapeutic form of the formulation (*e.g.*, whether the system is designed for immediate-release or sustained-, controlled-, or extended-release). In certain variations, a sustained-release formulation can further comprise an immediate-release component to quickly deliver a priming dose following drug delivery, as well as a sustained-release component. Thus, sustained-release formulations can be combined with immediate-release formulations to provide a rapid "burst" of drug into the system as well as a longer, gradual release. For example, a core sustained-release formulation may be coated with a highly soluble layer incorporating the drug. Alternatively, a sustained-release formulation and an immediate-release formulation may be included as alternate layers in a tablet or as separate granule types in a capsule. Other combinations of different types of drug formulations can be used to achieve the desired therapeutic plasma profile.

[0265] Exemplary sustained-release dosage formulations (discussed in *Remington's Pharmaceutical Sciences* 20th Edition, *supra*) can include a wide variety of drug delivery systems, including those that employ: (a) a reservoir system in which the drug is encapsulated in a polymeric membrane, permitting water to diffuse through the membrane to dissolve the drug, which then diffuses out of device; (b) a matrix system (gradient or monolithic) in which the drug is suspended in a polymeric matrix and gradually diffuses out as the matrix dissolves or disintegrates; (c) micro-encapsulation and coated granule systems in which particles of drug (or

particles of drug and polymer) as small as 1 micrometer ("μm"; 10^{-6} m) in diameter are coated in a polymeric membrane, including embodiments in which particles coated with polymers having different release characteristics (*e.g.*, pH-dependent or non-pH-dependent polymers, compounds with different degrees of water solubility, and the like) are delivered together in a single capsule; (d) solvent-activated systems, including (i) osmotically controlled devices (*e.g.*, OROS®, Alza Corp., Mountain View, CA) in which an osmotic agent and a drug are encapsulated in a semi-permeable membrane, such that an osmotic gradient pulls water into the device, and increased pressure drives drug out of device via pores in the membrane; (ii) a hydrogel swelling system in which drug is dispersed in a polymer and/or a polymer is coated onto a particle of drug, wherein the polymer swells on contact with water (in certain embodiments, swelling can be pH-dependent, pH-independent, or dependent on other physical or chemical characteristics), allowing diffusion of drug out of the device; (iii) a microporous membrane system in which drug is encapsulated in a membrane that has a component that dissolves on contact with water (in certain embodiments, swelling can be pH-dependent, pH-independent, or dependent on other physical or chemical characteristics), producing pores in the membrane through which the drug diffuses; and (iv) a wax matrix system in which the drug and an additional soluble component are dispersed in wax, such that, when water dissolves the soluble component, diffusion of drug from the system is allowed; and (e) polymeric degradation systems, including (i) bulk degradation, in which drug is dispersed in a polymeric matrix, and degradation occurs throughout the polymeric structure in a random fashion, allowing drug release; and (ii) surface erosion, in which drug is dispersed in a polymeric matrix and delivered as the surface of the polymer erodes.

E. Methods of Treatment

[0266] The invention provides methods for treating or preventing inflammatory bowel disease (IBC) in an individual comprising administering to the individual an effective amount of an IL-27 antagonist. In some embodiments, the individual is a human. In some embodiments, the individual has IBD or is at risk of developing IBD.

[0267] The IBD may be ulcerative colitis (UC) or Crohn's disease. In some embodiments, an individual having IBD is one that is experiencing or has experienced one or more signs, symptoms, or other indicators of IBD or has been diagnosed with IBD. An individual having IBD may have may have steroid-refractory and/or steroid dependent IBD, steroid-refractory

and/or steroid dependent UC or steroid-refractory and/or steroid dependent Crohn's disease. "Steroid-refractory" IBD is IBD which progresses, or worsens, even though steroid is being administered to the subject with IBD. An individual with "steroid-dependent" IBD is dependent on steroid use, and cannot taper or withdraw steroid administration due to persistent symptoms.

[0268] In some embodiments, the IBD may be T cell dependent or T cell mediated. In some embodiments, the individual with IBD has a breakdown in regulatory T-cell mediated tolerance. For example, T cells may be detected at the intestinal lesion site in the individual with IBD. Biopsy samples may be taken from the pathological lesion sites from the individual with IBD. The presence of T cells may be detected by methods known in the art (such as immunohistochemistry techniques). For example, antibodies directed against markers specifically expressed on T-cells, such as CD4 or CD3, may be used to detect the presence and amount of T cells. The amount of T cells detected in the biopsy sample may be compared to the amount of T cells in the biopsy sample from a healthy individual or from an intestinal site without inflammation from the same individual.

[0269] The administration of the IL-27 antagonist may result in a clinical response and/or disease remission. As used herein, "clinical response" refers to an improvement in the symptoms of disease. “Disease remission” indicates substantially no evidence of the symptoms of disease. The clinical response or disease remission may be achieved within a certain time frame, for example, within or at about 8 weeks from the start of treatment with, or from the initial dose of, the antagonist. Clinical response may also be sustained for a period of time; such as for ≥24 weeks, or ≥48 weeks.

[0270] Symptoms associated with IBD includes abdominal pain, vomiting, diarrhea, hematochezia (bright red blood in stools), and weight loss. Further tests may be carried out for diagnosing IBD. For example, complete blood cell count, electrolyte panel, liver function tests (LFT), fecal occult blood test, X-rays (including barium enema and upper gastrointestinal series), sigmoidoscopy, colonoscopy, and upper endoscopy may be used. Various scoring system known in the art may be used for quantitatively assessing severity of the disease.

[0271] For the prevention or treatment of disease, the appropriate dosage of an active agent (i.e., an IL-27 antagonist), will depend on the type of disease to be treated, the severity and course of the disease, whether the agent is administered for preventive or therapeutic purposes, previous therapy, the patient’s clinical history and response to the agent, and the discretion of the

attending physician. The particular dosage regimen, i.e., dose, timing, and repetition, will depend on the particular individual and that individual's medical history as assessed by a physician. Typically the clinician will administer an IL-27 antagonist, such as an anti-IL-27 antibody, an anti-IL-27p28 antibody, an anti-IL-27Ebi3 antibody, an anti-IL-27 receptor antibody, or an anti-IL-27Ra antibody, until a dosage is reached that achieves the desired result.

[0272] Methods of the present invention are useful for treating, ameliorating or palliating the symptoms of IBD (such as ulcerative colitis, or Crohn's disease) in an individual, or for improving the prognosis of an individual suffering from IBD. The quality of life in individuals suffering from IBD may be improved, and the symptoms of IBD may be reduced or eliminated following treatment with IL-27 antagonists. For example, weight loss associated with IBD may be reduced and/or eliminated. Methods of the present invention are also useful for delaying development of or preventing IBD in an individual at risk of developing IBD.

[0273] Any IL-27 antagonists described herein may be administered to the individual. In certain embodiments, the IL-27 antagonist is an anti-IL-27 antibody. In certain embodiments, the IL-27 antagonist is an anti-IL-27p28 antibody. In certain embodiments, the IL-27 antagonist is an anti-IL-27Ebi3 antibody. In certain embodiments, the IL-27 antagonist is an anti-IL-27 receptor antibody. In certain embodiments, the IL-27 antagonist is an anti-IL-27Ra antibody.

F. Combination Therapies

[0274] The methods of the invention can be combined with known methods of treatment for IBD (such as such as ulcerative colitis or Crohn's disease), either as combined or additional treatment steps or as additional components of a therapeutic formulation. Alternatively, different IL-27 antagonists may be administered in combination (*e.g.*, an anti-IL-27Ra antibody may be administered with an IL-27-specific aptamer, or an anti-IL-27 antibody may be administered with an siRNA directed to IL-27Ra). The type of combination therapy selected will depend on the clinical manifestations of the disease.

[0275] IBD (such as ulcerative colitis or Crohn's disease) can be treated by combination therapy comprising administration of IL-27 antagonists in conjunction with a second medicament for IBD. The type of such second medicament depends on various factors, including the type of IBD, the severity of the IBD, the condition and age of the subject, the type and dose of first medicament employed, etc. In some embodiments, the second medicament includes one or more of an aminosalicylate, a corticosteroid, and an immunosuppressive agent.

In some embodiments, the aminosalicylate is one of sulfasalazine, olsalazine, mesalamine, balsalazide, and asacol. In some embodiments, multiple aminosalicylates are co-administered, such as a combination of sulfasalazine and olsalazine. In some embodiments, the corticosteroid is budesonide, prednisone, prednisolone, methylprednisolone, 6-mercaptopurine (6-MP), azathioprine, methotrexate, or cyclosporin. In some embodiments, the second medicament is an antibiotic, such as ciprofloxacin and/or metronidazole; or an antibody-based agent such as infliximab (Remicade®).

[0276] All these second medicaments may be used in combination with each other or by themselves with the first medicament, so that the expression "second medicament" as used herein does not mean it is the only medicament besides the first medicament, respectively. Thus, the second medicament need not be one medicament, but may constitute or comprise more than one such drug.

[0277] These second medicaments as set forth herein are generally used in the same dosages and with administration routes as used hereinbefore or about from 1 to 99% of the heretofore-employed dosages. If such second medicaments are used at all, optionally, they are used in lower amounts than if the first medicament were not present, especially in subsequent dosings beyond the initial dosing with the first medicament, so as to eliminate or reduce side effects caused thereby.

[0278] Combined administration herein includes co-administration, using separate formulations or a single pharmaceutical formulation, and consecutive administration in either order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities.

G. Pharmaceutical Dosages

[0279] Dosages and desired drug concentration of pharmaceutical compositions of the present invention may vary depending on the particular use envisioned. The determination of the appropriate dosage or route of administration is well within the skill of an ordinary artisan. Animal experiments provide reliable guidance for the determination of effective doses for human therapy. Interspecies scaling of effective doses can be performed following the principles laid down by Mordenti, J. and Chappell, W. "The Use of Interspecies Scaling in Toxicokinetics," In *Toxicokinetics and New Drug Development*, Yacobi et al., Eds, Pergamon Press, New York 1989, pp.42-46.

[0280] For *in vivo* administration of the polypeptides or antibodies described herein, normal dosage amounts may vary from about 10 ng/kg up to about 100 mg/kg of an individual's body weight or more per day, preferably about 1 mg/kg/day to 10 mg/kg/day, depending upon the route of administration. For repeated administrations over several days or longer, depending on the severity of the disease or disorder to be treated, the treatment is sustained until a desired suppression of symptoms is achieved.

[0281] An exemplary dosing regimen comprises administering an initial dose of IL-27 antagonist, such as an antagonist antibody, of about 2 mg/kg, followed by a weekly maintenance dose of about 1 mg/kg every other week. Other dosage regimens may be useful, depending on the pattern of pharmacokinetic decay that the physician wishes to achieve. For example, dosing an individual from one to twenty-one times a week is contemplated herein. In certain embodiments, dosing ranging from about 3 μg/kg to about 2 mg/kg (such as about 3 μg/kg, about 10 μg/kg, about 30 μg/kg, about 100 μg/kg, about 300 μg/kg, about 1 mg/kg, and about 2/mg/kg) may be used. In certain embodiments, dosing frequency is three times per day, twice per day, once per day, once every other day, once weekly, once every two weeks, once every four weeks, once every five weeks, once every six weeks, once every seven weeks, once every eight weeks, once every nine weeks, once every ten weeks, or once monthly, once every two months, once every three months, or longer. Progress of the therapy is easily monitored by conventional techniques and assays. The dosing regimen, including the IL-27 antagonist administered, can vary over time independently of the dose used.

[0282] Generally, a non-antibody IL-27 antagonist may be administered at a dose of about 0.1 mg/kg to about 300 mg/kg, in one to three doses per day. In certain embodiments, for an adult individual of normal weight, doses ranging from about 0.3 mg/kg to about 5.00 mg/kg may be administered. The particular dosage regimen, *e.g.*, dose, timing, and repetition, will depend on the particular individual being treated, that individual's medical history, and the properties of the IL-27 antagonist being administered (*e.g.*, the half-life of the antagonist, and other considerations known in the art).

[0283] Dosages for a particular IL-27 antagonist may be determined empirically in individuals who have been given one or more administrations of IL-27 antagonist. Individuals are given incremental doses of an IL-27 antagonist. To assess efficacy of an IL-27 antagonist, a clinical symptom of IBD (such as ulcerative colitis, and Crohn's disease) can be monitored.

[0284] Administration of an IL-27 antagonist according to the methods of the invention can be continuous or intermittent, depending, for example, on the recipient's physiological condition, whether the purpose of the administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The administration of an IL-27 antagonist (*e.g.*, an IL-27 antibody, an IL-27-p28 antibody, an IL-27Ebi3 antibody, an IL-27 receptor antibody, or an IL-27Ra antibody) may be essentially continuous over a preselected period of time or may be in a series of spaced doses, *e.g.*, either during or after development of IBD (such as ulcerative colitis, and Crohn's disease).

[0285] Guidance regarding particular dosages and methods of delivery is provided in the literature; see, for example, U.S. Patent Nos. 4,657,760; 5,206,344; or 5,225,212. It is within the scope of the invention that different formulations will be effective for different treatments and different disorders, and that administration intended to treat a specific organ or tissue may necessitate delivery in a manner different from that to another organ or tissue. Moreover, dosages may be administered by one or more separate administrations, or by continuous infusion. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

H. Administration of the Formulations

[0286] The formulations of the present invention (*e.g.*, formulations of IL-27 antagonists), including, but are not limited to reconstituted formulations, are administered to an individual in need of treatment with the IL-27 antagonist, preferably a human, in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerobrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes.

[0287] In preferred embodiments, the formulations are administered to the individual by subcutaneous (*i.e.* beneath the skin) administration. For such purposes, the formulation may be injected using a syringe. However, other devices for administration of the formulation are available such as injection devices (*e.g.* the INJECT-EASE™ and GENJECT™devices); injector pens (such as the GENPEN™); auto-injector devices, needleless devices (*e.g.* MEDIJECTOR™ and BIOJECTOR™); and subcutaneous patch delivery systems.

[0288] The appropriate dosage (an "effective amount") of the IL-27 antagonist will depend, for example, on the condition to be treated, the severity and course of the condition, whether the IL-27 antagonist is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the IL-27 antagonist, the type of IL-27 antagonist used, and the discretion of the attending physician. The IL-27 antagonist is suitably administered to the patient at one time or over a series of treatments and may be administered to the patient at any time from diagnosis onwards. The IL-27 antagonist may be administered as the sole treatment or as part of a combination therapy in conjunction with other drugs or therapies useful in treating IBD (such as ulcerative colitis, and Crohn's disease).

[0289] Where the IL-27 antagonist of choice is an antibody, from about 0.1 mg/kg to about 20 mg/kg is an initial candidate dosage for administration to an individual, whether, for example, by one or more separate administrations. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques.

[0290] Uses for an IL-27 antagonist formulation include the treatment or prophylaxis of IBD, for example. Depending on the severity of the disease to be treated, a therapeutically effective amount (*e.g.*, from about 1 mg/kg to about 15 mg/kg) of the IL-27 antagonist is administered to the individual.

Nucleic acid formulations

[0291] Targeted delivery of therapeutic compositions containing an antisense polynucleotide, an siRNA or other RNAi agent, expression vector, or subgenomic polynucleotides can also be used. Receptor-mediated DNA delivery techniques are described in, for example, Findeis et al., *Trends Biotechnol.* (1993) 11:202; Chiou et al., *Gene Therapeutics: Methods and Applications of Direct Gene Transfer* (J.A. Wolff, ed.) (1994); Wu et al., *J. Biol. Chem.* (1988) 263:621; Wu et al., *J. Biol. Chem.* (1994) 269:542; Zenke et al., *Proc. Nat'l Acad. Sci. USA* (1990) 87:3655; Wu et al., *J. Biol. Chem.* (1991) 266:338. Therapeutic compositions containing a polynucleotide are administered in a range of about 100 ng to about 200 mg of DNA for local administration in a gene therapy protocol. In certain embodiments, concentration ranges of about 500 ng to about 50 mg, about 1 μg to about 2 mg, about 5 μg to about 500 μg, and about 20 μg to about 100 μg/of DNA or more can also be used during a gene therapy protocol. The therapeutic polynucleotides and polypeptides of the present invention can be delivered using gene delivery vehicles. The gene delivery vehicle can be of viral or non-viral origin (*see generally* Jolly,

Cancer Gene Therapy (1994) 1:51; Kimura, *Human Gene Therapy* (1994) 5:845; Connelly, *Human Gene Therapy* (1995) 1:185; and Kaplitt, *Nature Genet.* (1994) 6:148). Expression of such coding sequences can be induced using endogenous mammalian or heterologous promoters and/or enhancers, such as those discussed above. Expression of the coding sequence can be either constitutive or regulated.

[0292] Viral-based vectors for delivery of a desired polynucleotide and expression in a desired cell are well-known in the art. Exemplary viral-based vehicles include, but are not limited to, recombinant retroviruses (*see, e.g.*, PCT Publication Nos. WO 90/07936; WO 94/03622; WO 93/25698; WO 93/25234; WO 93/11230; WO 93/10218;, WO 91/02805; U.S. Patent Nos. 5,219,740 and 4,777,127; GB Patent No. 2,200,651; and EP Patent No. 0 345 242), alphavirus-based vectors (*e.g.*, Sindbis virus vectors, Semliki forest virus (ATCC VR-67; ATCC VR-1247), Ross River virus (ATCC VR-373; ATCC VR-1246) Venezuelan equine encephalitis virus (ATCC VR-923; ATCC VR-1250; ATCC VR-1249; ATCC VR-532), and adeno-associated virus ("AAV") vectors (*see, e.g.*, PCT Publication Nos. WO 94/12649; WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984; and WO 95/00655). Administration of DNA linked to killed adenovirus as described in Curiel, *Hum. Gene Ther.* (1992) 3:147, can also be used.

[0293] Non-viral delivery vehicles and methods can also be used, including, but not limited to, polycationic condensed DNA linked or unlinked to killed adenovirus alone (*see, e.g.*, Curiel, 1992), ligand-linked DNA (*see, e.g.*, Wu, *J. Biol. Chem.* (1989) 264:16985), eukaryotic cell delivery vehicles (*see, e.g.*, U.S. Patent No. 5,814,482; PCT Publication Nos. WO 95/07994; WO 96/17072; WO 95/30763; and WO 97/42338), and nucleic acid neutralization or fusion with cell membranes. Naked DNA can also be used. Exemplary methods using naked DNA are described in PCT Publication No. WO 90/11092 and U.S. Patent No. 5,580,859. Liposomes that can be used as gene delivery vehicles are described in U.S. Patent No. 5,422,120; PCT Publication Nos. WO 95/13796; WO 94/23697; WO 91/14445; and EP Patent No. 0 524 968. Additional approaches are described in Philip, *Mol. Cell Biol.* (1994) 14:2411, and in Woffendin, *Proc. Nat'l Acad. Sci. USA* (1994) 92:1581.

I. Articles of Manufacture

[0294] In another aspect, an article of manufacture is provided which contains an IL-27 antagonist formulation and preferably provides instructions for its use in the methods of the invention. Thus, in certain embodiments, the article of manufacture comprises instructions for

the use of an IL-27 antagonist in methods for treating or preventing IBD (such as ulcerative colitis, and Crohn's disease) in an individual comprising administering to the individual an effective amount of an IL-27 antagonist. In certain embodiments, the individual is a human.

[0295] The article of manufacture further comprises a container. Suitable containers include, for example, bottles, vials (*e.g.*, dual chamber vials), syringes (such as single or dual chamber syringes) and test tubes. The container may be formed from a variety of materials such as glass or plastic. The container holds the formulation. The article of manufacture may further comprises a label or a package insert, which is on or associated with the container, may indicate directions for reconstitution and/or use of the formulation. The label or package insert may further indicate that the formulation is useful or intended for subcutaneous or other modes of administration for treating or preventing IBD (such as ulcerative colitis, and Crohn's disease) in an individual. The container holding the formulation may be a single-use vial or a multi-use vial, which allows for repeat administrations (*e.g.* from 2-6 administrations) of the reconstituted formulation. The article of manufacture may further comprise a second container comprising a suitable diluent (*e.g.*, BWFI). Upon mixing the diluent and the lyophilized formulation, the final protein, polypeptide, or small molecule concentration in the reconstituted formulation will generally be at least 50 mg/ml. The article of manufacture may further include other materials desirable from a commercial, therapeutic, and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

[0296] The article of manufacture herein optionally further comprises a container comprising a second medicament, wherein the IL-27 antagonist is a first medicament, and which article further comprises instructions on the package insert for treating the subject with the second medicament, in an effective amount. The second medicament may be any of those set forth above, with an exemplary second medicament being an aminosalicylate, an oral corticosteroid, 6-mercaptopurine (6-MP), and azathioprine.

[0297] In another embodiment, the invention provides for an article of manufacture comprising the formulations described herein for administration in an auto-injector device. An auto-injector can be described as an injection device that upon activation, will deliver its contents without additional necessary action from the patient or administrator. They are particularly suited for self-medication of therapeutic formulations when the delivery rate must be constant and the time of delivery is greater than a few moments.

[0298] The invention will be more fully understood by reference to the following examples. They should not, however, be construed as limiting the scope of the invention. All citations throughout the disclosure are hereby expressly incorporated by reference.

EXAMPLES

[0299] Interleukin-27 (IL-27) is a cytokine known to have both pro-inflammatory and immunoregulatory functions. The latter appear to dominate *in vivo*, where IL-27 suppresses T helper type 17 (TH17) responses and promote the differentiation of Foxp3 negative, IFN-γ and IL-10 expressing Tr1 cells. Accordingly, IL-27 receptor (*Il27ra*) deficient mice suffer from exacerbated immune pathology when infected with various parasites or challenged with autoantigens. The role of IL-27 in human and experimental murine colitis is underexplored and controversial. The consequences of *Il27ra* deletion in T cell transfer colitis were studies and a pro-inflammatory role of IL-27 was discovered in this context. Absence of *Il27ra* on the transferred T-cells resulted in diminished weight loss and reduced colonic inflammation. A far greater fraction of the transferred T-cells assumed a $Foxp3^+$ phenotype in the absence of *Il27ra*, suggesting that IL-27 functions to restrain T_{reg} development. Indeed, IL-27 suppressed Foxp3 induction *in vitro* and in an ovalbumin dependent tolerization model *in vivo*. Taken together, the examples below describe a novel mechanism of how IL-27 overrides an immune suppressive pathway to promote intestinal inflammation and provide a rationale for targeting this cytokine in pathologic situations that result from a breakdown in peripheral immune tolerance.

Example 1: IL-27Ra deficient $CD4^+CD45Rb^{hi}$ T-cells fail to induce fulminant colitis.

[0300] To determine whether IL-27 responsiveness of T-cells was required for the establishment of the colitis phenotype, the $CD4^+CD45Rb^{hi}$ transfer model of colitis was used (Powrie et al., *Int Immunol* 5:1461-1471 (1993)). First, the IL-27Ra deficient allele (Chen et al., *Nature* 407:916-920 (2000)) was crossed into the *balb/c* background for 12 generations. As described previously in C57BL/6 mice, IL-27Ra deficiency causes no overt abnormalities in the *balb/c* background (data not shown). However, transfer of FACS purified *balb/c* IL-$27Ra^{-/-}$ $CD45Rb^{hi}$ T-cells into CB.17-SCID recipient mice surprisingly resulted in partial protection against weight loss compared with transfer of IL-$27Ra^{+/+}$ $CD45Rb^{hi}$ T-cells (Figures 1 A and B), suggesting that IL-27 plays a pro-inflammatory role in this model. While weight loss can be the

consequence of colitis, it has also been demonstrated in a related model that weight loss predominantly depends on IL-12, whereas development of colitis requires IL-23, and thus the two phenomena are not always tightly linked (Uhlig et al., I*mmunity* 25:309-318 (2006)). Since IL-27 suppresses the TH17 axis, which *in vivo* is driven by IL-23, colitis might still be exacerbated in recipients of IL-27Ra$^{-/-}$ CD45Rb^{hi} cells and performed histological analysis of the colons at 12 weeks post cell transfer. However, recipients of IL-27Ra$^{-/-}$ CD45Rb^{hi} cells also displayed a reduced amount of colonic shortening (Figure 1 C) and had significantly reduced histological scores (Figures 1 D and E). Therefore, presence of IL-27Ra on T-cells is required in this model for the development of both fulminant colitis and maximal weight loss. It is noteworthy that the CB.17-SCID recipients used are wild-type for IL-27Ra, and thus expression of IL-27Ra in the innate compartment is insufficient to confer protection in the CD45Rb^{hi} transfer model, as was observed by Troy et al in the DSS (dextran sulfate sodium) model (Troy et al., *J Immunol* 183:2037-2044 (2009)).

Example 2: IL-27Ra$^{-/-}$ T-cells produce less IFN-γ and more IL-17 and IL-22 than their WT counterparts.

[0301] In order to better understand how IL-27Ra signaling in T-cells promoted transfer colitis, lymphocytes isolated from the spleen, the mesenteric lymph node, and the colonic lamina propria of CD45Rb^{hi} recipient animals at week 12 post transfer were re-stimulated. In accordance with prior observations that IL-27 is required for efficient TH1 responses (Chen et al., *Nature* 407:916-920 (2000); Yoshida et al., *Immunity* 15:569-578 (2001)), cells isolated from IL-27Ra$^{-/-}$ CD45Rb^{hi} recipients produced significantly less IFN-γ as assessed by intracellular staining (Figures 2 A and B). However, while IFN-γ plays a pathogenic role in this model as shown through neutralization experiments (Powrie et al, *Immunity* 1:553-562 (1994)), IFN-γ that is specifically derived from T-cells is not required for colitis induction (Simpson et al., *J Exp Med* 187:1225-1234 (1998)), and, thus, reduced IFN-γ production alone can not explain why IL-27Ra$^{-/-}$ CD45Rb^{hi} T-cells fail to induce maximal disease. Conversely, these cells produced slightly higher amounts of the TH17 cytokines IL-17 and IL-22 (Figures 2 C and D), suggesting partial de-repression of TH17 responses due to IL-27 unresponsiveness. Interestingly, while the TH17 hallmark transcription factor RORγT is required for pathogenicity of CD45Rb^{hi} T-cells (Leppkes et al., *Gastroenterology* 136:257-267 (2009)), both IL-17 (O'Connor et al., *Nat Immunol* 10:603-609 (2009)) and IL-22 (Zenewicz et al., *Immunity* 29:947-957 (2008)) have

been shown to exert protective effects in the context of this model. Therefore, elevated IL-17 and IL-22 production by IL-27Ra$^{-/-}$ CD45Rb^{hi} cells is consistent with protection against colitis. However, the magnitude of change observed for IL-17 and IL-22 was relatively small and did not translate into increased infiltration by neutrophils (Figure 3). Thus, it is unlikely that IL-27 promotes colitis simply by changing the cytokine secretion profile of the transferred CD45Rb^{hi} cells.

Example 3: IL-27Ra signaling limits conversion of naïve T-cells into Foxp3$^+$ T_{regs}.

[0302] Control animals transferred with total CD4$^+$ cells from either genotype neither suffered from weight loss nor from colitis due to the presence of regulatory T-cells (T_{regs}) in the cell graft (Figure 1). A difference in the presence of Foxp3$^+$ cells might account for the observed phenotype was investigated. As shown in Figure 3A, elevated T_{reg} cells were detected in the absence of *Il27ra* on T-cells as early as five weeks after transfer of CD45Rb^{hi} cells. Furthermore, when mice were sacrificed at the end of the study, it was found that recipients of Il27ra$^{-/-}$ CD45Rb^{hi} cells contained about 2 to 3 times the normal proportion of Foxp3$^+$ T-cells in blood, spleen, mesenteric lymph node, and lamina propria (Figures 3 A and B and Figure 7 A). In agreement with enhanced suppressive activity, it was found that Il27ra$^{-/-}$ recipients contained fewer total CD4$^+$ T-cells (Figure 7 B and C).

[0303] Previous studies and experiments revealed no reduction in the frequency of Foxp3$^+$ cells in naïve Il27ra$^{-/-}$ mice (Figure 14 and (Batten et al., *Nat Immunol* 7:929-936 (2006)). Furthermore, the suppressive capacity of these cells in vitro is unaffected by the absence of Il27ra (Figure 4A and (Batten et al., *Nat Immunol* 7:929-936 (2006)). However, because FACS sorted WT and Il27ra$^{-/-}$ CD4$^+$CD45Rb^{hi} cells contained approximately 0.5% nT_{regs} (Figure 5), it remained possible that the increased frequency of Foxp3$^+$ cells in Il27ra$^{-/-}$ CD45Rb^{hi} recipients resulted from preferential in vivo expansion of or enhanced in vivo suppressive capacity by Il27ra$^{-/-}$ nT_{regs}. To address this concern, purified CD4$^+$CD25$^+$ cells from WT or IL-27Ra$^{-/-}$ mice were transferred into Rag2 deficient C57BL/6 mice that had been transferred with WT CD4$^+$CD45Rb^{hi} cells 7 weeks prior. nT_{regs} from either genotype were fully capable of rescuing their hosts from systemic wasting disease (Figure 4 B and C). Furthermore, similar frequencies of Foxp3$^+$ cells of both genotypes were observed in the blood, spleen, and mesenteric lymph

nodes of the rescued recipient animals, suggesting that the *in vivo* expansion rate of nT_{regs} was not affected by the IL-27Ra genotype (Figure 4 D).

[0304] Inducible T_{reg} develop from naïve $CD4^+$ T-cells upon stimulation in the presence of TGF-β. It has been demonstrated in the context of transfer colitis that this type of conversion occurs *in vivo* in a small fraction of the transferred cells (Sun et al., *J Exp Med* 204:1775-1785 (2007)); however, the resulting number of $Foxp3^+$ cells is insufficient to afford the host full protection, and colitis ensues nevertheless. T_{reg} conversion is restrained by IL-23 signaling. When $CD45Rb^{hi}$ cells are transferred into IL-23p19 deficient hosts, the fraction of $Foxp3^+$ cells is increased, while disease activity is suppressed (Izcue et al., *Immunity* 28:559-570 (2008)). To determine whether IL-27Ra signaling also acts to limit the conversion of naïve cells into the T_{reg} phenotype, T-cells from peripheral blood were studied at different times during the experiment. As shown in Figure 6A, elevated T_{reg} conversion was detected in the absence of IL-27Ra as early as five weeks after transfer of $CD45Rb^{hi}$ cells. Furthermore, when mice were sacrificed at the end of the study, $IL\text{-}27Ra^{-/-}$ cells were found to be converted to a $Foxp3^+$ phenotype at about 2 to 3 times the normal rate in blood, spleen, mesenteric lymph node, and lamina propria (Figure 6 B and C). In agreement with enhanced suppressive activity, $IL\text{-}27Ra^{-/-}$ recipients also contained fewer total $CD4^+$ T-cells (Figure 6 D).

Example 4: IL-27 does not act by limiting IL-2 supply

[0305] As an *in vitro* correlate to the homeostatic proliferation model for inducible T_{reg} generation, the ability of IL-27 to antagonize Foxp3 expression by naïve $CD4^+$ T cells was tested. Robust induction of Foxp3 was observed when naïve T-cells were stimulated in the presence of TGF-β, and this Foxp3 induction was efficiently suppressed when recombinant IL-27 was added to the culture (Figure 8 A), confirming that IL-27 can suppress the induction of $Foxp3^+$ cells (Huber et al., *Int Immunol* 20:223-234 9 (2008)). In addition to TGF-β, conversion of naïve cells into a $Foxp3^+$ T_{reg} phenotype also requires IL-2 (Knoechel et al., *J Exp Med* 202:1375-1386 (2005)), which is endogenously produced as a result of T-cell activation. Since IL-27 is known to suppress IL-2 production (Owaki et al., *J Immunol* 176:2773-2780 (2006), Villarino et al., *J Immunol* 176:237-247 (2006)), this may be the mechanism by which it constrains T_{reg} conversion. If this were true, then addition of recombinant IL-2 should reverse the suppressive effect of IL-27. However, addition of IL-2 did not affect Foxp3 expression (Figure 8

A and B). Furthermore, IL-27 dose dependently suppressed Foxp3 induction, and the IC_{50} of this effect was not changed upon addition of exogenous IL-2 to the culture (Figure 8 B). Finally, no impact of IL-27 on CD25 expression was observed (Figure 8 A), and thus IL-27 did not act to render T-cells insensitive to IL-2 stimulation in this *in vitro* system.

Example 5: IL-27 limits T_{reg} conversion in an OVA dependent tolerization model in vivo.

[0306] *In vivo*, T-cells receive co-stimulatory signals from antigen presenting cells, whose stimulatory capacity depends on their microenvironment. While splenic dendritic cells (DCs) are typically highly pro-inflammatory, DC isolated from the gut lamina propria allow for the development of a $Foxp3^+$ phenotype when used to stimulate naïve T-cells (Sun et al., *J Exp Med* 204:1775-523 1785 (2007)). Inducible T_{reg} develop from naïve $CD4^+$ T-cells upon stimulation in the presence of TGF-β. It has been demonstrated in the context of transfer colitis that this type of conversion occurs in vivo in a small fraction of the transferred cells (Sun et al. (2007)); however, the resulting number of $Foxp3^+$ cells is insufficient to afford the host full protection, and colitis ensues nevertheless. Prior reports have suggested that IL-27 can suppress the TGF-β driven induction of $Foxp3^+$ cells in vitro (Huber et al., *Int. Immunol.* 20:223-234 (2008); Neufert et al., *Eur. J. Immunol.* 37:1809-1816 (2007)), and whether IL-27 normally restrains T_{reg} conversion in vivo was investigated. To enable experiments that are not encumbered by nT_{reg} contamination, the IL-27Ra deficient allele was bred into a $DO11.10^+$ and $Rag2^{-/-}$ background. Such mice contain a pristine population of naïve, ovalbumin specific T-cells that is devoid of nT_{regs} (Figure 9 A), and hence represent an ideal system to study T_{reg} conversion. First, $DO11.10^+Rag2^{-/-}$ T-cells were cultured in the presence of lamina propria DC, Ova peptide and TGF-β (Figure 8 C). Consistent with published reports and Figure 8 A, lamina propria DC allowed for $Foxp3^+$ conversion, and IL-27 suppressed T_{reg} conversion (Figure 8 C). However, in this experimental system IL-27 also affected CD25 expression and generally suppressed T-cell activation as measured by CD69 surface expression. IL-27 acted directly on T-cells to downregulate Foxp3, CD25 and CD69, because it had no effect when $IL\text{-}27Ra^{-/-}$ T-cells were used in the experiment.

[0307] Next it was investigated whether IL-27 signals naturally limit T_{reg} conversion at mucosal sites *in vivo*. To this end, purified $DO11.10^+Rag2^{-/-}$ T-cells were transferred into naïve

balb/c recipients who were then exposed to ovalbumin in the drinking water. Exposure to antigen lead to a highly significant increase in $Foxp3^+$ cells in the spleens and mesenteric lymph nodes. (Figure 9B-C). Consistent with data obtained from the colitis model, IL-27Ra deficiency significantly augmented peripheral T_{reg} development, indicating that IL-27 limits T_{reg} conversion even in a non-inflammatory environment. This effect was further accentuated when the absolute numbers of $Foxp3^+$ $DO11.10^+rag2^{-/-}$ cells were measured (Figure 10A), and persisted when the experiment was repeated under inflammatory conditions in mice that had received WT $CD45Rb^{hi}$ cells 4 weeks earlier (Figure 11). Because only naïve, Foxp3 negative cells were transferred into recipients, this experiment also indicated that IL-27 signaling limits T_{reg} conversion rather

than expansion of nT_{regs}. Consistent with prior observations by others (Villarino et al., *J. Immunol.* 176:237-247 (2006)), $Il27ra^{-/-}$ DO11.10 cells produced more IL-2 (Figure 10 B), while IFN-γ production was minimal irrespective of *Il27ra* expression in the non-inflammatory environment of unchallenged *balb/c* mice (Figure 10 C). However, increased production of IL-2 is not responsible for enhanced T_{reg} conversion, because IL-2 does not override the suppressive effect of IL-27 on Foxp3 induction (Neufert et al., *Eur. J. Immunol.* 37:1809-1816 (2007) and unpublished observations), which has been shown in vitro to be a direct, STAT3 mediated effect of IL-27 on T-cells (Huber et al., *Int. Immunol.* 20:223-234 (2008)).

Example 6: $Il27ra^{-/-}$ effector T-cells have a different cytokine secretion profile compared to their WT counterparts.

[0308] Despite the increased conversion rate of $Il27ra^{-/-}$ $CD45Rb^{hi}$ cells, the majority of the transferred cells remained $Foxp3^-$. Therefore, cytokine production was examined in the colon by RT-PCR and in re-stimulated lymphocytes isolated from the spleen, the mesenteric lymph node, and the colonic lamina propria of $CD45Rb^{hi}$ recipient animals by intracellular staining. While IL-12, IL-23, IL2, IL-6 and IL-27 were all found to be induced in colitic mice, no statistically significant changes in were noted between WT and $Il27ra^{-/-}$ $CD45Rb^{hi}$ recipients (Figure 12). In accordance with prior observations (Artis et al., *J. Immunol.* 173:5626-5634 (2004); Batten et al., *Nat. Immunol.* 7:929-936 (2006); Chen et al., *Nature* 407:916-920 (2000); Stumhofer et al., *Nat. Immunol.* 7:937-945 (2006), T-cells isolated from $Il27ra^{-/-}$ $CD45Rb^{hi}$ recipients produced significantly less IFN-γ as assessed by intracellular staining (Figure 13 B), mimicking decreased

production of IFN- γ in naïve mice (Figure 14B). The overall decrease of $CD4^+$ cells (Figure 7 C) further accentuates the decrease in absolute numbers of IFN- γ producing cells (data not shown). Therefore, the question whether diminished IFN- γ production is partially responsible for the protective effect of *Il27ra* deficiency merits consideration. Using IFN- γ neutralizing antibodies, others have previously reported a pathogenic role for IFN- γ in this model (Powrie et al., *Immunity* 1:553-562 (1994)). IFN- $\gamma^{-/-}$ $CD45Rb^{hi}$ cells elicited less severe colitis, but also caused highly aggressive wasting disease requiring early termination of the experiment (Figure 15 A-C). Consistent with findings obtained by others (Wang et al., *J. Clin. Invest.* 116:2434-2441 (2006)), IFN- $\gamma^{-/-}$ $CD45Rb^{hi}$ cells had a diminished propensity to become $Foxp3^+$ and expanded more aggressively (Figures 15 D and E). Thus IFN-γ deficiency phenocopies only the reduced colitis but neither the improved wasting disease nor the increased T_{reg} conversion and reduced expansion observed with $Il27ra^{-/-}$ $CD45Rb^{hi}$ cells and thus can not fully explain the $Il27ra^{-/-}$ phenotype.

[0309] Conversely, and consistent with prior reports (Artis et al., 2004; Batten et al., 2006; Stumhofer et al., 2006; Yang et al., *Eur. J. Immunol.* 38:1204-1214 (2008)), mild elevations in TH17 (IL-17 A and IL-22) and TH2 (IL-5 and IL-13) cytokines produced by $Il27ra^{-/-}$ $CD45Rb^{hi}$ cells were observed (Figure 13 C-E and data not shown). However, these changes were effectively neutralized in absolute terms by the lower total number of $CD4^+$ cells (Figure 7C and data not shown). Increased neutrophil infiltration was not observed, as one would expect as a consequence of IL-17 overexpression (Figure 16). Therefore, the minor relative changes in IL-17 and IL-22 production by $Il27ra^{-/-}$ $CD45Rb^{hi}$ cells are unlikely to contribute significantly to disease protection, even though IL-17 (Izcue et al., *Immunity* 28:559-570 (2008); Leppkes et al., *Gastroenerol.* 136:257-267 (2009); O'Connor et al., *Nat. Immunol.* 10"603-609 (2009)) has been shown to either be neutral or exert protective effects in the context of this model, and IL-22 is protective (Zenewicz et al., *Immunity* 29:947-957 (2008)).

[0310] Earlier reports showed that IL-27 can induce IL-10 production in T-cells and lead to the development of Foxp3 negative, $IFN\gamma^+IL\text{-}10^+$ Tr1 cells (Awasthi et al., *Nat. Immunol.* 8:1380-1389 (2007); Batten et al., *J. Immunol.* 180:2752-2756 (2008); Fitzgerald et al., *Nat. Immunol.* 8:1372-1379 (2007); Stumhofer et al., *Nat. Immunol.* 8:1363-1371 (2007)). Because Tr1 cells have potent immunoregulatory effects (Anderson et al., *J. Exp. Med.* 204:285-297 (2007); Jankovic et al., *J. Exp. Med.* 204:273-283 (2007); Trinchieri, *J. Exp. Med.* 204:239-243 (2007)),

it has been postulated that this is a mechanism by which IL-27 exerts immune suppression in infectious and autoimmune disease. In transfer colitis, however, IL-10 production by cells originating from the Foxp3 negative $CD45Rb^{hi}$ graft is minimal (Uhlig et al., *J. Immunol.* 177:5852-5860 (2006)). These observations have been confirmed and no *Il27ra* dependent difference was found in IL-10 production by transferred $CD45Rb^{hi}$ cells (data not shown). Thus the Foxp3 suppressing effects of IL-27 are not in contradiction with its Tr1 inducing effects; the dichotomy merely reflects the differences between the physiological contexts in which IL-27 stimulation occurs and may explain why the essential function of IL-27 in the regulation of T_{reg} differentiation has not been noted previously.

[0311] The majority of in vivo effects assigned to IL-2 have been inferred from the analysis of *Il27ra*$^{-/-}$ mice, and some have been confirmed by studies of IL-27p28$^{-/-}$ or *ebi3*$^{-/-}$ mice. To date, IL-27 is the only confirmed ligand for IL-27Ra. However, IL-35 has been described as an IL-27 related heterodimer consisting of IL-12p35 and Ebi3 (Collison et al., *Nature* 450:566-569 (2007)), and its receptor has not been identified. Another recently described IL-27 related heterodimer consists of IL-27p28 and cytokine like factor (CLF) and appears to bind to IL-27Ra, but conclusive proof that IL-27Ra is required for signaling is currently not available (Crabe et al., *J. Immunol.* 183:7692-7702 (2009)). Thus it remains possible that IL- 27Ra has ligands other than IL-27 which might contribute further to the apparent complexity and dichotomous nature of IL-27 biology in vivo.

[0312] Taken together, IL-27 exerts pro-inflammatory effects in the T-cell transfer colitis model. IL-27 acts to suppress iT_{reg} development and thus reveal a hitherto unrecognized pro-inflammatory mechanism. Targeting of IL-27 in situations where pathology results from a breakdown in T_{reg} mediated tolerance may result in significant therapeutic benefit.

Materials and Methods

[0313] The following materials and methods were used in Examples 1-6:

[0314] *Mice.* All mice were maintained under pathogen-free conditions and experiments were approved by the Institutional Animal Care and Use Committee of Genentech, Inc. The IL-27Ra$^{-/-}$ allele (Chen et al., *Nature* 407:916-920 (2000)) was backcrossed onto the *balb/c* background for 12 generations. This strain was crossed further to the DO11.10^{+}Rag2$^{-/-}$ background (Taconic

Farms). CB17-SCID mice were purchased from Charles River Laboratories. *balb/c* and $Rag2^{-/-}$ C57BL6 mice were from Taconic.

[0315] *Cytokines.* Unless otherwise indicated, all cytokines including IL-27 were purchased from R&D Systems and all antibodies used in flow cytometry or culture experiments were from BD Biosciences. Anti-IL-22 (clone 3F11, isotype mouse IgG2a, Genentech) was directly conjugated to Alexa Fluor 647 (Molecular Probes).

[0316] *Induction of colitis with naïve $CD4^+CD45Rb^{hi}$ T cells.* Naïve $CD4^+CD45Rb^{hi}$ cells were isolated from spleens of female $IL\text{-}27R^{+/+}$ or $IL\text{-}27Ra^{-/-}$ mice by FACS sorting. Briefly, single cell suspensions were depleted of red blood cells by hypotonic lysis and $CD4^+$ cells were purified by MACS positive L3T4 selection (Miltenyi). After staining with Pacific Blue-conjugated anti-CD4, PE-conjugated anti-CD45Rb, and PECy5-conjugated anti-CD44, $CD4^+CD44^{lo}CD45Rb^{hi}$ naïve T cells were purified (>98%) by cell sorting (FACSAria, BD Biosciences). Female CB17-SCID mice were injected with $3x10^5$ $CD45Rb^{hi}$ cells or unsorted $CD4^+$ cells I.V. Mice were monitored for weight loss and sacrificed by CO_2 asphyxiation 11-12 weeks after initiation of the experiment. Blood was obtained by retro-orbital bleeds under isoflurane anesthesia at the indicated time points.

[0317] *Assessment of intestinal inflammation.* At the time of sacrifice, mouse colons were removed, flushed, and the length was measured from rectum to cecum. Tissues were immediately fixed in 10% buffered formalin and 4-5 μm paraffin-embedded sections were stained with hematoxylin and eosin. Colitis severity was scored in the proximal colon, medial colon, distal colon, and rectum on a scale of 0-5, with 0 and 5 representing a normal colon and severe colitis, respectively. The scores of 4 anatomical regions were summed for each mouse to yield a total histological score.

[0318] *Flow cytometry.* Single cell suspensions were obtained from spleens and mesenteric lymph nodes. Lamina propria leukocytes were isolated as described previously (Zheng et al., *Nat Med* 14:282-289 (2008)). Cell counts were measured by Viacount analysis on the Guava PCA-96 (Millipore). Anti-CD4 and anti-CD25 were used for surface staining of lymphocytes. For intracellular cytokine staining, single cell suspensions from spleens, mesenteric lymph nodes and lamina propria were restimulated for 4 hours in RPMI containing 10% FBS with 50 ng/ml of phorbol 12-myristate 13-acetate and 500 ng/ml of ionomycin in the presence of 5 μg/ml of brefeldin A (Sigma). Cells were then fixed in 1% paraformaldehyde in PBS, permeabilized with

0.5% saponin in flow cytometry buffer (0.5% BSA in PBS), and stained intracellularly with PE-conjugated anti-IL-17, PECy5-conjugated anti-mouse IFN-γ, and Alexa 647-conjugated anti-mouse IL-22 (Genentech) and PE-conjugated anti-mouse IL-13. Alternatively, Foxp3 staining was performed according to manufacturer's protocol (eBioscience).

[0319] *Induction of T regulatory cells.* $CD4^+CD25^-$ cells were isolated from spleens of IL-$27Ra^{+/+}$ or IL-$27Ra^{-/-}$ mice by flow cytometry. Cells were stimulated in flat-bottom 96-well plates (10^5 cells/well) for 72 h with plate-bound anti-CD3 (5 μg/ml), soluble anti-CD28 (2 μg/ml), anti-IFN-γ (5 μg/mL), anti-IL-4 (5 μg/mL), hTGF-β (5 ng/ml), IL-2 (20 ng/ml) as indicated, and IL-27 (0 ng/ml – 20 ng/ml). Cells were stained with APC-conjugated anti-CD25 and PE-conjugated anti-Foxp3 according to manufacturer's protocol (eBioscience).

[0320] *In vitro conversion of DO11.10 T cells.* Lamina propria (LP) and splenic (Sp) DCs were purified following collagenase treatment by sorting of $CD11c^+MHCII^+$ cells. $DO11.10^+CD4^+$ cells were MACS purified and incubated (5 x 10^4 cells/well) with SpDCs or LPDCs (5 x 10^3 cells/well) in the presence of $OVA_{323-339}$ (0.3 μM), TGF-β (3 ng/mL), and IL-27 (20 ng/mL) as indicated. Cells were stimulated for 5 days in round-bottom 96-well plates and intracellular Foxp3 was analyzed as described above.

[0321] *In vivo conversion of DO11.10 T-cells.* CD4+ T lymphocytes were enriched (~85% purity) from the spleens and mesenteric lymph nodes of female IL-$27Ra^{+/+}$ or IL-$27Ra^{-/-}$ $DO11.10^+Rag2^{-/-}$ mice by negative MACS selection (Miltenyi). Female *balb/c* recipient mice (Taconic) were injected with 1 × 10^6 cells I.V. on day 0. On day 1, mice were given 1.5% ovalbumin (Grade V, Sigma-Aldrich) dissolved in drinking water for 5 days as described (Sunet al., *J Exp Med* 204:1775-1785 (2007)). Ovalbumin water was replaced every 48 hours and control mice received normal drinking water. On day 6, mice were sacrificed and spleens, mesenteric lymph nodes, Peyer's patches, and lamina propria lymphocytes were assessed for Foxp3 expression. Cells were surface stained with PE-conjugated anti-KJI-26 for the DO-11.10 TCR then stained for intracellular Foxp3 as described above.

[0322] *Suppression assay.* $CD4^+CD25^-$ and $CD4^+CD25^+$ cells were FACS-sorted from spleens of WT and $IL27Ra^{-/-}$ mice. Splenocyte samples magnetically depleted of T cells with anti-CD90 (Thy-1.2) MACS Micro Beads (Miltenyi Biotec) were irradiated (2,600 rads) and used as APCs. WT $CD4^+CD25^-$ cells ($5x10^4$/well) were incubated with irradiated APCs ($2.5x10^5$/well), 0.5 mg/mL soluble anti-CD3, and WT or IL-$27Ra^{-/-}$ $CD4^+CD25^+$ cells as indicated. Cells were

stimulated for 72 h in round-bottomed 96-well plates and proliferation was assessed by measurement of the incorporation of ^{3}H-thymidine during the final 8 h of culture.

[0323] *$CD4^{+}CD25^{+}$ rescue of wasting disease in $Rag2^{-/-}$ recipients.* $Rag2^{-/-}$ mice on a C57BL/6 background (Taconic) were injected I.V. with $4x10^{5}$ WT FACS-sorted $CD45Rb^{hi}$ cells. At 7 weeks post-transfer, mice were randomized according to percentage of initial weight and either received no further treatment or were injected I.V. with 10^{6} $CD4^{+}CD25^{+}$ cells that were purified with the MACS T regulatory cell kit (Miltenyi Biotech). Mice were weighed weekly and sacrificed 12 weeks after $CD45Rb^{hi}$ transfer. Single cell suspensions of spleens and lymph nodes were analyzed for Foxp3 expression as per manufacturer's protocol (eBioscience).

WHAT IS CLAIMED IS:

1. A method for treating or preventing inflammatory bowel disease in an individual comprising administering to the individual an effective amount of an IL-27 antagonist.

2. The method of claim 1, wherein T cells are detected in a biopsy sample from the intestinal lesion site in the individual.

3. The method of claim 2, wherein the T cells detected are $CD4^+$ T cells.

4. The method of any one of claims 1-3, wherein the individual has Crohn's disease.

5. The method of any one of claims 1-3, wherein the individual has ulcerative colitis.

6. The method of any one of claims 1-5, wherein the individual is a human.

7. The method of any one of claims 1-6, wherein the IL-27 antagonist is an anti-IL-27 antibody that specifically binds to IL-27.

8. The method of claim 7, wherein the IL-27 antagonist is an anti-IL-27 antibody that specifically binds to the p28 subunit of IL-27 ("IL-27p28").

9. The method of claim 7, wherein the IL-27 antagonist is an anti-IL-27 antibody that specifically binds to the Epstein Barr virus induced protein 3 (Ebi3) subunit of IL-27 ("IL-27Ebi3").

10. The method of any one of claims 7-9, wherein the anti-IL-27 antibody inhibits binding between IL-27 and its receptor.

11. The method of any one of claim 7-10, wherein the anti-IL-27 antibody inhibits IL-27 signal transduction.

12. The method of claim 11, wherein the anti-IL-27 antibody inhibits IL-10 production.

13. The method of claim 11, wherein the anti-IL-27 antibody inhibits IL-21 production.

14. The method of any one of claims 7-13, wherein the anti-IL-27 antibody is a monoclonal antibody.

15. The method of claim 14, wherein the anti-IL-27 antibody is an antibody fragment selected from the group consisting of Fab, Fab'-SH, Fv, scFv, and $(Fab')_2$ fragments.

16. The method of any one of claims 7-13, wherein the anti-IL-27 antibody is a humanized antibody.

17. The method of any one of claims 7-13, wherein the anti-IL-27 antibody is a human antibody.

18. The method of any one of claims 7-13, wherein the anti-IL-27 antibody is a bispecific antibody.

19. The method of any one of claims 1-6, wherein the IL-27 antagonist is an anti-IL-27Ra antibody that specifically binds to IL-27Ra.

20. The method of claim 19, wherein the anti-IL-27Ra antibody is a monoclonal antibody.

21. The method of claim 19, wherein the anti-IL-27Ra antibody is an antibody fragment selected from the group consisting of Fab, Fab'-SH, Fv, scFv, and $(Fab')_2$ fragments.

22. The method of claim 19, wherein the anti-IL-27Ra antibody is a humanized antibody.

23. The method of claim 19, wherein the anti-IL-27Ra antibody is a human antibody.

24. The method of any one of claims 19-23, wherein the anti-IL-27Ra antibody inhibits binding between IL-27 and its receptor.

25. The method of any one of claims 1-6, wherein the IL-27 antagonist is a small molecule that inhibits binding between IL-27 and its receptor.

26. The method of any one of claims 1-6, wherein the IL-27 antagonist is a polypeptide that inhibits binding between IL-27 and its receptor.

27. The method of any one of claims 1-6, wherein the IL-27 antagonist is a DNA or RNA aptamer that inhibits binding between IL-27 and its receptor.

28. The method of any one of claims 1-6, wherein the IL-27 antagonist is a short interfering RNA that inhibits expression of IL-27, IL-27p28, IL-27Ebi3, or IL-27Ra.

29. The method of any one of claims 1-28, wherein the IL-27 antagonist is administered intravenously, intramuscularly, subcutaneously, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally.

30. The method of any one of claims 1-29, where in the wasting disease associated with ulcerative colitis in the individual is treated.

31. An article of manufacture comprising an IL-27 antagonist and a package insert comprising instructions for using the IL-27 antagonist to treat or prevent inflammatory bowel disease in an individual.

Figure 1

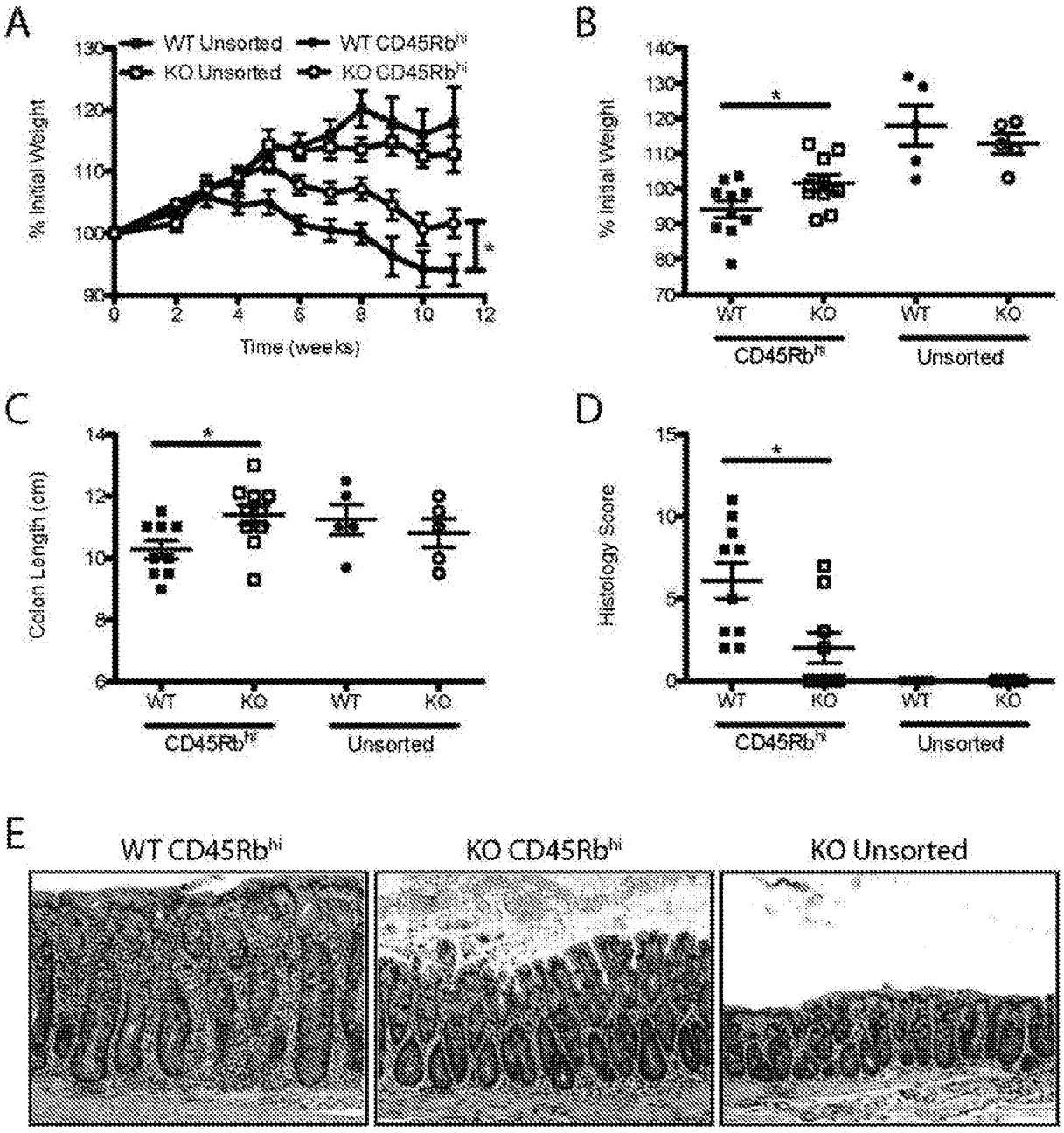

A
WT Unsorted
WT CD45Rb^hi
KO Unsorted
KO CD45Rb^hi
% Initial Weight
Time (weeks)
B
% Initial Weight
WT
KO
WT
KO
CD45Rb^hi
Unsorted
C
Colon Length (cm)
D
Histology Score
E
WT CD45Rb^hi
KO CD45Rb^hi
KO Unsorted

Figure 2

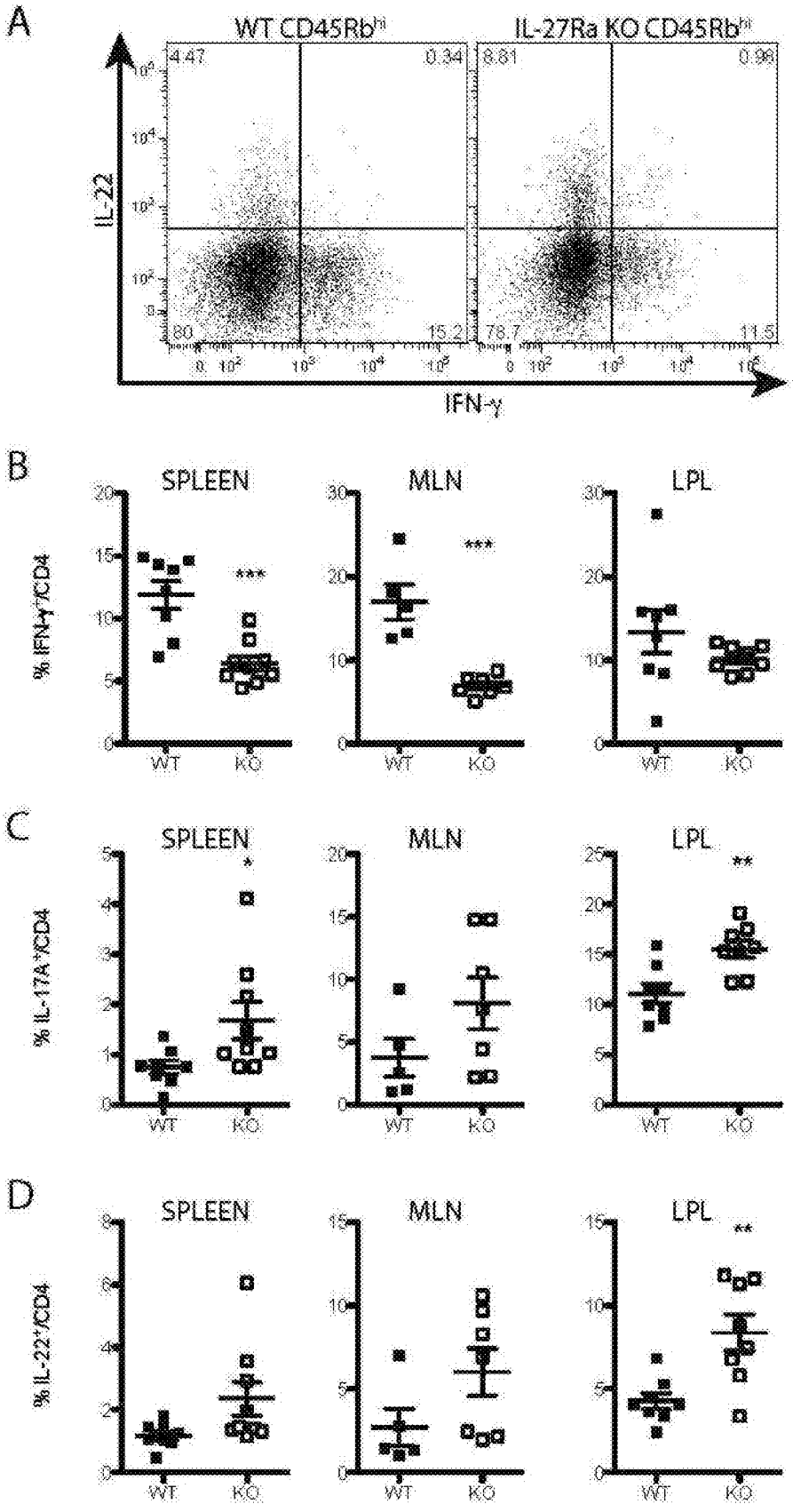

A
WT CD45Rb^hi
IL-27Ra KO CD45Rb^hi
4.47
0.34
80
15.2
8.81
0.98
78.7
11.5
IL-22
IFN-γ
B
SPLEEN
MLN
LPL
% IFN-γ+/CD4
***
***
WT
KO
C
SPLEEN
MLN
LPL
% IL-17A+/CD4
*
**
D
SPLEEN
MLN
LPL
% IL-22+/CD4
**

Figure 3

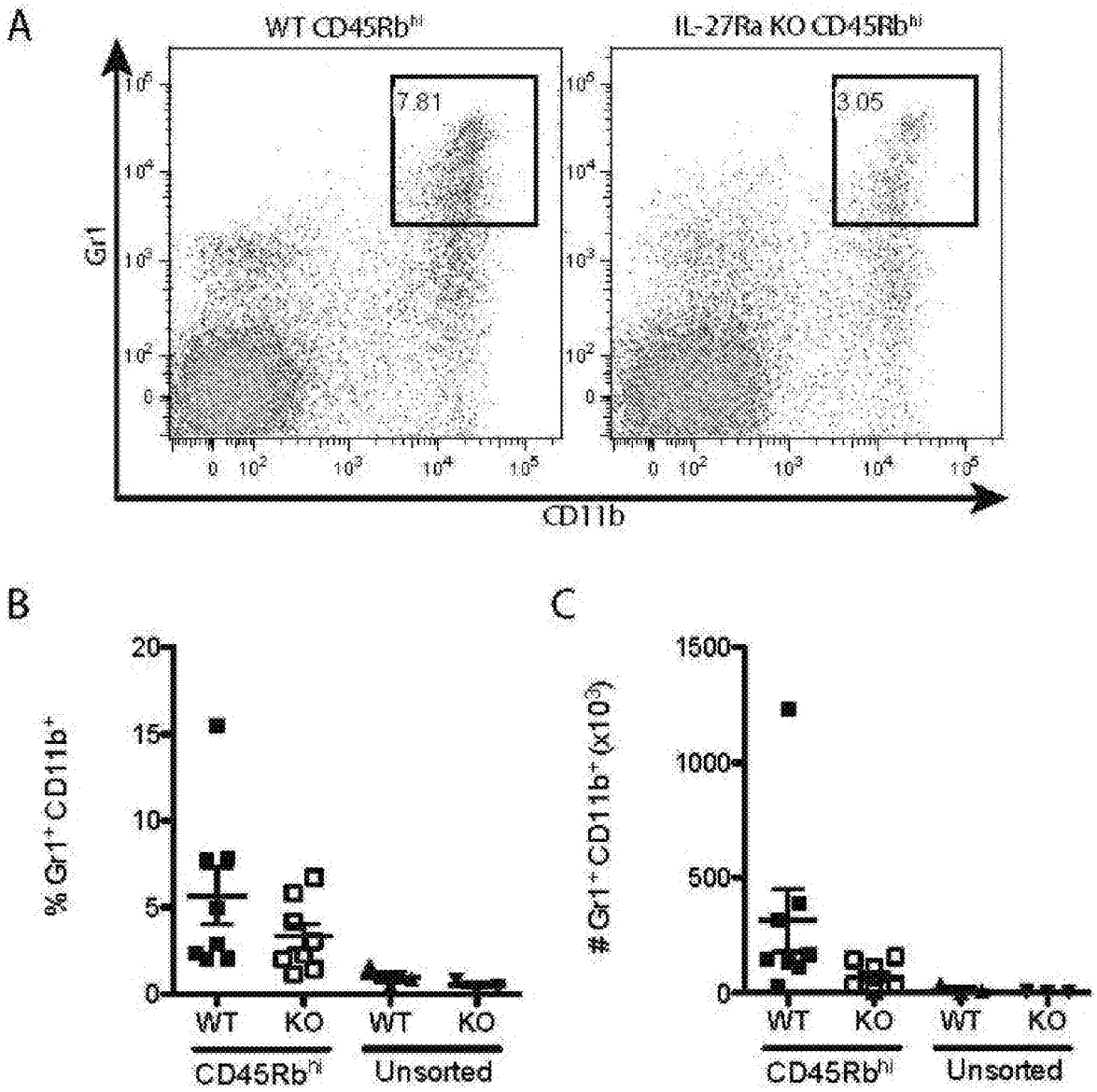

A
WT CD45Rb^hi
IL-27Ra KO CD45Rb^hi
7.81
3.05
Gr1
CD11b
B
% Gr1+ CD11b+
WT
KO
WT
KO
CD45Rb^hi
Unsorted
C
Gr1+ CD11b+ (x10^3)
WT
KO
WT
KO
CD45Rb^hi
Unsorted

Figure 4

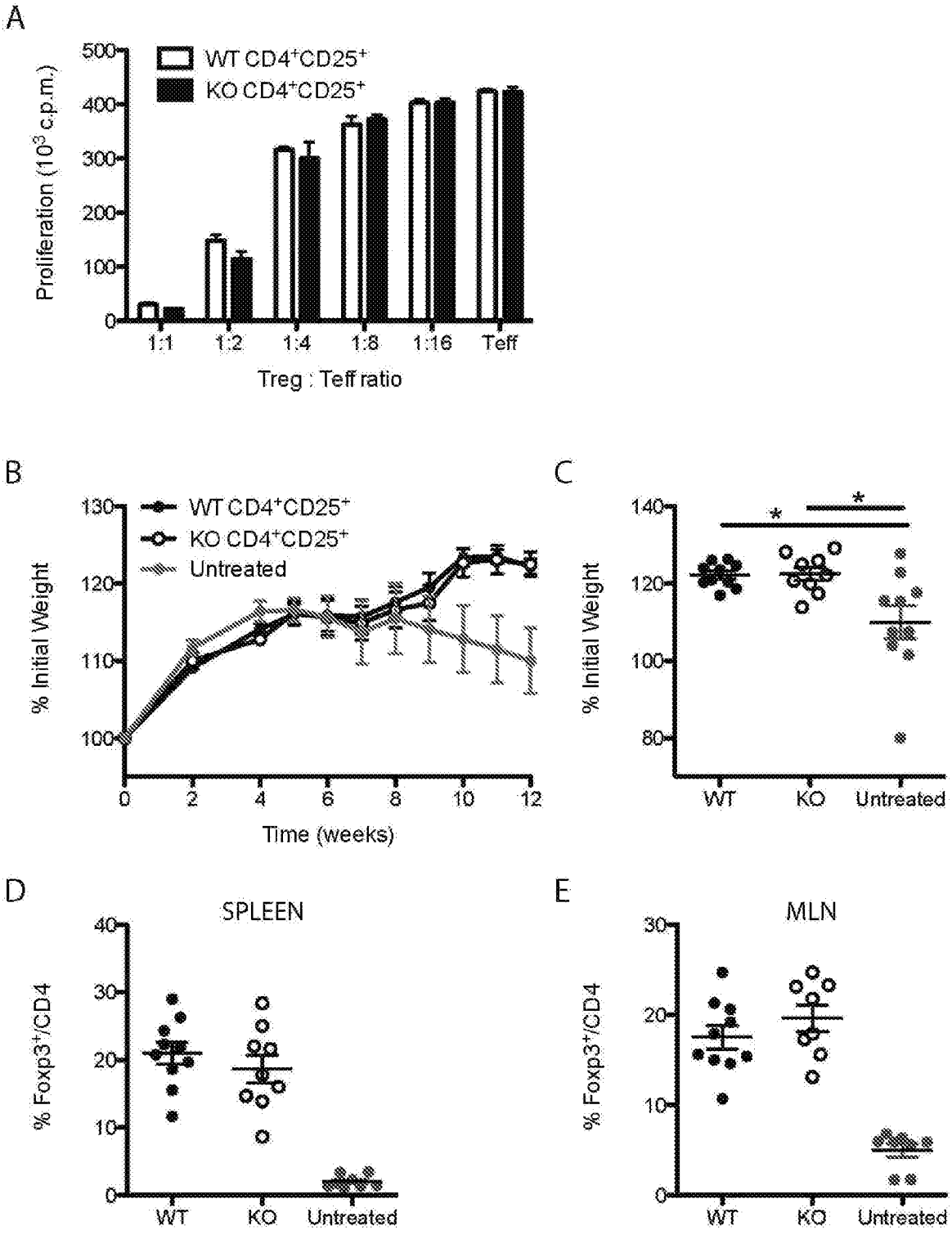

A
WT CD4+CD25+
KO CD4+CD25+
Proliferation (10^3 c.p.m.)
500
400
300
200
100
0
1:1
1:2
1:4
1:8
1:16
Teff
Treg : Teff ratio
B
WT CD4+CD25+
KO CD4+CD25+
Untreated
% Initial Weight
130
120
110
100
0
2
4
6
8
10
12
Time (weeks)
C
% Initial Weight
140
120
100
80
*
*
WT
KO
Untreated
D
SPLEEN
% Foxp3+/CD4
40
30
20
10
0
WT
KO
Untreated
E
MLN
% Foxp3+/CD4
30
20
10
0
WT
KO
Untreated

Figure 5

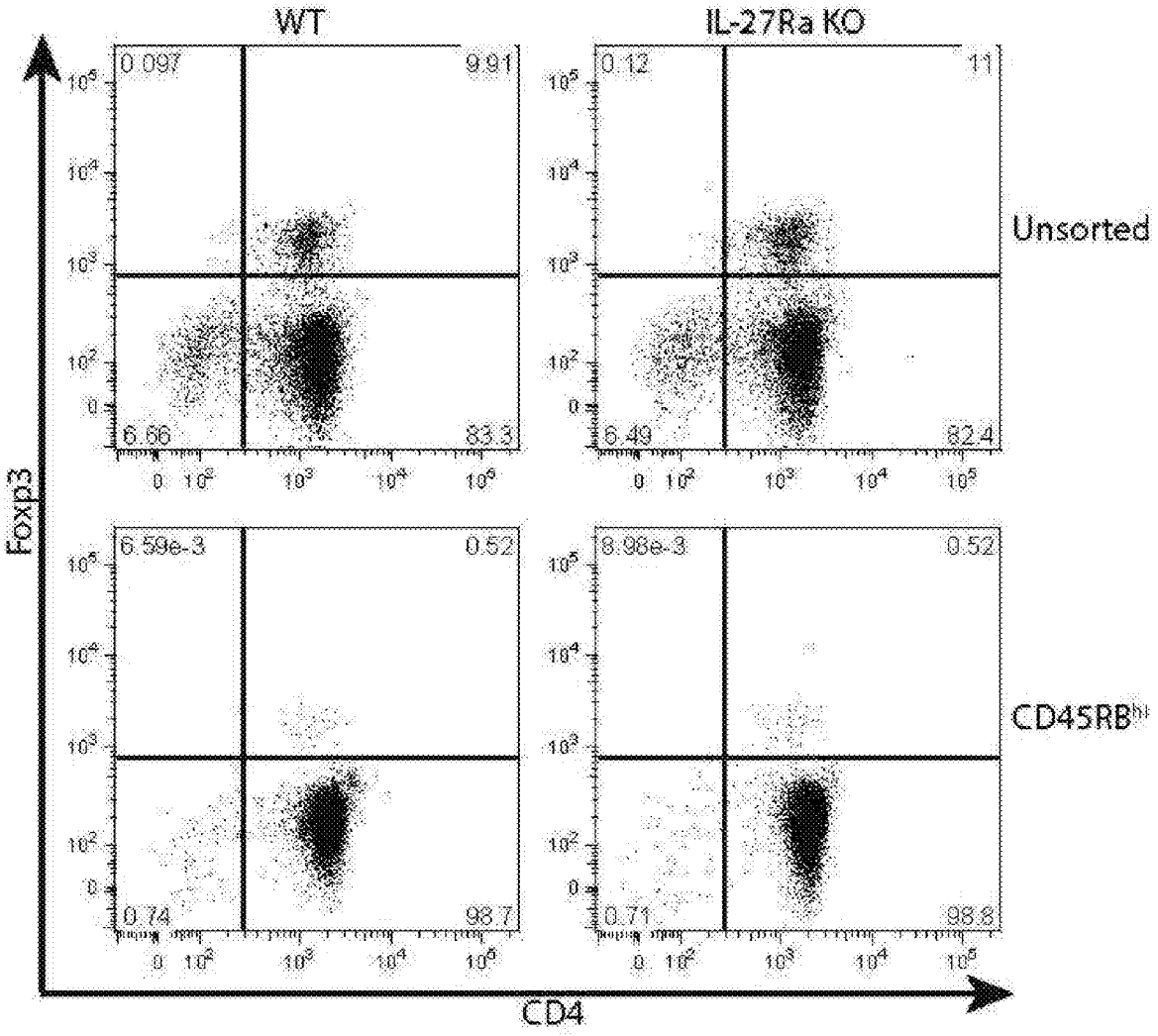

WT
IL-27Ra KO
Unsorted
CD45RB^hi
Foxp3
CD4
0.097
9.91
6.66
83.3
0.12
11
6.49
82.4
6.59e-3
0.52
0.74
98.7
8.98e-3
0.52
0.71
98.8

Figure 6

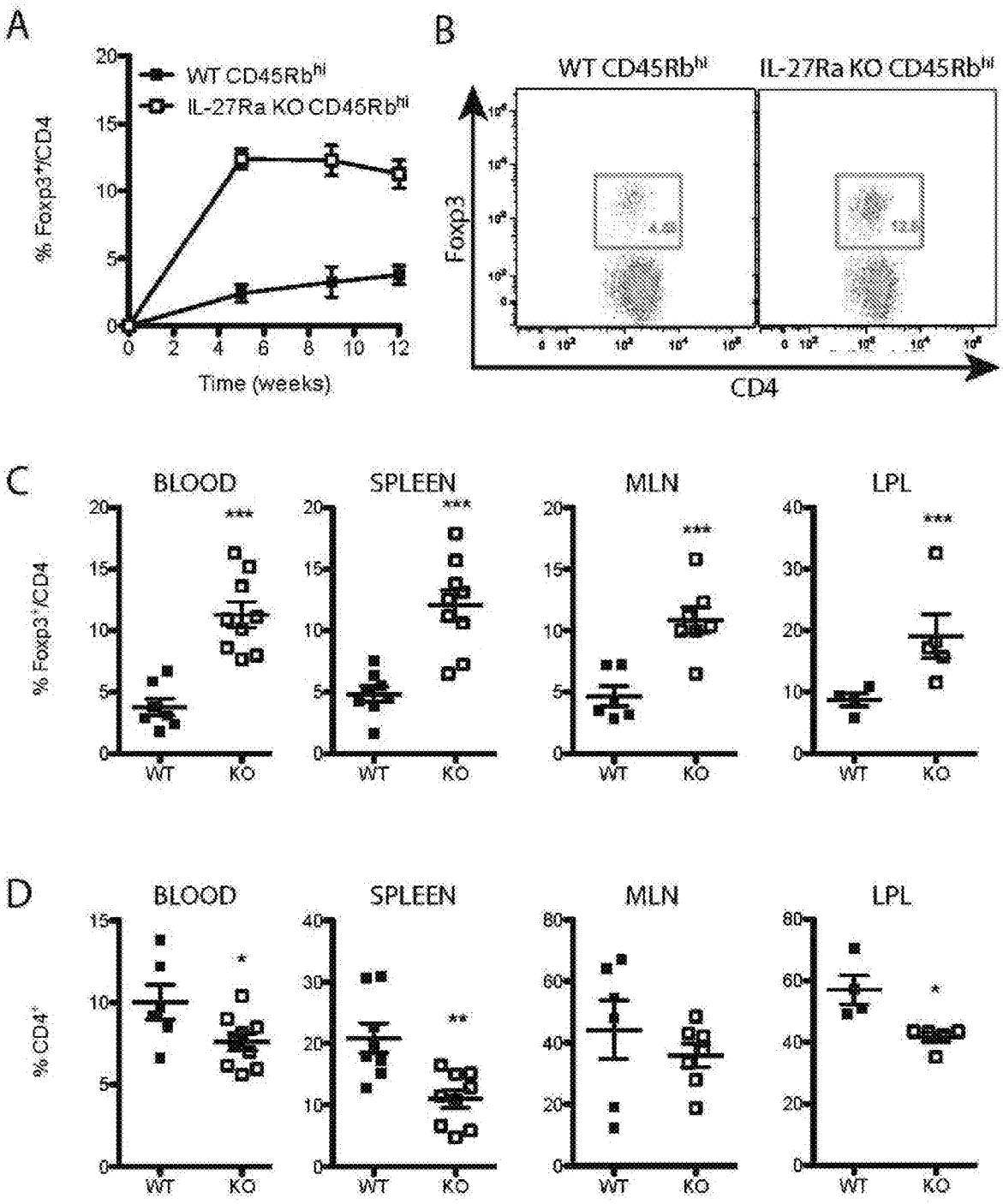

A
% Foxp3+/CD4
WT CD45Rb^hi
IL-27Ra KO CD45Rb^hi
Time (weeks)
B
WT CD45Rb^hi
IL-27Ra KO CD45Rb^hi
Foxp3
CD4
C
BLOOD
SPLEEN
MLN
LPL
% Foxp3+/CD4
WT
KO
D
BLOOD
SPLEEN
MLN
LPL
% CD4+
WT
KO

Figure 7

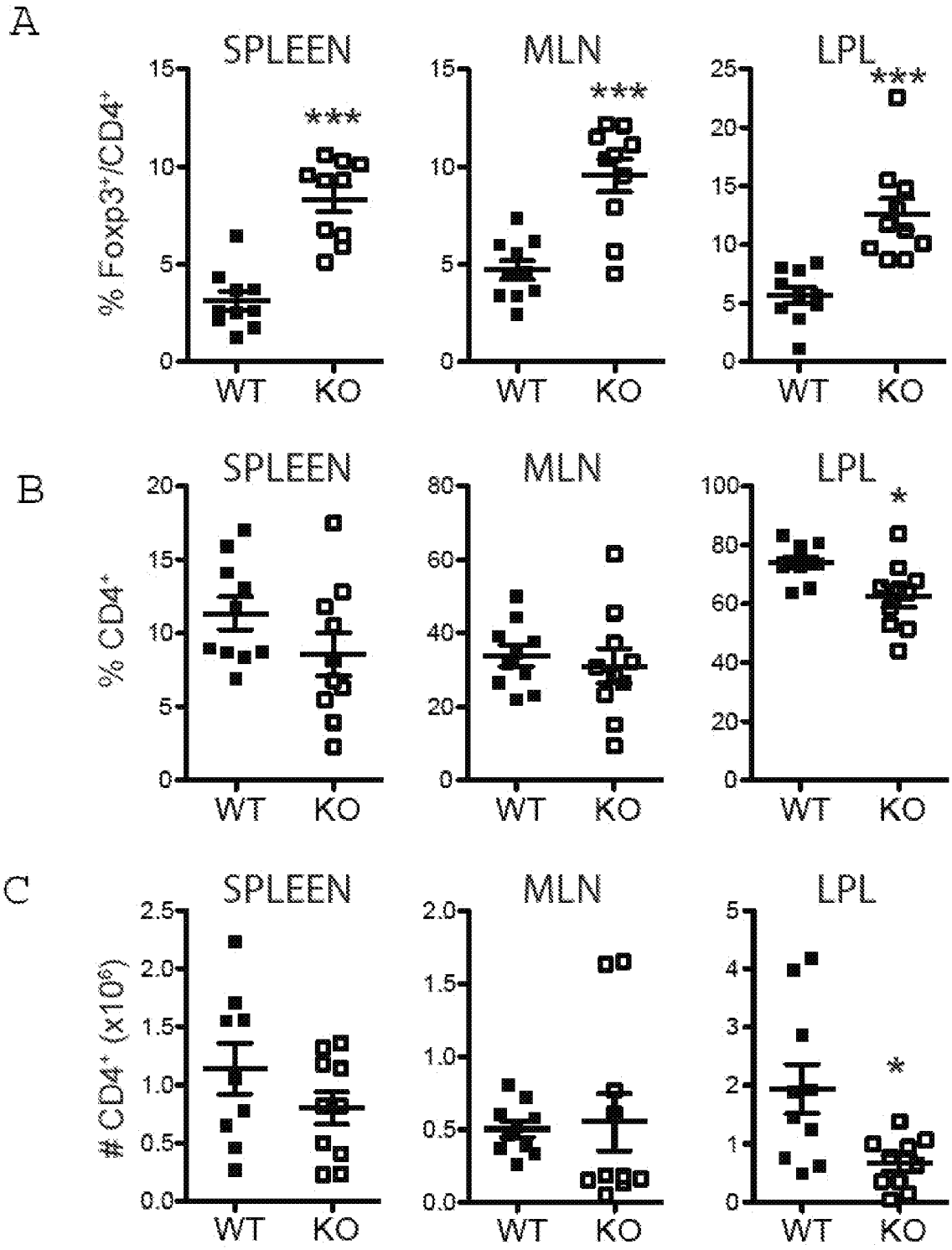

A
SPLEEN
MLN
LPL
% Foxp3+/CD4+
***
***
***
WT
KO
B
SPLEEN
MLN
LPL
% CD4+
*
C
SPLEEN
MLN
LPL
CD4+ (x10^6)
*

Figure 8

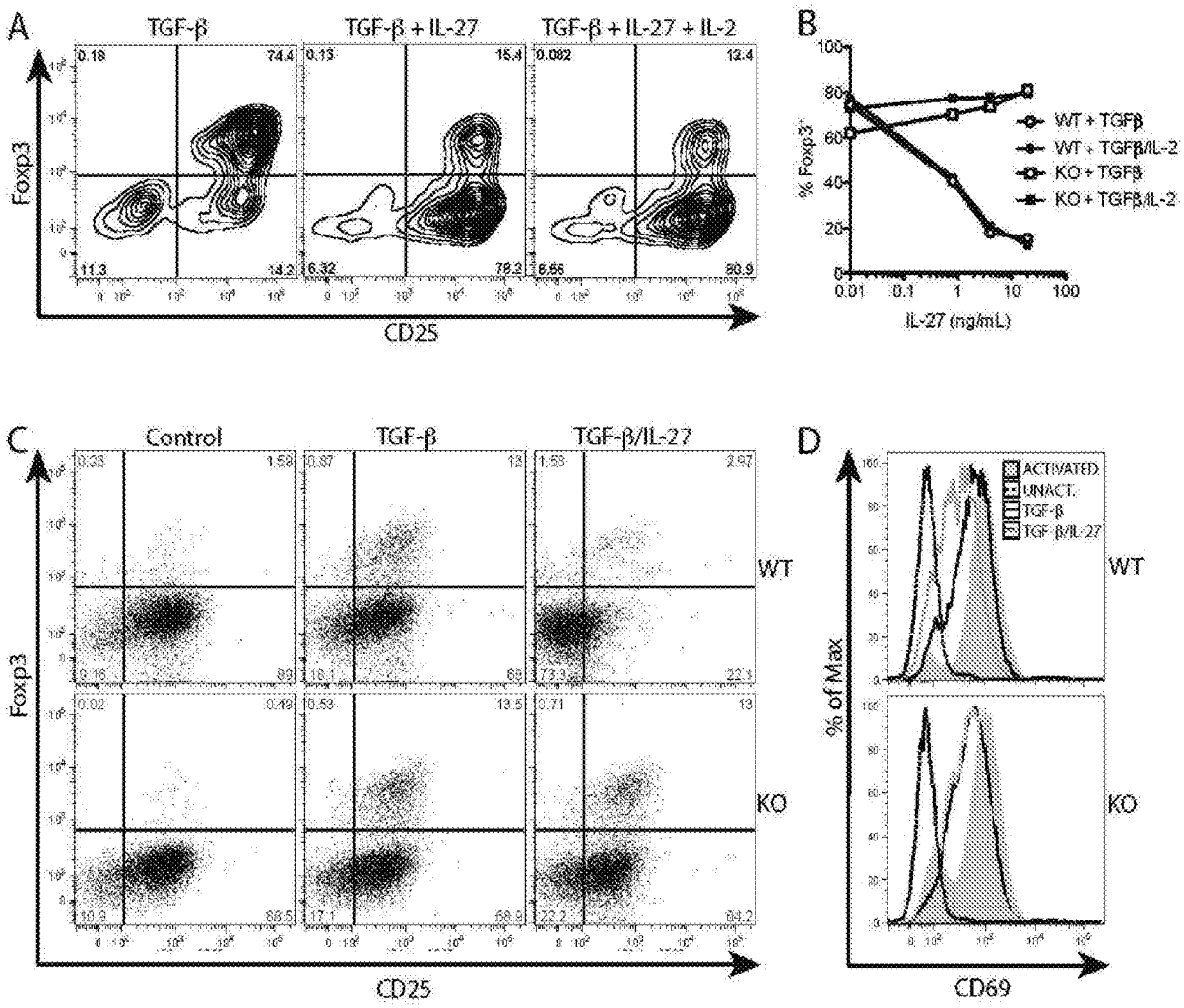

A
TGF-β
TGF-β + IL-27
TGF-β + IL-27 + IL-2
Foxp3
CD25
B
% Foxp3+
IL-27 (ng/mL)
WT + TGFβ
WT + TGFβ/IL-2
KO + TGFβ
KO + TGFβ/IL-2
C
Control
TGF-β
TGF-β/IL-27
WT
KO
Foxp3
CD25
D
ACTIVATED
UNACT.
TGF-β
TGF β/IL-27
WT
KO
% of Max
CD69

Figure 9

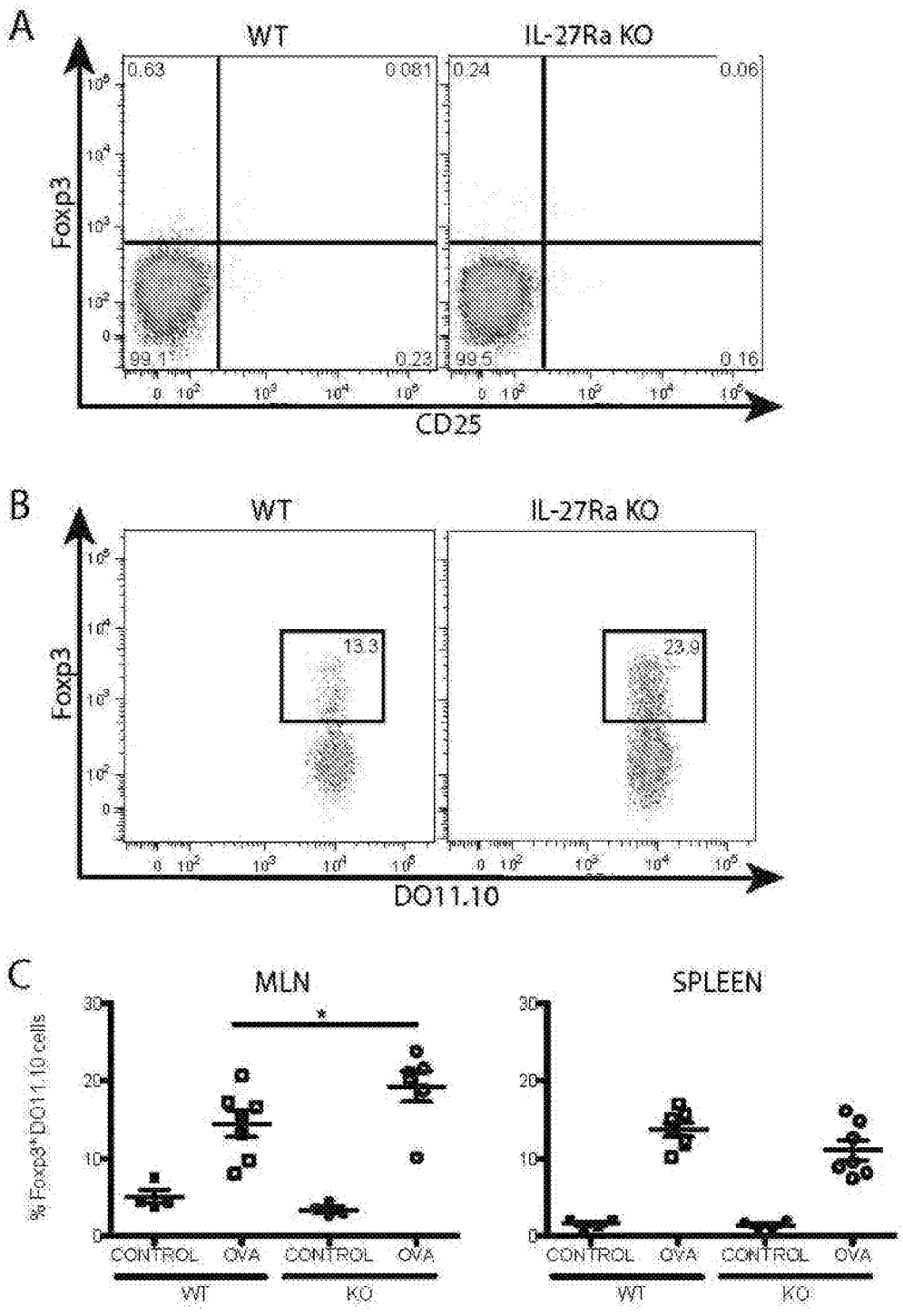

A
WT
IL-27Ra KO
0.63
0.081
0.24
0.06
99.1
0.23
99.5
0.16
Foxp3
CD25
B
WT
IL-27Ra KO
13.3
23.9
Foxp3
DO11.10
C
MLN
SPLEEN
% Foxp3+ DO11.10 cells
CONTROL
OVA
CONTROL
OVA
WT
KO

Figure 10

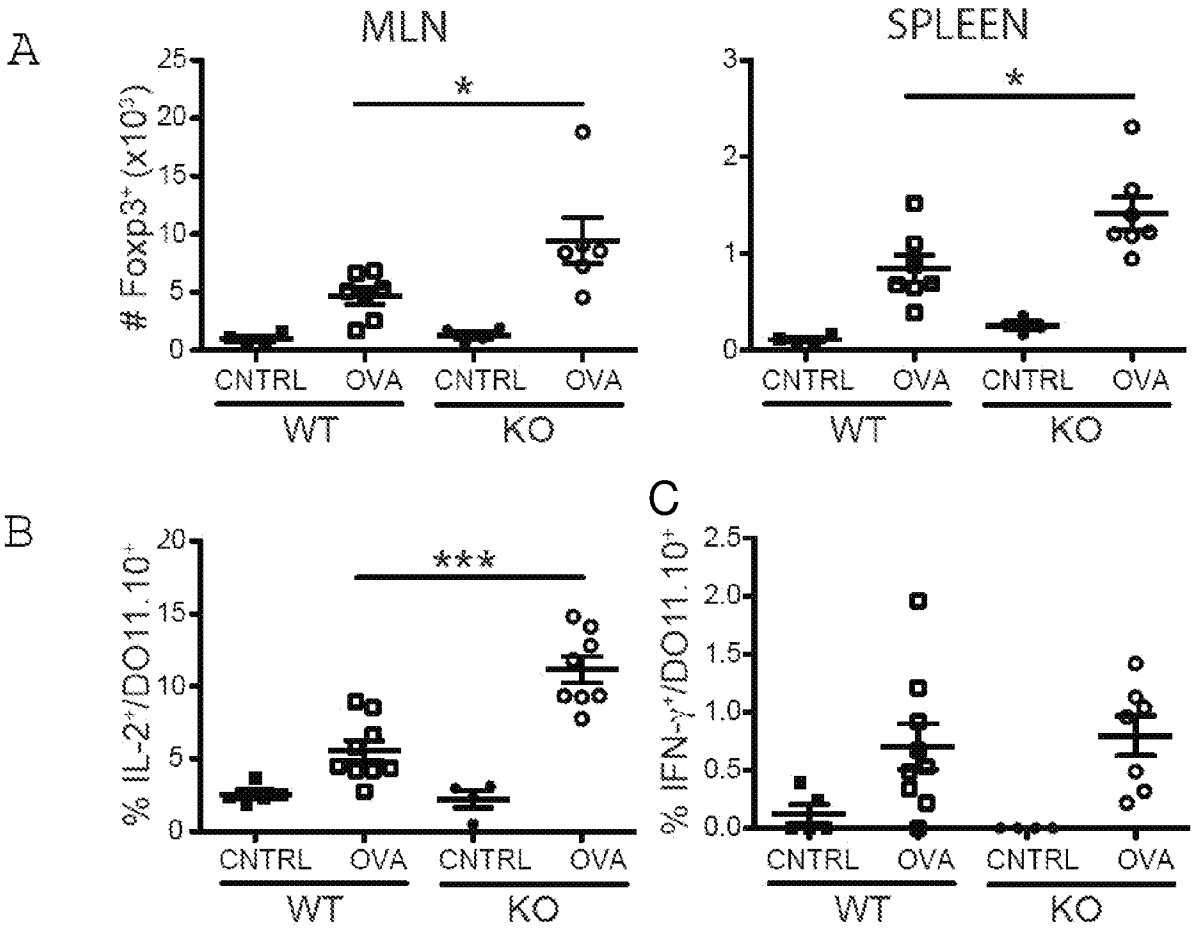

A
MLN
SPLEEN
Foxp3+ (x10³)
*
*
CNTRL
OVA
CNTRL
OVA
WT
KO
B
% IL-2+/DO11.10+
***
C
% IFN-γ+/DO11.10+

Figure 11

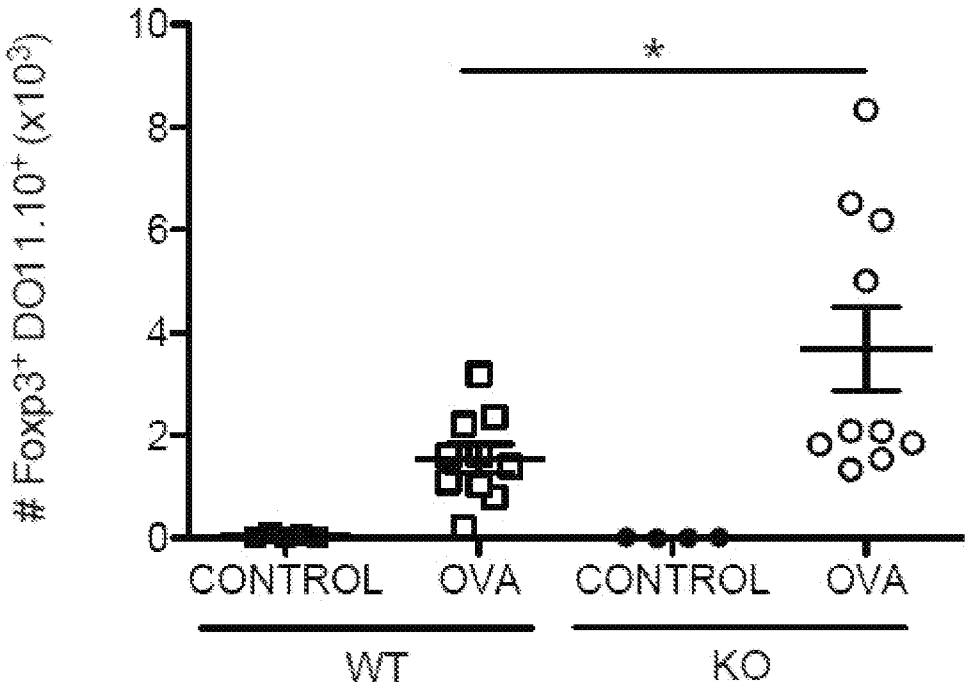
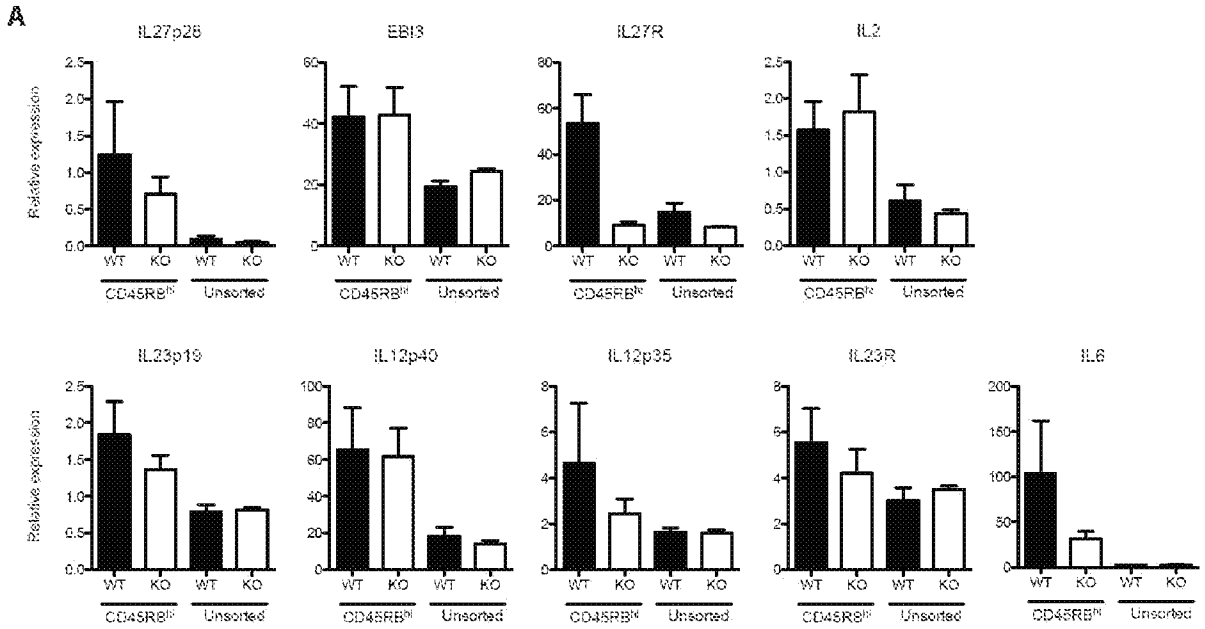


Figure 12

A



B

Target	*Forward Primer*	*Reverse Primer*	*Probe*
Rpl19	AGCGCATCCTCATGGAGC	GTCAGCCAGGAGCTTCTTGC	GCCTTGTCTGCCTTCAGCTTGTGGA
IL27p28	ABI Mm00461164_m1	ABI Mm00461164_m1	ABI Mm00461164_m1
EBI3	GGCCTGTCCTGAGTCTGAATA	AGTCAAGTGAATTATCCAGTGCTT	CTTTCCATGTACTGGGCTGCTCCG
IL27R	TGGTCTCTCCTGGCAACAGC	AGCCAAGCACACCAGAGACA	CAGCTGGGTGCCTCCCACCAA
IL23p19	TCCGTTCCAAGATCCTTCGA	TCCGTGGGCAAAGACCC	CCAGGCCTTTTTGGCCATAGCTGC
IL12p40	ACATCTACCGAAGTCCAATGCA	GGAATTGTAATAGCGATCCTGAGC	TGCACGCAGACATTCCCGCCT
IL12p35	TCTGAATCATAATGGCGAGACT	TCACTCTGTAAGGGTCTGCTTCT	TGCGCCAGAAACCTCCTGTGG
IL2	AAAGGGCTCTGACAACACATT	TCAGAAAGTCCACCACAGTTG	TGACTCATCATCGAATTGGCACTCA
IL6	TCCAATGCTCTCCTAACAGATAAG	CAAGATGAATTGGATGGTCTTG	TCCTTAGCCACTCCTTCTGTGACTCCA
IL23R	TGGACTTTTGTCGGGAATGG	TCGAAGTGATCTGTTAAATATCCCAA	TGGCCATCATGTTGCCGATTTTTTCT

Figure 13

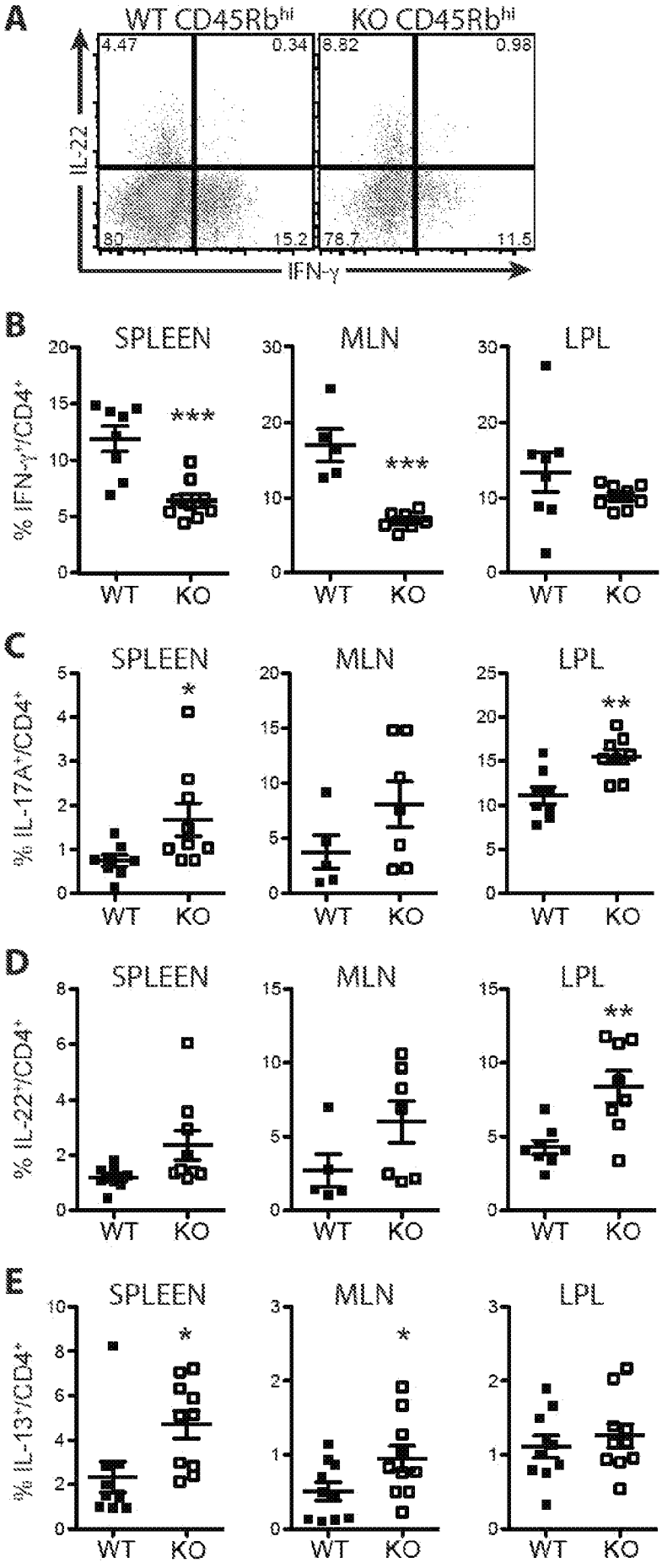

A
WT CD45Rb^hi
KO CD45Rb^hi
4.47
0.34
8.82
0.98
80
15.2
78.7
11.5
IL-22
IFN-γ
B
SPLEEN
MLN
LPL
% IFN-γ+/CD4+
***
***
WT
KO
C
% IL-17A+/CD4+
*
**
D
% IL-22+/CD4+
**
E
% IL-13+/CD4+
*
*

Figure 14

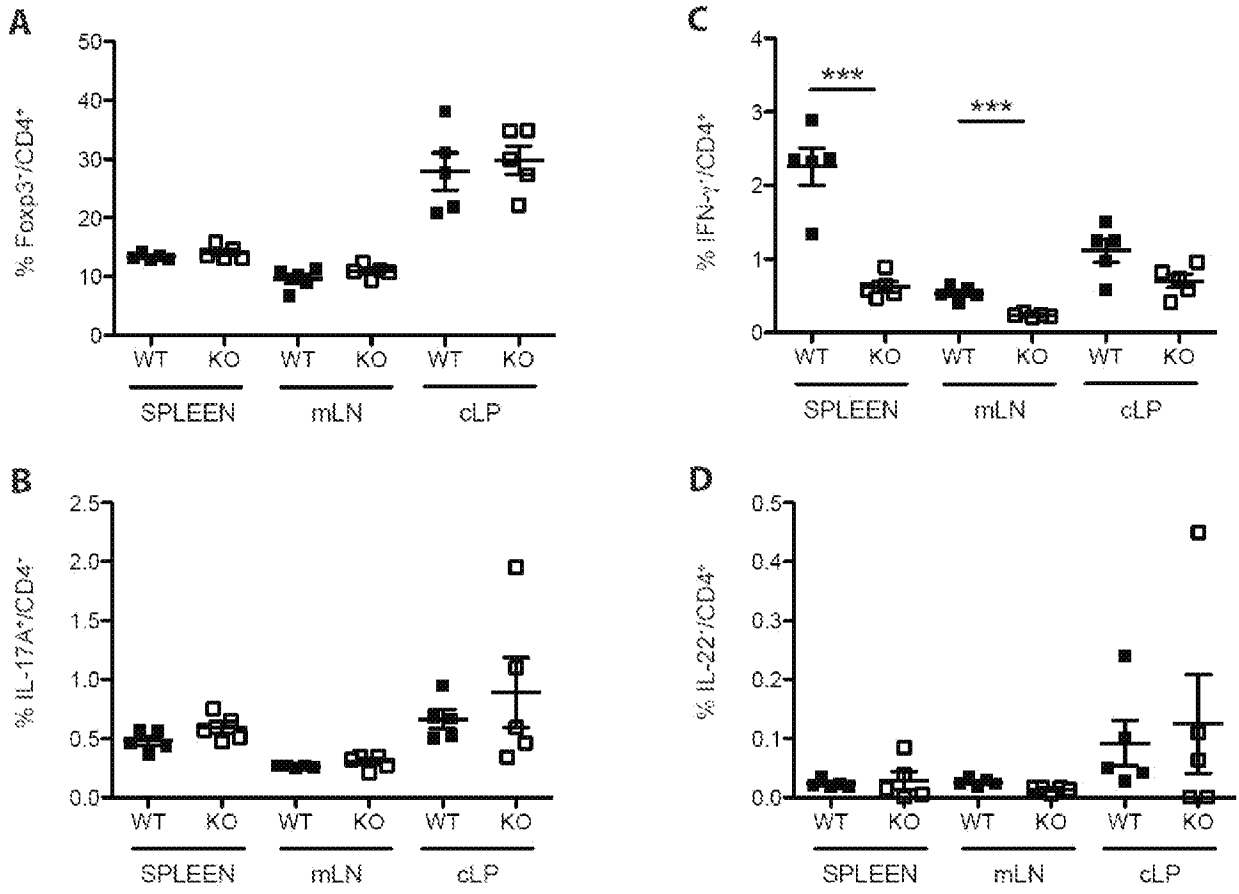

A
% Foxp3+/CD4+
50
40
30
20
10
0
WT KO WT KO WT KO
SPLEEN mLN cLP
C
% IFN-γ+/CD4+
4
3
2
1
0
***
***
WT KO WT KO WT KO
SPLEEN mLN cLP
B
% IL-17A+/CD4+
2.5
2.0
1.5
1.0
0.5
0.0
WT KO WT KO WT KO
SPLEEN mLN cLP
D
% IL-22+/CD4+
0.5
0.4
0.3
0.2
0.1
0.0
WT KO WT KO WT KO
SPLEEN mLN cLP

Figure 15

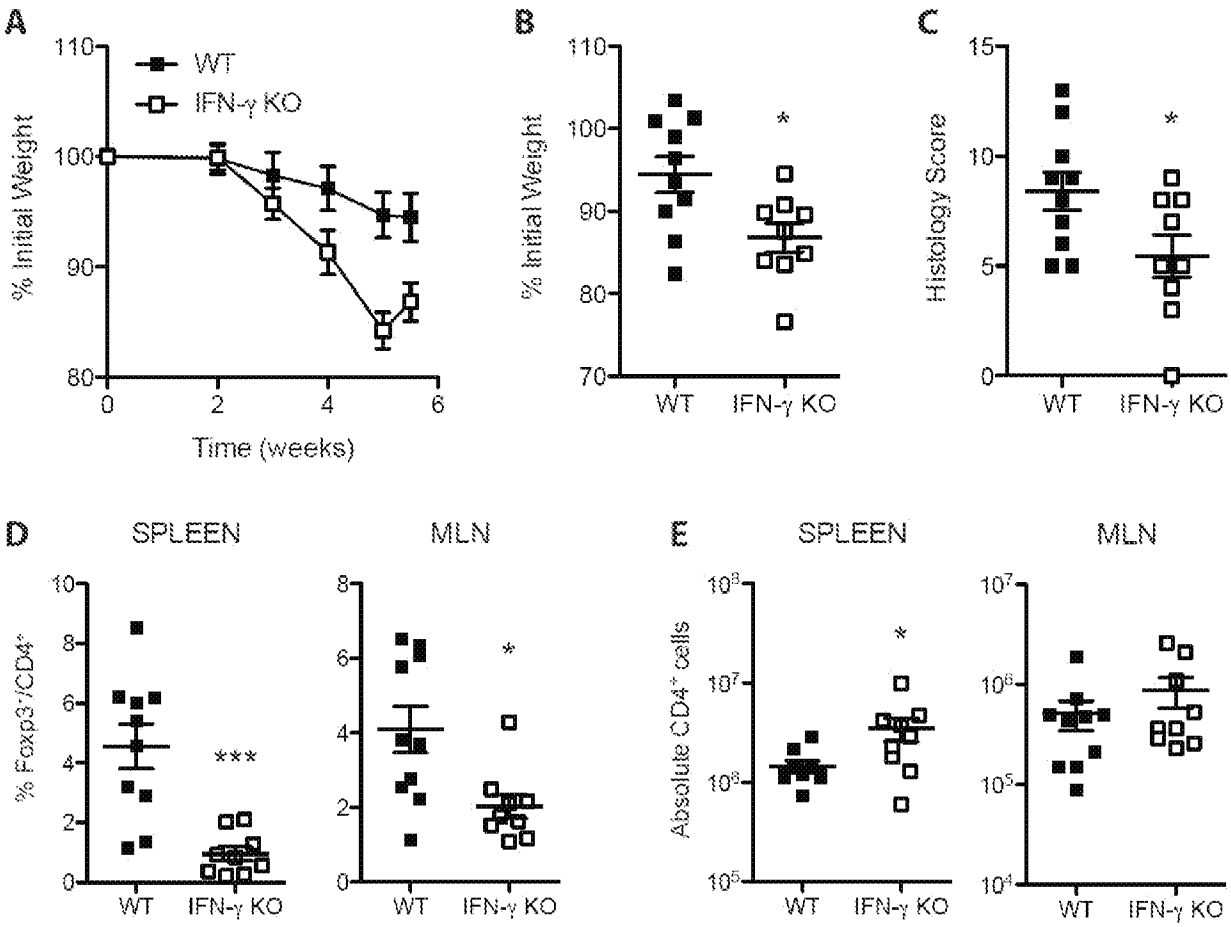
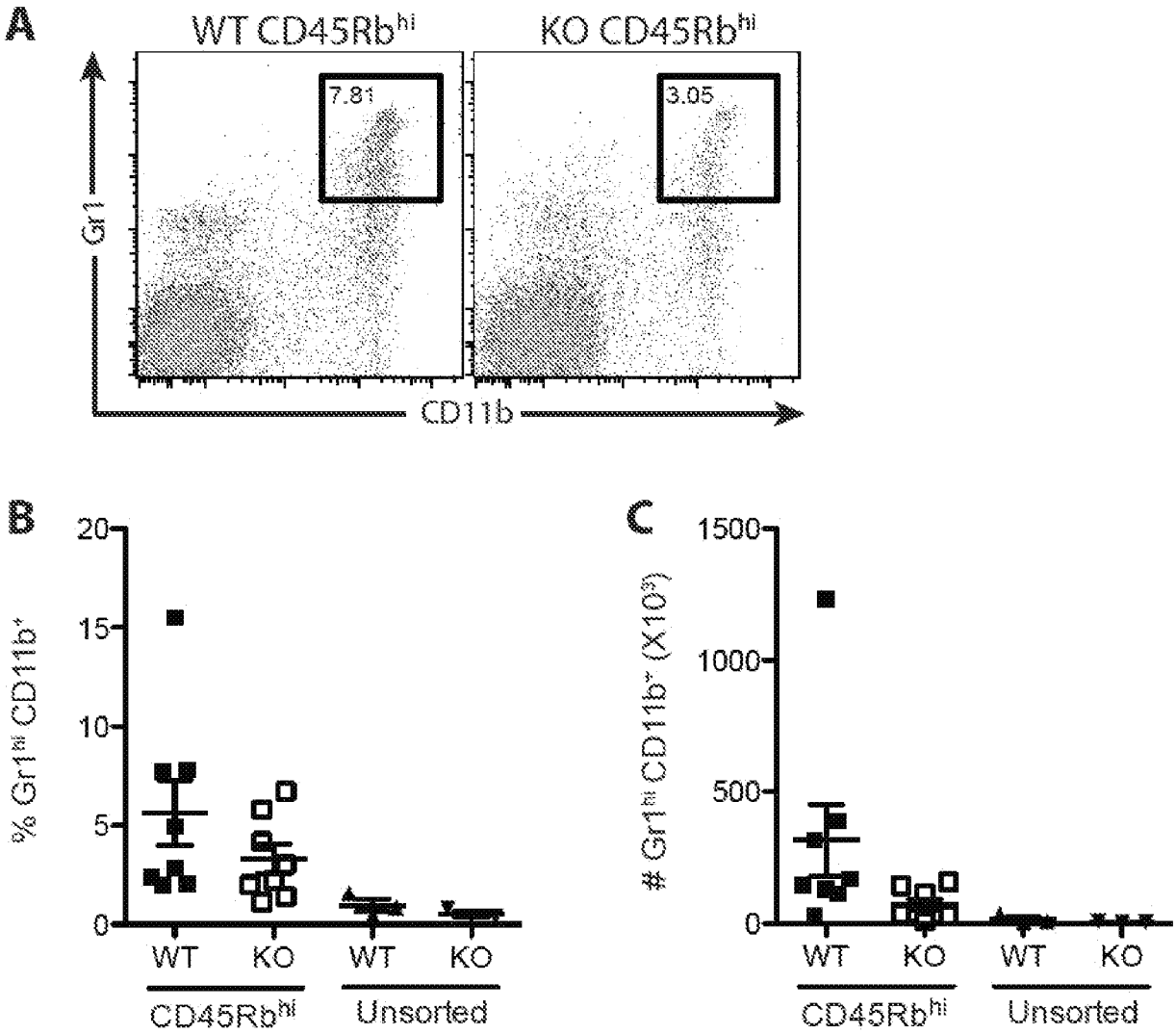

A
% Initial Weight
WT
IFN-γ KO
Time (weeks)
B
% Initial Weight
WT
IFN-γ KO
C
Histology Score
WT
IFN-γ KO
D
SPLEEN
MLN
% Foxp3+/CD4+
WT
IFN-γ KO
E
SPLEEN
MLN
Absolute CD4+ cells
WT
IFN-γ KO

Figure 16


A
WT CD45Rb^hi
KO CD45Rb^hi
7.81
3.05
Gr1
CD11b
B
% Gr1^hi CD11b^+
20
15
10
5
0
WT
KO
WT
KO
CD45Rb^hi
Unsorted
C
Gr1^hi CD11b^+ (X10^3)
1500
1000
500
0
WT
KO
WT
KO
CD45Rb^hi
Unsorted

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/033667

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/395 A61P37/00 C07K16/24
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/079848 A2 (SCHERING CORP [US]; KASTELEIN ROBERT A [US]; MCCLANAHAN TERRILL K [US]) 1 September 2005 (2005-09-01) paragraphs [0008] - [0010], [0 50], [0 51], [0 63], [0 93] - [0095]; claims 1, 2, 9, 12-14, 16, 20	1-31

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 July 2011	02/08/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Cilensek, Zoran

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/033667

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	A. V. VILLARINO ET AL: "IL-27R deficiency delays the onset of colitis and protects from helminth-induced pathology in a model of chronic IBD", INTERNATIONAL IMMUNOLOGY, vol. 20, no. 6, 1 January 2008 (2008-01-01), pages 739-752, XP55003318, ISSN: 0953-8178, DOI: 10.1093/intimm/dxn032 figures 1-7	1-31
X,P	COX JENNIFER H ET AL: "IL-27 promotes T cell-dependent colitis through multiple mechanisms", JOURNAL OF EXPERIMENTAL MEDICINE, vol. 208, no. 1, January 2011 (2011-01), pages 115-123, XP007919123, ISSN: 0022-1007 the whole document	1-31
X,P	COX J H ET AL: "PS1-20 IL-27 promotes T-cell dependent colitis through restriction of regulatory T-cell differentiation", CYTOKINE, ACADEMIC PRESS LTD, PHILADELPHIA, PA, US, vol. 52, no. 1-2, 1 October 2010 (2010-10-01), page 21, XP027261669, ISSN: 1043-4666 [retrieved on 2010-09-02] the whole document	1-31

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2011/033667

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005079848 A2	01-09-2005	AU 2005215771 A1	01-09-2005
		BR PI0507776 A	10-07-2007
		CA 2555421 A1	01-09-2005
		CN 1921886 A	28-02-2007
		EP 1755641 A2	28-02-2007
		JP 2007523169 A	16-08-2007
		ZA 200606833 A	28-05-2008