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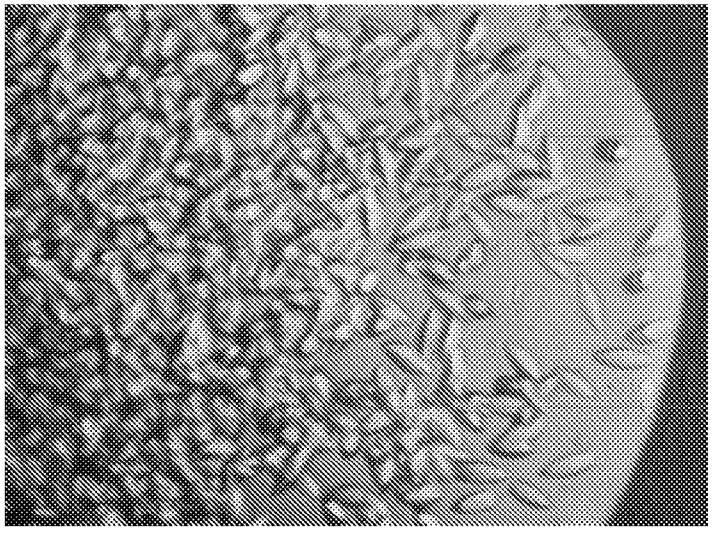
[Continued on next page]

(54) Title: CRYSTALLINE ANTI-HUMAN IL-23 ANTIBODIES



(57) Abstract: Crystalline forms of antibodies to human IL-23, such as antibodies to human IL- 23p19, are provided, as well as methods of producing such crystalline forms, and uses of such crystalline forms, *e.g.* in treatment of inflammatory, autoimmune, and proliferative disorders.

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CRYSTALLINE ANTI-HUMAN IL-23 ANTIBODIES

FIELD OF THE INVENTION

[0001] The present invention relates generally to crystalline forms of antibodies specific for human interleukin-23 and uses thereof. More specifically, the invention relates to crystalline forms of antibodies that recognize human IL-23p19 and modulate its activity, particularly in inflammatory, autoimmune and proliferative disorders.

BACKGROUND OF THE INVENTION

[0002] The immune system functions to protect individuals from infective agents, *e.g.*, bacteria, multi-cellular organisms, and viruses, as well as from cancers. This system includes several types of lymphoid and myeloid cells such as monocytes, macrophages, dendritic cells (DCs), eosinophils, T cells, B cells, and neutrophils. These lymphoid and myeloid cells often produce signaling proteins known as cytokines. The immune response includes inflammation, *i.e.*, the accumulation of immune cells systemically or in a particular location of the body. In response to an infective agent or foreign substance, immune cells secrete cytokines which, in turn, modulate immune cell proliferation, development, differentiation, or migration. Immune response can produce pathological consequences, *e.g.*, when it involves excessive inflammation, as in the autoimmune disorders (see, *e.g.*, Abbas *et al.* (eds.) (2000) *Cellular and Molecular Immunology*, W.B. Saunders Co., Philadelphia, PA; Oppenheim and Feldmann (eds.) (2001) *Cytokine Reference*, Academic Press, San Diego, CA; von Andrian and Mackay (2000) *New Engl. J. Med.* 343:1020-1034; Davidson and Diamond (2001) *New Engl. J. Med.* 345:340-350).

[0003] Interleukin-12 (IL-12) is a heterodimeric molecule composed of p35 and p40 subunits. Studies have indicated that IL-12 plays a critical role in the differentiation of naïve T cells into T-helper type 1 $CD4^+$ lymphocytes that secrete IFNγ. It has also been shown that IL-12 is essential for T cell dependent immune and inflammatory responses *in vivo*. *See, e.g.*, Cua *et al.* (2003) *Nature* 421:744-748. The IL-12 receptor is composed of IL-12Rβ1 and IL-12Rβ2 subunits.

[0004] Interleukin-23 (IL-23) is a heterodimeric cytokine comprised of two subunits, p19 which is unique to IL-23, and p40, which is shared with IL-12. The p19 subunit is

structurally related to IL-6, granulocyte-colony stimulating factor (G-CSF), and the p35 subunit of IL-12. IL-23 mediates signaling by binding to a heterodimeric receptor, comprised of IL-23R and IL-12Rβ1, which is shared by the IL-12 receptor. A number of early studies demonstrated that the consequences of a genetic deficiency in p40 (p40 knockout mouse; p40KO mouse) were more severe than those found in a p35KO mouse. Some of these results were eventually explained by the discovery of IL-23, and the finding that the p40KO prevents expression of not only IL-12, but also of IL-23 (*see, e.g.*, Oppmann *et al.* (2000) *Immunity* 13:715-725; Wiekowski *et al.* (2001) *J. Immunol.* 166:7563-7570; Parham *et al.* (2002) *J. Immunol.* 168:5699-708; Frucht (2002) *Sci STKE* 2002, E1-E3; Elkins *et al.* (2002) *Infection Immunity* 70:1936-1948).

[0005] Recent studies, through the use of p40 KO mice, have shown that blockade of both IL-23 and IL-12 is an effective treatment for various inflammatory and autoimmune disorders. However, the blockade of IL-12 through p40 appears to have various systemic consequences such as increased susceptibility to opportunistic microbial infections. Bowman *et al.* (2006) *Curr. Opin. Infect. Dis.* 19:245.

[0006] Therapeutic antibodies may be used to block cytokine activity. Subcutaneous administration is the preferred method of administration of many such antibodies, at least in part because it enables self-administration. Therapeutic antibodies are traditionally prepared in lyophilized form or in solution. Lyophilized forms may exhibit enhanced long-term stability, but require reconstitution prior to use, making them less than ideal for self-administration. Solution formulations do not require reconstitution, but may suffer from reduced stability and typically require cold storage prior to use. Both lyophilized and solution formulations may fail to provide sufficiently high concentrations to allow for high dose delivery by subcutaneous administration. High concentration solution formulations, if achievable, may also be prone to dropping out of solution, or may be too viscous to be delivered in a narrow gauge needle, *e.g.* as required for subcutaneous administration, particularly self-administration.

[0007] Exemplary engineered antibodies to IL-23p19 are disclosed in commonly-assigned U.S. Patent Application Publication Nos. 2010/0272731 and 2010/0111966, in U.S. Patent Application Publication Nos. 2007/0009526 and 2007/0048315, and in International Patent Publication Nos. WO 2007/076524, WO 2007/024846 and WO 2007/147019. One IL-23 antagonist antibody, ustekinumab (STELARA®), is commercially available, but it binds

to the p40 subunit of IL-23 rather than the p19 subunit. As a result, ustekinumab also inhibits the activity of IL-12, which is generally an undesired side effect that can lead to increased susceptibility of certain infections and tumors. Bowman *et al.* (2006) *Curr. Opin. Infect. Dis.* 19:245. Briakinumab, another anti-p40 antibody that has entered clinical trials, suffers from the same safety concerns. Anti-human IL-23p19 antibody guselkumab (CNTO-1959) has entered clinical trials.

[0008] The need exists for improved formulations of anti-huIL-23p19 antibodies for use, *e.g.*, in treatment of inflammatory, autoimmune, and proliferative disorders. Preferably, such antibody formulations will not require reconstitution prior to administration. In addition, such formulations will enable higher concentration administration of the antibody than would be readily achievable using typical solution formulations, and will preferably support high concentrations with sufficiently low viscosity to be conveniently delivered subcutaneously. Such formulations may also provide for sustained release, and will also be more stable than typical solution formulations.

SUMMARY OF THE INVENTION

[0009] The present invention satisfies these needs and more by providing crystalline forms of anti-huIL-23 antibodies, such as antibodies that bind to the p19 subunit of human IL-23 (anti-human IL-23p19 antibodies). In one aspect, the invention relates to crystalline forms of anti-huIL-23 antibodies, such as anti-huIL-23p19 antibodies. In another aspect, the invention relates to suspensions of these crystalline forms of anti-huIL-23 antibodies, such as anti-huIL-23p19 antibodies, *e.g.* as crystalline slurries. In yet another aspect, the invention relates to pharmaceutical formulations comprising suspensions of these crystalline forms of anti-huIL-23 antibodies, such as anti-huIL-23p19 antibodies. In various embodiments, the crystalline form of the anti-huIL-23 antibodies, such as anti-huIL-23p19 antibodies, is used to facilitate purification, storage, and therapeutic administration of the anti-huIL-23 antibody.

[0010] In some embodiments, the anti-huIL-23 antibody crystals, such as anti-huIL-23p19 antibody crystals, of the present invention have an average particle size between five and 200 microns. In other embodiments, the anti-huIL-23 antibody crystals, such as anti-huIL-23p19 antibody crystals, are characterized by unit cell dimensions of $a = b = 192$ Å, $c = 106$ Å, $\alpha = \beta = \gamma = 90°$ and are in space group I4. In some embodiments, the anti-huIL-23p19 antibody crystals can diffract X-rays to a resolution of at least about 5 Å.

[0011] In various embodiments, the anti-huIL-23 antibody crystals, such as anti-huIL-23p19 antibody crystals, of the present invention are obtainable by batch crystallization methods, vapor diffusion methods, liquid-liquid diffusion methods, and dialysis.

[0012] In other aspects, the invention relates to suspensions of the crystalline anti-huIL-23 antibodies, such as anti-human IL-23p19 antibodies, of the present invention, including those at higher concentrations and lower viscosities than would be possible with a corresponding non-crystalline solution at the same concentration of antibody. In some embodiments the crystalline suspension of anti-huIL-23 antibody crystals, such as anti-huIL-23p19 antibody crystals, has an antibody concentration of greater than about 50, 100, 150, 200 or 250 mg/ml. In one aspect, the invention relates to suspensions of crystalline anti-human IL-23p19 antibodies that are substantially less viscous than a corresponding non-crystalline solution at the same concentration of antibody. In one embodiment the suspension has a viscosity of less than half that of a corresponding solution formulation, and has an antibody concentration of at least about 150 or 200 mg/ml.

[0013] In other embodiments, the anti-huIL-23 antibody crystals, such as anti-huIL-23p19 antibody crystals, of the present invention have increased stability, *i.e.* they maintain biological activity of the anti-huIL-23 antibody, such as anti-huIL-23p19 antibody, longer than corresponding solution formulations. In some embodiments, the increased stability is at room temperature, enabling storage of the crystalline suspensions of the present invention at room temperature rather than at 4°C, such as room temperature (e.g. 20°C - 25°C).

[0014] In some embodiments, the anti-huIL-23 antibody comprises the CDR sequences of ustekinumab or briakinumab. In various other embodiments, the anti-huIL-23p19 antibody comprises the CDR sequences of antibodies 13B8-a, 13B8-b or 13B8-c of commonly assigned Int'l Pat. App. Pub. WO 08/103432, or the CDR sequences of antibodies LY2525623 or CNTO 1959 (guselkumab).

[0015] In a preferred embodiment, the crystalline anti-huIL-23p19 antibody comprises a light chain variable domain in which CDRL1 comprises the sequence of SEQ ID NO: 10; CDRL2 comprises the sequence of SEQ ID NO: 11; and CDRL3 comprises the sequence of SEQ ID NO: 12, and a heavy chain variable domain in which CDRH1 comprises the sequence of SEQ ID NO: 5; CDRH2 comprises a sequence selected from the group consisting of SEQ ID NOs: 6 – 8; and CDRH3 comprises the sequence of SEQ ID NO: 9. In a preferred refinement of the preceding embodiment, CDRH2 comprises the sequence of SEQ ID NO: 7.

[0016] In a further embodiment the crystalline anti-huIL-23p19 antibody comprises the light chain variable domain (residues 1 – 108) of SEQ ID NO: 4 and a heavy chain variable domain (residues 1 – 116) of a sequence selected from the group consisting of SEQ ID NOs: 1 – 3. In a preferred refinement of the preceding embodiment, the heavy chain variable domain is from SEQ ID NO: 2. In various embodiments the crystalline anti-huIL-23p19 antibody comprises a heavy chain comprising a γ1 human heavy chain constant region or a γ4 human heavy chain constant region. In another embodiment the binding compound comprises the light chain sequence of SEQ ID NO: 4 and heavy chain sequence selected from the group consisting of SEQ ID NOs: 1 – 3. In a preferred refinement of the preceding embodiment, the heavy chain sequence is SEQ ID NO: 2.

[0017] In various embodiments, the invention relates use of the crystalline formulations of the present invention in the treatment of, or in methods of treatment of, or in the manufacture of medicaments for the treatment of, disorders including but not limited to inflammatory disease, autoimmune disease, proliferative disorders, cancer, and infectious disease (*e.g.* bacterial, mycobacterial, viral or fungal infection, including chronic infections), such as psoriasis, psoriatic arthritis, rheumatoid arthritis (RA), inflammatory bowel diseases (IBD) such a Crohn's disease (CD) and ulcerative colitis (UC), ankylosing spondylitis (AS), graft-versus-host disease (GVHD), multiple sclerosis (MS), uveitis, systemic lupus erythematosus (SLE) and diabetes (IDDM). The invention also relates to pharmaceutical compositions comprising the crystalline suspension of the present invention for treating these disorders.

[0018] In some embodiments, the crystalline suspension of the present invention is delivered intravenously. In other embodiments, the crystalline suspension of the present invention is delivered subcutaneously. In yet other embodiments, the crystals of the present invention are delivered, *e.g.*, by inhalation or insufflation.

[0019] In some embodiments, the crystalline suspension or pharmaceutical compositions of the present invention provide prolonged pharmacokinetics of the anti-human IL-23 antibody, such as the anti-human IL-23p19 antibody. In various embodiments, crystalline formulations or pharmaceutical compositions of the present invention are dosed at intervals of 12-, 13-, 16-, 20-, 24-, 26-, 30-, 39-, or 52-weeks, or longer.

[0020] In another aspect, the invention relates to methods of preparing the anti-huIL-23 antibody crystals, such as the anti-human IL-23p19 antibody crystals, of the present

invention. In one embodiment, the crystalline anti-human IL-23 antibody, such as the anti-human IL-23p19 antibody, of the present invention is made by a batch crystallization method comprising the steps of mixing a solution of the antibody (antibody solution) with a precipitant solution to form a crystallization solution, incubating that crystallization solution for a time sufficient for crystal formation (crystallization), and harvesting the crystals from the solution. In some embodiments, an equal volume of precipitant solution is added to form the crystallization solution.

[0021] In some embodiments, the precipitant solution comprises a buffer with a pH between 4 and 8, such as 4.5, 5.5, 7.5, or any other suitable value. In various embodiments, the buffers are sodium citrate, sodium acetate, HEPES, or BisTris.

[0022] In some embodiments, the precipitant solution comprises 5 – 70% 1,2 propanediol, such as 10%, 20%, 30% or any other suitable value. In related embodiments, the crystallization solution comprises 2.5 – 35% 1,2 propanediol, such as 5%, 10%, 15% or any other suitable value.

[0023] In some embodiments, the precipitant solution comprises 5 – 70% PEG 300, such as 14%, 40%, 70% or any other suitable value. In related embodiments, the crystallization solution comprises 2.5 – 35% PEG 400, such as 7%, 20%, 35% or any other suitable value. In other embodiments, the precipitant solution comprises 5 – 70% PEG 400, such as 20%, 25%, 27%, 30% or any other suitable value. In related embodiments, the crystallization solution comprises 2.5 – 35% PEG 300, such as 10%, 12.5%, 13.5%, 15% or any other suitable value.

[0024] In one embodiment, the precipitant solution comprises 1,2 propanediol; HEPES (pH 7.5); and PEG 400, *e.g.* 30% 1,2 propanediol; 0.1 M HEPES (pH 7.5); and 20% PEG 400. In a related embodiment, the crystallization solution may comprise, *e.g.*, 15% 1,2 propanediol; 0.05 M HEPES (pH 7.5); and 10% PEG 400.

[0025] In another embodiment, the precipitant solution comprises PEG 300; sodium acetate (pH 4.5); and NaCl, *e.g.* 70% PEG 300; 0.1 M sodium acetate (pH 4.5); and 0.2 M NaCl. In a related embodiment, the crystallization solution may comprise, *e.g.*, 35% PEG 300; 0.05 M sodium acetate (pH 4.5); and 0.1 M NaCl.

[0026] In some embodiments, the antibody solution (*i.e.* the solution that is mixed with the precipitation solution to create the crystallization solution) comprises the anti-human IL-23 (*e.g.* anti-human IL-23p19) antibody, sodium citrate (pH 4 – 6), 4 – 10% sucrose, 0.1 –

0.5% polysorbate 80. In one embodiment, the antibody solution comprises about 100 mg/ml anti-human IL-23 (*e.g.* anti-human IL-23p19) antibody, about 10 mM sodium citrate (about pH 4.8), about 7% sucrose, and about 0.25% polysorbate 80.

[0027] In another embodiment, the precipitant solution comprises PEG 300 or PEG 400 and sodium citrate (pH 4 - 6), *e.g.* 57.4% PEG 300 and 0.1 M sodium citrate (pH 5.1). In a related embodiment, the crystallization solution may comprise, *e.g.*, about 28.7% PEG 300; 0.055 M sodium citrate (pH 4.8 – 5.1). In this embodiment the crystallization solution also comprises about 50 mg/ml antibody, about 3.5% sucrose, and about 0.125% polysorbate 80.

[0028] In some embodiments anti-human IL-23 (or anti-human IL-23p19) antibody is present at 5 – 70 mg/ml in the antibody solution, *e.g.* about 60 mg/ml. In a related embodiment, anti-human IL-23p19 antibody is present at 2.5 – 35 mg/ml in the crystallization solution. In other embodiments anti-human IL-23 (or anti-human IL-23p19) antibody is present at about 100 mg/ml in the antibody solution. In a related embodiment, anti-human IL-23p19 antibody is present at about 50 mg/ml in the crystallization solution.

[0029] In some embodiments the incubation is performed between 10 – 40°C, *e.g.* at room temperature (*e.g.* 22°C), for 18 hours, 1 day, 5 days or 10 days, or any other time sufficient to allow crystal formation. In other embodiments, the temperature is ramped-up during the incubating step, *e.g.* from 4°C up to 10 – 40°C. In other embodiments, incubation is performed at about 30°C for 24 hours, optionally with agitation, such as on a rotating platform or nutator.

[0030] In some embodiments, the crystallization solution is seeded with crystals (pre-existing crystals of the antibody to be crystallized) during the incubating step.

[0031] In another embodiment, the crystalline anti-huIL-23 antibody crystals, such as the anti-human IL-23p19 antibody crystals, of the present invention are prepared by a bulk dialysis crystallization method comprising the steps of dialyzing a solution of anti-human IL-23 antibody, such as the anti-human IL-23p19 antibody, against a dialysis solution for a time sufficient for crystal formation (crystallization), and harvesting the crystals from the retentate.

[0032] In some embodiments, the dialysis solution comprises a buffer with a pH between 4 and 8, such as 4.5, 5.5, 7.5, or any other suitable value.

[0033] In some embodiments, the dialysis solution comprises 2.5 – 35% 1,2 propanediol, such as 5%, 10%, 15% or any other suitable value. In some embodiments, the dialysis solution comprises 2.5 – 35% PEG 400, such as 10%, 12.5%, 13.5%, 15%, 20%,

35% or any other suitable value. In one embodiment, the dialysis solution comprises 1,2 propanediol; HEPES (pH 7.5); and PEG 400, *e.g.* 10% 1,2 propanediol; 0.1 M HEPES (pH 7.5); and 20% PEG 400.

[0034] In other embodiments, the dialysis solution comprises 2.5 – 35% PEG 300, such as 7%, 14%, 20%, 35% or any other suitable value. In another embodiment, the dialysis solution comprises PEG 300; sodium citrate (pH 5.5); and NaCl, *e.g.* 14% PEG 300; 0.1 M sodium citrate (pH 5.5); and 0.2 M NaCl.

[0035] In some dialysis-based embodiments the anti-human IL-23 antibody, such as the anti-human IL-23p19 antibody, is present at 5 – 70 mg/ml in the antibody solution, *e.g.* about 60 mg/ml.

[0036] In some embodiments of the bulk dialysis method, the dialysis is performed between 10 – 40°C, *e.g.* at room temperature (*e.g.* 22°C), for 18 hours, 1 day, 5 days or 10 days, or any other time sufficient to allow crystal formation. In other embodiments, the temperature is ramped-up during dialysis, *e.g.* from 4°C up to 10 – 40°C.

[0037] In some embodiments of the bulk dialysis method, the crystallization solution is agitated during the dialysis. In other embodiments, the antibody solution/retentate is seeded with crystals at some point during the dialysis.

[0038] In one aspect, the invention relates to crystalline anti-human IL-23 antibodies, such as anti-human IL-23p19 antibodies, made by the methods of the present invention.

[0039] In a further aspect, the invention provides methods of purifying anti-huIL-23 antibodies, such as anti-human IL-23p19 antibodies, comprising crystallizing the antibody, using methods of the present invention, and then re-dissolving the antibody prior to use. In another aspect, the invention provides preparations of anti-huIL-23 antibodies such as anti-human IL-23p19 antibodies, that have been purified by the crystallization methods described herein. In yet a further aspect, the invention provides methods of preparing crystals of anti-huIL-23 antibodies, such as anti-human IL-23p19 antibodies, for use in structure determination, *e.g.* by X-ray diffraction methods.

BRIEF DESCRIPTION OF THE DRAWINGS

[0040] FIG. 1 is a photomicrograph of crystals within an anti-huIL-23p19 mAb crystalline suspension, obtained by vapor diffusion. Anti-huIL-23p19 mAb 13B8-b was crystallized from a solution comprising 30% 1,2 propanediol, 0.1 M HEPES (pH 7.5), and

20% PEG 400. The photomicrograph, at 70 X magnification, was taken after 10 days at 18°C. *See* Example 2.

[0041] FIG. 2 is a photomicrograph of crystals within an anti-huIL-23p19 mAb crystalline suspension, obtained by vapor diffusion. Anti-huIL-23p19 mAb 13B8-b was crystallized from a solution comprising 0.1 M sodium acetate (pH 4.5), 0.2 M NaCl, and 40% PEG 300. The photomicrograph, at 70 X magnification, was taken after 10 days at 18°C. *See* Example 2.

[0042] FIG. 3 is a photomicrograph of crystals within an anti-huIL-23p19 mAb crystalline suspension, obtained by vapor diffusion. Anti-huIL-23p19 mAb 13B8-b was crystallized from a solution comprising 30% 1,2 propanediol, 0.1 M HEPES (pH 7.5), and 20% PEG 400. The photomicrograph, at 70 X magnification, was taken after 10 days at 4°C, and then two days at 22°C. *See* Example 2.

[0043] FIG. 4 is a photomicrograph of crystals within an anti-huIL-23p19 mAb crystalline suspension, obtained by vapor diffusion. Anti-huIL-23p19 mAb 13B8-b was crystallized from a solution comprising 0.1 M sodium acetate (pH 4.5), 0.2 M NaCl, and 40% PEG 300. The photomicrograph, at 70 X magnification, was taken after 10 days at 4°C, and then two days at 22°C. *See* Example 2.

[0044] FIG. 5 is a photomicrograph of crystals within an anti-huIL-23p19 mAb crystalline suspension, obtained by batch crystallization at pH 7.5. Anti-huIL-23p19 mAb 13B8-b was crystallized after mixture with a solution comprising 30% 1,2 propanediol, 0.1 M HEPES (pH 7.5), and 20% PEG 400, as discussed at Example 3. The photomicrograph, at 70 X magnification, was taken after 18 hours at 22°C. Particle sizes range from about 3 – 5 microns.

[0045] FIG. 6 is a photomicrograph of crystals within an anti-huIL-23p19 mAb crystalline suspension, obtained by batch crystallization at pH 4.5, as discussed at Example 3. Anti-huIL-23p19 mAb 13B8-b was crystallized after mixture with a solution comprising 58% PEG 300, 0.1 M sodium acetate (pH 4.5), and 0.2 M NaCl. The photomicrograph, at 400 X magnification, was taken after 18 hours at 22°C. Particle sizes range from about 30 – 80 microns.

[0046] FIG. 7 is a photomicrograph of crystals within an anti-huIL-23p19 mAb crystalline suspension, obtained by bulk dialysis at pH 7.5, as discussed at Example 4. Anti-huIL-23p19 mAb 13B8-b was crystallized after dialysis against a solution comprising 10%

1,2 propanediol, 0.1 M HEPES (pH 7.5), and 20% PEG 400. The photomicrograph, at 400 X magnification, was taken after 18 hours at 22°C. Particle sizes range from about 5 – 10 microns.

[0047] FIG. 8 is a photomicrograph of crystals within an anti-huIL-23p19 mAb crystalline suspension, obtained by bulk dialysis at pH 4.5, as discussed at Example 4. Anti-huIL-23p19 mAb 13B8-b was crystallized after dialysis against a solution comprising 14% PEG 300; 0.1 M sodium acetate (pH 4.5); and 0.2 M NaCl. The photomicrograph, at 400 X magnification, was taken after 18 hours at 22°C. Particle sizes range from about 5 – 10 microns.

[0048] FIG. 9 is a photomicrograph (at 170 X magnification) of crystals within an anti-huIL-23p19 mAb crystalline suspension, obtained by batch crystallization as discussed at Example 4 (last paragraph).

[0049] FIG. 10 presents a photograph of an SDS-PAGE gel comparing solution anti-huIL-23p19 mAb 13B8-b with the same material after it has been re-dissolved from the crystalline anti-huIL-23p19 mAb 13B8-b of the present invention. Details are provided at Example 6, and as indicated in the Legend. Lane 1 is MW markers, and lanes 2, 5, 8 and 11 are empty. Lanes 3 and 4 were loaded with 10 and 20 μg of solution antibody, respectively. Lanes 6 and 7 were loaded with 20 and 10 μg of re-dissolved crystalline antibody, respectively. Lanes 9 and 10 were loaded with 20 and 10 μg of the mother liquor aspirated from the crystalline suspension, and lane 12 was loaded with a portion of the wash.

[0050] FIGS. 11A and 11B presents photographs of crystals obtained by seeding methods, as described in Example 8. Crystals were obtained by mixing equal volumes of anti-huIL-23p19 mAb 13B8-b in 10 mM sodium citrate (pH 5.5) with an equal volume of 27% PEG 400, 0.1 M sodium citrate (pH 4.83), 0.2 M NaCl at 22°C. Samples were incubated five days at 22°C and then streaked with a pipette tip. Photographs were taken 24 hours after streaking (FIG. 11A), and again at 10 days after streaking (FIG. 11B). FIGS. 11A and 11B are at 70 X and 200 X magnification, respectively.

DETAILED DESCRIPTION

[0051] As used herein, including the appended claims, the singular forms of words such as "a," "an," and "the," include their corresponding plural references unless the context clearly dictates otherwise. Table 5 below provides a listing of sequence identifiers used in

this application. All references cited herein are incorporated by reference to the same extent as if each individual publication, patent application, or patent, was specifically and individually indicated to be incorporated by reference. Citation of the references herein is not intended as an admission that any of the foregoing is pertinent prior art, nor does it constitute any admission as to the contents or date of these publications or documents.

I. Definitions

[0052] "Administration" and "treatment," as it applies to an animal, human, experimental subject, cell, tissue, organ, or biological fluid, refers to contact of an exogenous pharmaceutical, therapeutic, diagnostic agent, or composition to the animal, human, subject, cell, tissue, organ, or biological fluid. "Administration" and "treatment" can refer, *e.g.*, to therapeutic, pharmacokinetic, diagnostic, research, and experimental methods. Treatment of a cell encompasses contact of a reagent to the cell, as well as contact of a reagent to a fluid, where the fluid is in contact with the cell. "Administration" and "treatment" also include *in vitro* and *ex vivo* treatments, *e.g.*, of a cell, by a reagent, diagnostic, binding composition, or by another cell.

[0053] As used herein, the term "hypervariable region" refers to the amino acid residues of an antibody that are responsible for antigen-binding. The hypervariable region comprises amino acid residues from a "complementarity determining region" or "CDR" (*e.g.* residues 24-34 (CDRL1), 50-56 (CDRL2) and 89-97 (CDRL3) in the light chain variable domain and residues 31-35 (CDRH1), 50-65 (CDRH2) and 95-102 (CDRH3) in the heavy chain variable domain (Kabat *et al.* (1991) Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md.) and/or those residues from a "hypervariable loop" (*i.e.* residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain (Chothia and Lesk (1987) *J. Mol. Biol.* 196: 901-917). As used herein, the term "framework" or "FR" residues refers to those variable domain residues other than the hypervariable region residues defined herein as CDR residues. The residue numbering above relates to the Kabat numbering system and does not necessarily correspond in detail to the sequence numbering in the accompanying Sequence Listing.

[0054] "Conservatively modified variants" or "conservative substitution" refers to substitutions of amino acids are known to those of skill in this art and may be made generally

without altering the biological activity of the resulting molecule, even in essential regions of the polypeptide. Such exemplary substitutions are preferably made in accordance with those set forth in Table 1 as follows:

Table 1
Exemplary Conservative Amino Acid Substitutions

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

[0055] In addition, those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide do not substantially alter biological activity. *See, e.g.*, Watson *et al.* (1987) *Molecular Biology of the Gene*, The Benjamin/Cummings Pub. Co., p. 224 (4th Edition).

[0056] The phrase "consists essentially of," or variations such as "consist essentially of" or "consisting essentially of," as used throughout the specification and claims, indicate the inclusion of any recited elements or group of elements, and the optional inclusion of other

elements, of similar or different nature than the recited elements, that do not materially change the basic or novel properties of the specified dosage regimen, method, or composition. As a non-limiting example, a binding compound that consists essentially of a recited amino acid sequence may also include one or more amino acids, including substitutions of one or more amino acid residues, that do not materially affect the properties of the binding compound.

[0057] "Immune condition" or "immune disorder" encompasses, *e.g.*, pathological inflammation, an inflammatory disorder, and an autoimmune disorder or disease. "Immune condition" also refers to infections, persistent infections, and proliferative conditions, such as cancer, tumors, and angiogenesis, including infections, tumors, and cancers that resist eradication by the immune system. "Cancerous condition" includes, *e.g.*, cancer, cancer cells, tumors, angiogenesis, and precancerous conditions such as dysplasia.

[0058] "Inflammatory disorder" means a disorder or pathological condition where the pathology results, in whole or in part, from, *e.g.*, a change in number, change in rate of migration, or change in activation, of cells of the immune system. Cells of the immune system include, *e.g.*, T cells, B cells, monocytes or macrophages, antigen presenting cells (APCs), dendritic cells, microglia, NK cells, NKT cells, neutrophils, eosinophils, mast cells, or any other cell specifically associated with the immunology, for example, cytokine-producing endothelial or epithelial cells.

[0059] As used herein, "inhibit" or "treat" or "treatment" includes a postponement of development of the symptoms associated with autoimmune disease or pathogen-induced immunopathology and/or a reduction in the severity of such symptoms that will or are expected to develop. The terms further include ameliorating existing uncontrolled or unwanted autoimmune-related or pathogen-induced immunopathology symptoms, preventing additional symptoms, and ameliorating or preventing the underlying causes of such symptoms. Thus the terms encompass, but are not limited to, circumstances in which a beneficial result has been conferred on a vertebrate subject with an autoimmune or pathogen-induced immunopathology disease or symptom, or with the potential to develop such a disease or symptom.

[0060] As used herein, the term "therapeutically effective amount" or "effective amount" refers to an amount of an IL-23p19 specific antibody that when administered alone or in combination with an additional therapeutic agent to a cell, tissue, or subject is effective

to prevent or ameliorate the autoimmune disease or pathogen-induced immunopathology associated disease or condition or the progression of the disease. A therapeutically effective dose further refers to that amount of the compound sufficient to result in amelioration of symptoms, *e.g.,* treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to an individual active ingredient administered alone, a therapeutically effective dose refers to that ingredient alone. When applied to a combination, a therapeutically effective dose refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously. An effective amount of therapeutic will decrease the symptoms typically by at least 10%; usually by at least 20%; preferably at least about 30%; more preferably at least 40%, and most preferably by at least 50%. *See, e.g.*, Maynard *et al.* (1996) *A Handbook of SOPs for Good Clinical Practice*, Interpharm Press, Boca Raton, FL; Dent (2001) *Good Laboratory and Good Clinical Practice*, Urch Publ., London, UK.

[0061] To examine the extent of inhibition of IL-23 activity, for example, samples or assays comprising a given, *e.g.*, protein, gene, cell, or organism, are treated with a potential inhibiting agent, such as an anti-huIL-23p19 mAb, and are compared to control samples without the agent. Control samples, *i.e.*, not treated with agent, are assigned a relative activity value of 100%. Inhibition is achieved when the activity value relative to the control is about 90% or less, typically 85% or less, more typically 80% or less, most typically 75% or less, generally 70% or less, more generally 65% or less, most generally 60% or less, typically 55% or less, usually 50% or less, more usually 45% or less, most usually 40% or less, preferably 35% or less, more preferably 30% or less, still more preferably 25% or less, and most preferably less than 25%. Activation is achieved when the activity value relative to the control is about 110%, generally at least 120%, more generally at least 140%, more generally at least 160%, often at least 180%, more often at least 2-fold, most often at least 2.5-fold, usually at least 5-fold, more usually at least 10-fold, preferably at least 20-fold, more preferably at least 40-fold, and most preferably over 40-fold higher.

[0062] "Specifically" or "selectively" binds, when referring to a ligand/receptor, antibody/antigen, or other binding pair, indicates a binding reaction which is determinative of the presence of the protein in a heterogeneous population of proteins and other biologics. Thus, under designated conditions, a specified ligand binds to a particular receptor and does

not bind in a significant amount to other proteins present in the sample. As used herein, an antibody is said to bind specifically to a polypeptide *comprising* a given sequence (in this case IL-23p19) if it binds to polypeptides comprising the sequence of IL-23p19 but does not bind to proteins lacking the sequence of IL-23p19. For example, an antibody that specifically binds to a polypeptide comprising IL-23p19 may bind to a FLAG®-tagged form of IL-23p19 but will not bind to other FLAG®-tagged proteins.

[0063] "Interleukin-23" (or "IL-23") means a protein consisting of two polypeptide subunits, p19 and p40. The sequence of the human p19 subunit (also known as IL-23p19, IL-23A) is provided at NCBI Protein Sequence Database Accession Number NP_057668. The sequence of the human p40 subunit (also known as IL-12p40, IL-12B) is provided at NCBI Protein Sequence Database Accession Number NP_002178.

[0064] As used herein, "anti-human IL-23p19" and "anti-huIL-23p19" refer to antibodies that specifically bind to the p19 subunit of human interleukin (IL-23). Unless otherwise indicated, or clear from the context, "IL-23" and "IL-23p19" refer to their respective human forms. Unless otherwise indicated, or clear from the context, antibodies referred to herein are monoclonal antibodies.

[0065] As used herein, an "antibody solution" may be used to refer to a solution of an anti-huIL-23p19 antibody that is used to generate the crystalline antibody of the present invention. "Precipitant solution" refers to a second solution that is mixed with the antibody solution, typically at a 1:1 volume ration (*i.e.* equal volumes of the two solutions are mixed) to create a "crystallization solution" from which antibodies grow. The concentrations of the antibody and precipitant solutions are provided herein for a 1:1 mixture, for convenience, but one of skill in the art would recognize that the volume ratio used to make the mixture can be changed, and thus so can the concentrations of the solutions making up the mixture. Such modifications are within the scope of the invention if they generate the same crystallization conditions (*i.e.* the same crystallization solution) as the mixtures described herein.

[0066] As used herein, and with regard to crystallization methods based on dialysis, "dialysis solution" refers to the solution against which a solution of anti-huIL-23p19 antibody (the "antibody solution") is dialyzed to drive formation of the crystalline antibody of the present invention. "Retentate" refers to the antibody solution after dialysis, which may include crystals of the antibody, which are harvested. The antibody solution / retentate are on one side of the dialysis membrane, and the dialysis solution is on the opposite side.

[0067] A "precipitant" is a compound that decreases the solubility of a polypeptide, such as an antibody, in a concentrated solution. In batch crystallization methods the precipitant is included in the "precipitant solution," and in bulk dialysis methods the precipitant is included in the "dialysis solution." Precipitants induce crystallization by forming an energetically unfavorable precipitant-depleted layer around the polypeptide molecules. To minimize the relative amount of this depletion layer, the polypeptides form associations and, ultimately, crystals. This process is explained in Weber (1991) *Advances in Protein Chemistry* 41:1, which is incorporated by reference. Various precipitants are known in the art and include: ammonium sulfate, ethanol, isopropanol, 1,2 propanediol, 3-ethyl-2,4 pentanediol; and many of the polyglycols, such as polyethylene glycol (*e.g.* PEG 300 and PEG 400). In addition to precipitants, other materials are sometimes added to the polypeptide precipitant solution. These include buffers, such as Tris or HEPES, to adjust the pH of the solution (and hence surface charge on the peptide) and salts, such as sodium chloride, lithium chloride and sodium citrate, to reduce the solubility of the polypeptide.

[0068] When used with reference to a crystalline antibody suspension of the present invention, "concentration" refers to the amount of antibody (in this case anti-huIL-23p19 antibody) present in a given macroscopic unit volume of solution. The term concentration is used in its customary sense despite the inherent heterogeneity of the suspension, as compared to a traditional solution. The concentration of antibody in a crystalline suspension is equal to the concentration of an equivalent sample in which the antibody was not in crystalline form.

II. General

[0069] The present invention provides crystalline forms of anti-huIL-23p19 antibodies, suspensions of these crystals, and pharmaceutical formulations of these suspensions. Highly purified anti-human interleukin-23p19 monoclonal antibody hu13B8-b was used in high throughput (HT) vapor diffusion sparse matrix experiments. Crystalline suspensions were obtained at room temperature using low molecular weight PEGs (300 and 400) and 1,2 propanediol, or under other conditions as disclosed herein. Conditions were established to prepare crystalline suspensions by bulk crystallization methods (batch and dialysis) in high yield. The resulting crystalline suspensions have a particle size of 5-200 microns. Antibody obtained by re-dissolving the crystals have been characterized by ELISA, SDS PAGE, and dynamic light scattering. The properties of the re-dissolved crystalline antibody are similar to those of the original antibody starting sample in all biophysical characterization studies.

[0070] Crystalline anti-huIL-23p19 antibodies of the present invention have several advantageous properties for use in therapy. The crystalline suspensions of anti-huIL-23p19 antibodies of the present invention can also be prepared at higher concentrations that solution formulations. This high concentration can enable more efficient administration to subject, *e.g.* by subcutaneous injection. For example, solution formulations at 100 mg/ml cannot be used to deliver more than 100 mg to a subject with a single subcutaneous injection due to limitations of how much volume can be practically delivered at a single injection site. This limits dosing to approximately 1.5 mg/kg, unless the subject is willing to accept (and in some cases administer) multiple injections at multiple sites. The crystalline suspensions of the present invention, in contrast, can be used to prepare pharmaceutical formulations up to 200 mg/ml or more, enabling higher dosing with lower injection volume, and thus less discomfort. The crystalline suspensions of the present also have significantly lower viscosity than high concentration solution formulations, facilitating administration by syringe, and/or enabling use of a smaller needle for injection. Crystalline suspensions of the present invention may be delivered by subcutaneous injection using small bore needles, such as 28G insulin syringes. The reduced volume, decreased viscosity and use of a smaller needle are all likely to decrease patient discomfort accompanying subcutaneous administration of anti-huIL-23p19 antibodies, which is of particular concern when a drug of intended for self-administration (*e.g.* by prefilled syringe or autoinjector).

[0071] The crystalline suspensions of anti-huIL-23p19 antibodies of the present invention also exhibit superior properties with regard to the pharmacokinetics of drug delivery. Compared with the corresponding solution formulations, the crystalline suspensions of the present invention exhibit delayed bioavailability. This time-released delivery of the drug (anti-huIL-23p19 antibody) into the circulation in the subject can advantageously increase the time over which the drug is present at an effective dose for a given administration. This can reduce the initial spike in drug concentration that would otherwise occur soon after administration (*e.g.* subcutaneous delivery of a solution formulation), and may enable less frequent dosing.

[0072] Crystalline anti-huIL-23p19 antibodies of the present invention also have other advantageous properties. Suspensions of the crystalline anti-huIL-23p19 antibodies will likely have improved stability compared with corresponding solution formulations, *i.e.* the crystalline suspensions will retain anti-IL-23 biological activity for a longer time. Suspensions of the crystalline anti-huIL-23p19 antibodies of the present invention can even be stored at room temperature, whereas typical solution formulations would have to be stored at 4°C. The longer shelf-life, and the ability to store the suspensions of the present invention a room temperature, offer significant advantages in handling of drug product and supply chain management.

[0073] Re-dissolved crystalline anti-huIL-23p19 antibodies of the present invention are characterized at Example 6. These results demonstrate the re-dissolved crystalline antibodies of the present invention retain the binding affinity and biological activity (IL-23 neutralization) of the starting material, and thus that they are suitable for all purposes for which the original antibody was suited, *e.g.* therapeutic treatment of human subjects. Specifically, the re-dissolved crystalline anti-huIL-23p19 antibodies of the present invention are substantially similar as assessed by SDS-PAGE, and dynamic light scattering shows that the re-dissolved antibody remains monodisperse with approximately the same experimentally observed molecular weight. *See* Table 3, Example 6. The re-dissolved crystalline anti-huIL-23p19 antibodies of the present invention also exhibit substantially similar EC50 values when compared with starting material in an ELISA. *See* Table 4, Example 6.

[0074] Accordingly, crystalline anti-huIL-23p19 antibodies that retain the properties of the pre-crystallization starting material within acceptable tolerances are encompassed in embodiments of the present invention. Acceptable tolerances for the various functional

parameters may vary based on the intended use, but with regard to binding affinity or biological activity, may include retention of 100%, 90%, 75%, 50% or 25% of the original (non-crystallized) affinity or activity. For example, acceptable K_D or EC50 / IC50 values may include 1, 2, 3, 4 or 5X the original (non-crystallized) K_D or EC50 / IC50 values, since these increased numerical values correspond to decreased binding affinity and biological activity. Methods for determining binding affinity and biological (neutralizing) activity are found at Examples 9 – 12.

[0075] The anti-huIL-23p19 antibody crystals themselves are characterized in Example 7. X-ray diffraction demonstrates that the anti-huIL-23p19 antibody crystals are characterized by unit cell dimensions of a = 192 Å, b = 192 Å, c = 106 Å, $\alpha = 90°$, $\beta = 90°$, $\gamma = 90°$ and are in space group I4. Crystals of anti-huIL-23p19 antibodies exhibiting substantially similar properties are also encompassed in embodiments of the present invention.

[0076] The invention further provides various methods for making the crystalline anti-huIL-23p19 antibody of the present invention, as described in greater detail in Examples 2 – 4. Example 2 provides methods based on vapor diffusion, which are best suited for screening to determine preferred crystallization conditions, rather than for large scale crystal production. Such methods may also be suitable for generation of large crystals for use in X-ray diffraction studies, *e.g.* to determine the three dimensional structure of the anti-huIL-23p19 antibody.

[0077] With regard to commercial scale production of crystalline anti-huIL-23p19, *e.g.* for therapeutic use, Examples 3 and 4 provide crystallization protocols more suited to large-scale production, such as batch crystallization and bulk dialysis crystallization. Two independent sets of solution conditions are provided for each example, one at pH 7.5 and the other at pH 4.5. The methods disclosed in Examples 3 and 4 amenable to being scaled-up for commercial scale production. Example 5 provides a method of harvesting crystals of the present invention using centrifugation, but filtration methods know in the art, such as hollow fiber tangential flow filtration, may also be used to harvest crystals, *e.g.*, at commercial scale. Although the specific disclosed embodiments employ a 1:1 mixture of an antibody solution with a precipitant solution, any modification of the method that ends up with approximately the same concentrations of solution components in the final crystallization solution (from which crystals arise) would be equivalent. Specifically, the concentrations of the components

in the precipitant solution may be proportionally increased or decreased if the precipitant solution comprises less than or more than 50% of the final volume of the crystallization solution, respectively.

[0078] The crystallization methods of the present invention also provide a method of purifying anti-huIL-23p19 antibodies, even if such crystals are re-dissolved prior to use. In one embodiment, an anti-huIL-23p19 antibody is produced and at least partially purified by methods described elsewhere herein (Section VII) and known in the art. The antibody is then crystallized, *e.g.* by batch crystallization as described in Example 3, or by bulk dialysis as described in Example 4. The crystalline antibody was then recovered and washed, *e.g.* as described in Example 5 (or by filtration), and re-dissolved essentially as described in the first paragraph of Example 6 except that the resuspension buffer need not be 10 mM sodium citrate (pH 5.5), but can instead be any suitable buffer for the intended use of the purified antibody. For therapeutic uses, suitable pharmaceutically acceptable buffers and excipients would be used.

III. Protein Crystallization

[0079] Various methods of crystallization are known. Giege *et al.* (1994) *Acta Crystallogr.* D50:339; McPherson (1990) *Eur. J. Biochem.* 189:1. Such techniques include hanging drop vapor diffusion (McPherson (1976) *J. Biol. Chem.* 251:6300), sitting drop vapor diffusion, microbatch and dialysis. Both hanging drop and sitting drop vapor diffusion entail a droplet containing purified protein, buffer, and precipitant being allowed to equilibrate with a larger reservoir containing similar buffers and precipitants in higher concentrations. Initially, the droplet of protein solution contains an insufficient concentration of precipitant for crystallization, but as water vaporizes from the drop and transfers to the reservoir, the precipitant concentration increases to a level optimal for crystallization. Since the system is in equilibrium, these optimum conditions are maintained until the crystallization is complete. The hanging drop method differs from the sitting drop method in the vertical orientation of the protein solution drop within the system. In the microbatch method, polypeptide is mixed with precipitants to achieve supersaturation, and the vessel is sealed and set aside until crystals appear. In the dialysis method, polypeptide is retained on one side of a dialysis membrane which is placed into contact with a solution containing precipitant. Equilibration across the membrane increases the precipitant concentration thereby causing the polypeptide

to reach supersaturation levels. For the methods of the present invention, it is desirable to use an anti-huIL-23p19 antibody at an initial protein concentration of about 5 – 100 mg/ml, such as 60 mg/ml or 100 mg/ml.

[0080] Uniform crystalline suspensions of therapeutic proteins present opportunities for novel drug delivery systems. Crystalline insulin suspensions have been used as sustained release preparations for over fifty years (Brange *et al.* (1999) *Adv. Drug Deliv. Rev.* 35:307). Crystalline suspensions have also been proposed for the delivery of interferon (U.S. Pat. No. 5,972,331) and monoclonal antibodies (Yang *et al.* (2003) *Proc. Nat'l Acad. Sci. (USA)* 100:6934). Crystalline forms also enable non-injectable delivery systems such as pulmonary delivery for local or systemic delivery of protein therapeutics (U.S. Pat. No. 5,972,331).

[0081] Henry Bence Jones was the first to describe naturally occurring crystals of immunoglobulin light chains, the so-called "Bence-Jones protein," isolated the urine of a myeloma patient. Jones (1848) *Phil. Trans. Royal Soc. (London)* 55–62. Full-length, intact antibodies, however, are difficult to crystallize, likely due to the flexibility of their multiple (four) polypeptide chains. Although, there have been numerous reports of crystallization of intact antibodies over the last 30 years, only four structures have been deposited in the Research Collaboratory for Structural Bioinformatics Protein Databank (RCSB-PDB). In contrast, there have been over 800 structures deposited for antibody fragments, such as Fab fragments (either apo or complexed).

[0082] Some full-length therapeutic antibodies have been crystallized. Determination of the crystal structure for an anti-human IL-13 antibody is described at Int'l Pat. App. Pub. WO 2005/121177 (issued as U.S. Pat. No. 7,615,213) to Wyeth, and methods for the preparation of crystalline anti-human TNF-α crystals are described at Int'l Pat. App. Pub. WO 2008/057240 (issued as U.S. Pat. No. 8,034,906) to Abbott Biotechnology. Ltd. Methods for the preparation of crystalline anti-human IL-12 antibodies are described at Int'l Pat. App. Pub. WO 2008/121301 (issued as U.S. Pat. No. 8,168,760) to Abbott Laboratories.

[0083] Protein crystals, including antibodies, are also being developed as therapeutic compositions. Altus Pharmaceuticals, now a part of Althea Technologies, Inc., has put three crystalline protein formulations into human clinical trials: ALTU-238 (long-acting injectable formulation of somatropin); ALTU-237 (oral oxalate-degrading enzyme); and ALTU-236 (oral phenylalanine degrading enzyme). Researchers from Altus also crystallized three commercially available monoclonal antibodies (rituximab, trastuzumab and infliximab) by

vapor diffusion methods or batch crystallization. Yang *et al.* (2003) *Proc. Nat'l Acad. Sci. (USA)* 100:6934; Int'l Pat. App. Pub. WO 02/072636, issued as U.S. Pat. No. 7,833,525). The resulting high concentration, low viscosity crystals were obtained in high yield, and showed excellent physical and chemical stability, as well as retention of biological activity *in vitro*. Subcutaneous injection of trastuzumab and infliximab crystalline suspensions resulted in an extended serum pharmacokinetic profile and high bioavailability compared with the soluble forms of the antibodies delivered intravenously. The crystalline formulation of trastuzumab was also effective in a preclinical model of human breast cancer. Spherical protein particles of therapeutic antibodies are disclosed at U.S. Pat. No. 7,998,477.

[0084] Crystallization by methods of the present invention also provides an improved method of purification of anti-huIL-23p19 antibodies. Although macro-scale crystallization is frequently used in purification of small organic molecules, there are few examples of the use of crystallization in the preparation of proteins. An exception is the use of a crystallization step in the manufacture of interferon alpha-2b (IFN-α_{2b}), where a temperature induction method is used in the purification process on a multigram scale. The resulting crystalline suspension is harvested by centrifugation, washed and solubilized in a cold normal saline phosphate buffer. The crystallization and harvesting process removes small molecule, interferon-related and non-interferon impurities that may remain in the mother liquor or wash. Crystallization also confirms the purity of the therapeutic protein.

IV. IL-23 Biology

[0085] Interleukin-23 (IL-23) is a heterodimeric cytokine comprised of two subunits, p19 which is unique to IL-23, and p40, which is shared with IL-12. The p19 subunit is structurally related to IL-6, granulocyte-colony stimulating factor (G-CSF), and the p35 subunit of IL-12. IL-23 mediates signaling by binding to a heterodimeric receptor, comprised of IL-23R and IL-12β1, which is shared by the IL-12 receptor. A number of early studies demonstrated that the consequences of a genetic deficiency in p40 (p40 knockout mouse; p40KO mouse) were more severe than those found in a p35KO mouse. Some of these results were eventually explained by the discovery of IL-23, and the finding that the p40KO prevents expression of not only IL-12, but also of IL-23 (*see, e.g.*, Oppmann *et al.* (2000) *Immunity* 13:715-725; Wiekowski *et al.* (2001) *J. Immunol.* 166:7563-7570; Parham *et al.* (2002) *J. Immunol.* 168:5699-708; Frucht (2002) *Sci STKE* 2002, E1-E3; Elkins *et al.* (2002) *Infection*

Immunity 70:1936-1948). Additional information relating to IL-23 (*IL23A*)may be found at NCBI's Gene Database under GeneID 51561, and at the Online Mendelian Inheritance in Man database under MIM 605580, each of which is hereby incorporated by reference.

[0086] Recent studies, through the use of p40 KO mice, have shown that blockade of both IL-23 and IL-12 is an effective treatment for various inflammatory and autoimmune disorders. IL-23 is known to play a central role in psoriasis, and the IL-23/IL-12 antagonist antibody ustekinumab (anti-IL-12/23p40 mAb) has been approved in the U.S. and Europe for the treatment of psoriasis. However, the blockade of IL-12 through p40 appears to have various systemic consequences such as increased susceptibility to opportunistic microbial infections. Bowman *et al*. (2006) *Curr. Opin. Infect. Dis*. 19:245.

V. Anti-human IL-23 Antibody - 13B8-b

[0087] The present application discloses data relating to crystallization of the anti-human IL-23p19 antibody clone 13B8-b, as described and claimed in commonly assigned WO 2008/103432, the disclosure of which is hereby incorporated by reference in its entirety. WO 2008/103432 discloses a humanized anti-huIL-23p19 antibody, designated 13B8-a, and two variants differing only at residues in CDRH2, designated 13B8-b and 13B8-c. The CDRH2 sequences are provided at Table 2. The humanized light chain 13B8 sequence (with kappa constant region) is provided at SEQ ID NO: 4, and the light chain variable domain comprises residues 1-108 of that sequence. Three versions of the humanized heavy chain 13B8 sequence (with γ1 constant regions) are provided at SEQ ID NOs: 1 – 3, and the heavy chain variable domain comprises residues 1-116 of those sequences.

Table 2

Antibody 13B8 CDRH2 Variants

Antibody	CDRH2 Sequence	SEQ ID NO:
13B8-a	QIFPASGSADYNEMFEG	6
13B8-b	QIFPASGSADYNE**K**FEG	7
13B8-c	QIFPASGSADY**AQKLQ**G	8

[0088] A hybridoma expressing the parental (mouse) antibody 13B8 was deposited pursuant to the Budapest Treaty with American Type Culture Collection (ATCC - Manassas, Virginia, USA) on August 17, 2006 under Accession Number PTA-7803. All restrictions on

the accessibility of this deposit will be irrevocably removed upon the granting of a U.S. patent based on the present application.

VI. Additional Anti-human IL-23 Antibodies

[0089] The heterodimeric nature of IL-23 suggests that antagonist antibodies may bind exclusively to the p40 subunit, exclusively to the p19 subunit, or exclusively to the IL-23 complex (p40 and p19). IL-23 antagonist antibodies have been reported in all three of these categories. Although the results presented herein were obtained exclusively with humanized anti-human IL-23p19 antibody clone 13B8-b, it is possible that the methods used herein could be used to crystallize these other anti-IL-23 antagonist antibodies as well. In some such prophetic embodiments, the IL-23 antagonist antibody binds to the p40 subunit of IL-23 and IL-12. Examples include ustekinumab (CNTO 1275) and briakinumab (ABT-874, J-695). Ustekinumab is marketed by Centocor for the treatment of psoriasis, and is described at US 6,902,734 and US 7,166,285 (to Centocor, Inc.), the disclosures of which are hereby incorporated by reference in their entireties. Specifically, the sequences of SEQ ID NOs: 7 (heavy chain variable domain) and 8 (light chain variable domain), of US 6,902,734 are hereby incorporated by reference. SEQ ID NOs: 4-5-6 and 1-2-3 of US 6,902,734 are also incorporated by reference. Briakinumab was developed by Abbott, and is described at US 6,914,128 and US 7,504,485, the disclosures of which are hereby incorporated by reference in their entireties. Sequences for ustekinumab are also provided at SEQ ID NOs: 16 – 25 of the sequence listing of the present application. Specifically, the sequences of SEQ ID NOs: 31 (heavy chain variable domain), 32 (light chain variable domain) SEQ ID NOs; 30-28-26 (light chain CDRs 1-2-3, respectively) and 29-27-25 (heavy chain CDRs 1-2-3, respectively) of US 6,914,128 are hereby incorporated by reference. Sequences for briakinumab are also provided at SEQ ID NOs: 26 – 35 of the sequence listing of the present application. Examples also include an antibody that binds specifically to IL-23p40 but not IL-12p40 as disclosed in US 7,247,711 (to Centocor).

[0090] In other prophetic embodiments, the IL-23 antagonist antibody binds to the p19 subunit of IL-23. Examples include Eli Lilly's LY2525623 and Centocor's CNTO 1959, both of which have entered human clinical trials. Specifically, the sequences of SEQ ID NOs: 48 and 52 (heavy chain variable domains), 57 (light chain variable domain), 28-37-40 (light chain CDRs 1-2-3, respectively) and 3-8-19 (light chain CDRs 1-2-3, respectively) of

EP 1937721 B1 (to Eli Lilly and Company) are hereby incorporated by reference. In addition, the sequences of SEQ ID NOs: 106 (heavy chain variable domain), 116 (light chain variable domain), 50-56-73 (light chain CDRs 1-2-3, respectively) and 5-20-44 (light chain CDRs 1-2-3, respectively) of US 7,935,344 (to Centocor) are also hereby incorporated by reference. Further examples include monoclonal antibody FM303 (Femta Pharmaceuticals), and the antibodies disclosed at WO 2008/103432, US 2007/0048315 and WO 2008/103473 (to Schering Corp.); US 7,491,391, US 7,935,344 and EP 1971366 A2 (to Centocor Ortho Biotech, Inc.); US 7,872,102 (to Eli Lilly and Co.); WO 2007/147019, WO 2008/134659 and WO 2009/082624 (to Zymogenetics); US 2009/0311253 (to Abbott Bioresearch); and US 2009/0123479 and WO 2010/115786 (to Glaxo SmithKline), the disclosures of which are hereby incorporated by reference in their entireties.

[0091] In yet further prophetic embodiments, the IL-23 antagonist antibody binds to the IL-23 complex rather than to the individual subunits. Examples include an antibody that makes contacts with both the p19 and p40 subunits of IL-23 (WO 2011/056600 to Amgen, Inc.) and an antibody that binds to IL-23 but not significantly to either of its individual subunits (WO 2012/009760 to Cephalon Australia Pty., Ltd).

[0092] Further exemplary non-specific IL-23 antagonist antibodies that bind to the p40 subunit of IL-23 and IL-12 are disclosed at Clarke *et al.* (2010) *mAbs* 2:1-11 (Cephalon Australia, Pty., Ltd.). FM202 (Femta Pharmaceuticals) is also a monoclonal antibody that binds to the p40 subunit of both IL-12 and IL-23, as are the antibodies disclosed at WO 2010/017598 (Arana Therapeutics, Ltd.).

VII. Antibody Production

[0093] In one embodiment, for recombinant production of the antibodies of the present invention, the nucleic acids encoding the two chains are isolated and inserted into one or more replicable vectors for further cloning (amplification of the DNA) or for expression. DNA encoding the monoclonal antibody is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). Many vectors are available. The 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, an enhancer element, a promoter, and a transcription termination sequence. In one embodiment, both the light and

heavy chains of the humanized anti-huIL-23p19 antibody of the present invention are expressed from the same vector, *e.g.* a plasmid or an adenoviral vector.

[0094] Antibodies of the present invention may be produced by any method known in the art. In one embodiment, antibodies are expressed in mammalian or insect cells in culture, such as chinese hamster ovary (CHO) cells, human embryonic kidney (HEK) 293 cells, mouse myeloma NSO cells, baby hamster kidney (BHK) cells, *Spodoptera frugiperda* ovarian (Sf9) cells. In one embodiment, antibodies secreted from CHO cells are recovered and purified by standard chromatographic methods, such as protein A, cation exchange, anion exchange, hydrophobic interaction, and hydroxyapatite chromatography. Resulting antibodies are concentrated and stored in 20 mM sodium acetate (pH 5.5).

[0095] In another embodiment, the antibodies of the present invention are produced in yeast according to the methods described in WO 2005/040395. Briefly, vectors encoding the individual light or heavy chains of an antibody of interest are introduced into different yeast haploid cells, *e.g.* different mating types of the yeast *Pichia pastoris*, which yeast haploid cells are optionally complementary auxotrophs. The transformed haploid yeast cells can then be mated or fused to give a diploid yeast cell capable of producing both the heavy and the light chains. The diploid strain is then able to secret the fully assembled and biologically active antibody. The relative expression levels of the two chains can be optimized, for example, by using vectors with different copy number, using transcriptional promoters of different strengths, or inducing expression from inducible promoters driving transcription of the genes encoding one or both chains.

[0096] In one embodiment, the respective heavy and light chains of a plurality of different anti-IL-23p19 antibodies (the "original" antibodies) are introduced into yeast haploid cells to create a library of haploid yeast strains of one mating type expressing a plurality of light chains, and a library of haploid yeast strains of a different mating type expressing a plurality of heavy chains. These libraries of haploid strains can be mated (or fused as spheroplasts) to produce a series of diploid yeast cells expressing a combinatorial library of antibodies comprised of the various possible permutations of light and heavy chains. The combinatorial library of antibodies can then be screened to determine whether any of the antibodies has properties that are superior (*e.g.* higher affinity for IL-23) to those of the original antibodies. *See. e.g.*, WO 2005/040395.

VIII. Pharmaceutical Compositions

[0097] To prepare pharmaceutical or sterile compositions, the crystalline anti-human IL-23p19 antibodies of the present invention are mixed with a pharmaceutically acceptable carrier or excipient. *See, e.g.*, *Remington's Pharmaceutical Sciences* and *U.S. Pharmacopeia: National Formulary*, Mack Publishing Company, Easton, PA (1984).

[0098] Selecting an administration regimen for a therapeutic depends on several factors, including the serum or tissue turnover rate of the entity, the level of symptoms, the immunogenicity of the entity, and the accessibility of the target cells in the biological matrix. Preferably, an administration regimen maximizes the amount of therapeutic delivered to the patient consistent with an acceptable level of side effects. Accordingly, the amount of biologic delivered depends in part on the particular entity and the severity of the condition being treated. Guidance in selecting appropriate doses of antibodies, cytokines, and small molecules are available. *See, e.g.*, Wawrzynczak (1996) *Antibody Therapy*, Bios Scientific Pub. Ltd, Oxfordshire, UK; Kresina (ed.) (1991) *Monoclonal Antibodies, Cytokines and Arthritis*, Marcel Dekker, New York, NY; Bach (ed.) (1993) *Monoclonal Antibodies and Peptide Therapy in Autoimmune Diseases*, Marcel Dekker, New York, NY; Baert *et al.* (2003) *New Engl. J. Med.* 348:601-608; Milgrom *et al.* (1999) *New Engl. J. Med.* 341:1966-1973; Slamon *et al.* (2001) *New Engl. J. Med.* 344:783-792; Beniaminovitz *et al.* (2000) *New Engl. J. Med.* 342:613-619; Ghosh *et al.* (2003) *New Engl. J. Med.* 348:24-32; Lipsky *et al.* (2000) *New Engl. J. Med.* 343:1594-1602.

[0099] Determination of the appropriate dose is made by the clinician, *e.g.*, using parameters or factors known or suspected in the art to affect treatment or predicted to affect treatment. Generally, the dose begins with an amount somewhat less than the optimum dose and it is increased by small increments thereafter until the desired or optimum effect is achieved relative to any negative side effects. Important diagnostic measures include those of symptoms of, *e.g.*, the inflammation or level of inflammatory cytokines produced.

[00100] Crystalline antibody compositions of the present invention can be provided by continuous infusion, or by doses at intervals of, *e.g.*, one day, 1-7 times per week, one week, two weeks, monthly, bimonthly, quarterly, biannually, annually, etc. Doses may be provided intravenously, subcutaneously, intramuscularly, intracerebrally, intraspinally, or by inhalation. A preferred dose protocol is one involving the maximal dose or dose frequency that avoids significant undesirable side effects. A total weekly dose is generally at least 0.05 μg/kg, 0.2

μg/kg, 0.5 μg/kg, 1 μg/kg, 10 μg/kg, 100 μg/kg, 0.2 mg/kg, 1.0 mg/kg, 2.0 mg/kg, 10 mg/kg, 25 mg/kg, 50 mg/kg body weight or more. *See, e.g.*, Yang *et al.* (2003) *New Engl. J. Med.* 349:427-434; Herold *et al.* (2002) *New Engl. J. Med.* 346:1692-1698; Liu *et al.* (1999) *J. Neurol. Neurosurg. Psych.* 67:451-456; Portielji *et al.* (20003) *Cancer Immunol. Immunother.* 52:133-144.

[00101] Crystalline formulations of anti-huIL-23p19 antibodies of the present invention may enable administration at levels that are not otherwise practical using standard solution formulations, such as administration at 100, 15, 200, 250 mg/ml or higher concentrations. Such high concentration delivery enables delivery of high doses by subcutaneous injection, in which there exists a practical limit of approximately 1 ml for the volume that can be delivered in any given injection. Crystalline formulations of anti-huIL-23p19 antibodies of the present invention may also enable modes of administration that are not otherwise practical, such as oral, pulmonary or needle-free delivery.

IX. Uses

[00102] The present invention provides methods for using engineered anti-huIL-23 antibodies and fragments thereof for the treatment of inflammatory disorders and conditions, *e.g.*, of the central nervous system, peripheral nervous system, and gastrointestinal tract, as well as autoimmune and proliferative disorders.

[00103] Methods are provided for the treatment of, *e.g.*, multiple sclerosis (MS), including relapsing-remitting MS and primary progressive MS, Alzheimer's disease, amyotrophic lateral sclerosis (a.k.a. ALS; Lou Gehrig's disease), ischemic brain injury, prion diseases, and HIV-associated dementia. Also provided are methods for treating neuropathic pain, posttraumatic neuropathies, Guillain-Barre syndrome (GBS), peripheral polyneuropathy, and nerve regeneration.

[00104] Provided are methods for treating or ameliorating one or more of the following features, symptoms, aspects, manifestations, or signs of multiple sclerosis, or other inflammatory disorder or condition of the nervous system: brain lesions, myelin lesions, demyelination, demyelinated plaques, visual disturbance, loss of balance or coordination, spasticity, sensory disturbances, incontinence, pain, weakness, fatigue, paralysis, cognitive impairment, bradyphrenia, diplopia, optic neuritis, paresthesia, gait ataxia, fatigue, Uhtoff's symptom, neuralgia, aphasia, apraxia, seizures, visual-field loss, dementia, extrapyramidal

phenomena, depression, sense of well-being, or other emotional symptoms, chronic progressive myelopathy, and a symptom detected by magnetic resonance imaging (MRI), including gadolinium-enhancing lesions, evoked potential recordings, or examination of cerebrospinal fluid. *See, e.g.*, Kenealy *et al.* (2003) *J. Neuroimmunol.* 143:7-12; Noseworthy *et al.* (2000) *New Engl. J. Med.* 343:938-952; Miller *et al.* (2003) *New Engl. J. Med.* 348:15-23; Chang *et al.* (2002) *New Engl. J. Med.* 346:165-173; Bruck and Stadelmann (2003) *Neurol. Sci.* 24 Suppl.5:S265-S267.

[00105] Moreover, the present invention provides methods for treating inflammatory bowel disorders, *e.g.*, Crohn's disease, ulcerative colitis, celiac disease, and irritable bowel syndrome. Provided are methods for treating or ameliorating one or more of the following symptoms, aspects, manifestations, or signs of an inflammatory bowel disorder: malabsorption of food, altered bowel motility, infection, fever, abdominal pain, diarrhea, rectal bleeding, weight loss, signs of malnutrition, perianal disease, abdominal mass, and growth failure, as well as intestinal complications such as stricture, fistulas, toxic megacolon, perforation, and cancer, and including endoscopic findings, such as, friability, aphthous and linear ulcers, cobblestone appearance, pseudopolyps, and rectal involvement and, in addition, anti-yeast antibodies. *See, e.g.*, Podolsky, *supra*; Hanauer, *supra*; Horwitz and Fisher, *supra*.

[00106] Also contemplated are treatment of inflammatory disorders such as psoriasis, atopic dermatitis, arthritis, including rheumatoid arthritis, osteoarthritis, and psoriatic arthritis, ankylosing spondylitis, graft-versus-host disease, autoimmune disorders, such as systemic lupus erythematosus and type I diabetes, and proliferative disorders such as cancer. *See, e.g.*, PCT patent application publications WO 04/081190; WO 04/071517; WO 00/53631; and WO 01/18051. Also contemplated is treatment of chronic infections, such as chronic fungal infections, *e.g.* infections with *Candida spp.* and *Aspergillus spp.* *See* commonly assigned Int'l Pat. App. Pub. WO 2008/153610.

[00107] The IL-23p19 binding compounds of the present invention can also be used in combination with one or more antagonists of other cytokines (*e.g.* antibodies), including but not limited to, IL-17A, IL-17F, IL-1β, IL-6 and TGF-β. *See, e.g.*, Veldhoen (2006) *Immunity* 24:179-189; Dong (2006) *Nat. Rev. Immunol.* 6(4):329-333. In various embodiments, an IL-23p19 binding compound of the invention is administered before, concurrently with, or after administration of the another antagonist or antagonists, such as an anti-IL-17A antibody. In one embodiment, an IL-17A binding compound is used in treatment of the acute early phase

of an adverse immune response (*e.g.* MS, Crohn's Disease) alone or in combination with an IL-23 antagonist antibody of the present invention. In the latter case, the IL-17A binding compound may be gradually decreased and treatment with the antagonist of IL-23 alone is continued to maintain suppression of the adverse response. Alternatively, antagonists to IL-1β, IL-6 and/or TGF-β may be administered concurrently, before or after an IL-23p19 binding compound of the present invention. *See* Cua and Kastelein (2006) *Nat. Immunol.* 7:557-559; commonly assigned Int'l Pat. App. Pub. WO 2008/106131; Tato and O'Shea (2006) *Nature* 441:166-168; Iwakura and Ishigame (2006) *J. Clin. Invest.* 116:1218-1222.

[00108] The broad scope of this invention is best understood with reference to the following examples, which are not intended to limit the inventions to the specific embodiments. The specific embodiments described herein are offered by way of example only, and the invention is to be limited by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled.

EXAMPLES

Example 1

General Methods

[00109] Standard methods in molecular biology are described. Maniatis *et al.* (1982) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Sambrook and Russell (2001) *Molecular Cloning, 3rd ed.,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Wu (1993) *Recombinant DNA*, Vol. 217, Academic Press, San Diego, CA. Standard methods also appear in Ausbel *et al.* (2001) *Current Protocols in Molecular Biology, Vols. 1-4*, John Wiley and Sons, Inc. New York, NY, which describes cloning in bacterial cells and DNA mutagenesis (Vol. 1), cloning in mammalian cells and yeast (Vol. 2), glycoconjugates and protein expression (Vol. 3), and bioinformatics (Vol. 4).

[00110] Methods for protein purification including immunoprecipitation, chromatography, electrophoresis, centrifugation, and crystallization are described. Coligan *et al.* (2000) *Current Protocols in Protein Science, Vol. 1*, John Wiley and Sons, Inc., New York. Chemical analysis, chemical modification, post-translational modification, production of fusion proteins, glycosylation of proteins are described. *See, e.g.*, Coligan *et al.* (2000)

Current Protocols in Protein Science, Vol. 2, John Wiley and Sons, Inc., New York; Ausubel *et al.* (2001) *Current Protocols in Molecular Biology, Vol. 3*, John Wiley and Sons, Inc., NY, NY, pp. 16.0.5-16.22.17; Sigma-Aldrich, Co. (2001) *Products for Life Science Research*, St. Louis, MO; pp. 45-89; Amersham Pharmacia Biotech (2001) *BioDirectory*, Piscataway, N.J., pp. 384-391. Production, purification, and fragmentation of polyclonal and monoclonal antibodies are described. Coligan *et al.* (2001) *Current Protocols in Immunology, Vol. 1*, John Wiley and Sons, Inc., New York; Harlow and Lane (1999) *Using Antibodies*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Harlow and Lane, *supra*. Standard techniques for characterizing ligand/receptor interactions are available. *See, e.g.*, Coligan *et al.* (2001) *Current Protocols in Immunology, Vol. 4*, John Wiley, Inc., New York.

Example 2

Vapor Diffusion Crystallization of Anti-huIL-23p19 mAb 13B8-b

[00111] Conditions for preparing crystals of anti-huIL-23p19 mAb 13B8-b were determined as follows. A solution of anti-huIL-23p19 mAb 13B8-b was prepared at 61.9 mg/ml in 10 mM sodium citrate (pH 5.5). The solution was screened in hanging drop vapor diffusion experiments using commercially available screens from Emerald BioStructures (Bainbridge Island, Washington, USA), Qiagen Technologies (Gaithersburg, Maryland, USA) and Jena Bioscience (Jena, DE) in crystallization plates from Neuro Probe, Inc. (Gaithersburg, Maryland, USA). Antibody solution (0.5 µl) was mixed with complimentary reservoir solution (0.5 µl) and suspended over 80 µl of complimentary reservoir solution. Experiments were performed at both 4°C and 18°C and monitored microscopically over time. Crystals were observed in experiments at 18°C after 10 days in the Qiagen screening system using a first complimentary reservoir solution comprising 30% 1,2 propanediol; 0.1 M HEPES (pH 7.5); and 20% PEG 400, and also using a second complimentary reservoir solution comprising 0.1 M sodium acetate (pH 4.5); 40% PEG 300; and 0.2 M NaCl. Photomicrographs of the resulting crystals are provided at FIGS. 1 and 2, respectively.

[00112] Two analogous experiments at 4°C showed no evidence of crystals after 10 days, but both these experiments produced crystals within two days after the temperature was shifted to 22°C (room temperature). Photomicrographs of the crystals observed in the 4°C experiments are provided at FIGS. 3 and 4.

[00113] Additional experiments failed to generate any crystals. Crystals were not observed under any conditions in Emerald BioStructures or Jena Bioscience systems. Crystals were also not observed, at either 4°C or 18°C, under four sets conditions: PP(PEG/salt); HPPEG (PEG/salt); J1 (PEG/salt); J2 (salt).

Example 3

Batch Crystallization of Anti-huIL-23p19 mAb 13B8-b

[00114] In light of the results obtained in Example 2, batch crystallization of anti-huIL-23p19 mAb 13B8-b at pH 7.5 was performed as follows. A solution of anti-huIL-23p19 mAb 13B8-b was prepared at 60 mg/ml in 10 mM sodium citrate (pH 5.5). 500 µl of this solution was mixed with an equal volume of 30% 1,2 propanediol; 0.1 M HEPES (pH 7.5); and 20% PEG 400 at 4°C. The mixture was incubated for 18 hours at room temperature (22°C). A photomicrograph of the resulting crystals is provided at FIG. 5. The crystalline suspension was recovered as described in Example 5 at 85% yield.

[00115] Batch crystallization of anti-huIL-23p19 mAb 13B8-b was also performed at pH 4.5, as follows. A solution of anti-huIL-23p19 mAb 13B8-b was prepared at 60 mg/ml in 10 mM sodium citrate (pH 5.5). 500 µl of this solution was mixed with an equal volume of 70% PEG 300; 0.1 M sodium acetate (pH 4.5); and 0.2 M NaCl at 4°C. The mixture was incubated for 18 hours at room temperature (22°C). A photomicrograph of the resulting crystals is provided at FIG. 6. The crystalline suspension was recovered as described in Example 5 at 70% yield.

[00116] Alternatively, batch crystallization of anti-huIL-23p19 mAb 13B8-b was performed as follows. A solution of anti-huIL-23p19 mAb 13B8-b was prepared at 100 mg/ml in 10 mM sodium citrate (pH 4.8), 7% sucrose, 0.25% polysorbate 80. 500 µl of this solution was mixed with an equal volume of 100 mM sodium citrate (pH 5.1), 57.4% PEG 300 at 22°C. The mixture was placed on a ClayAdams nutator mixer (BD Diagnostics Part No. 421105) (VWR International LLC, Radnor, Penna., USA) and incubated 24 hours at 30°C. A photomicrograph of the resulting crystals is provided at FIG. 9. The crystalline suspension was recovered as described in Example 5 at 90% yield.

Example 4

Bulk Dialysis Crystallization of Anti-huIL-23p19 mAb 13B8-b

[00117] Bulk dialysis crystallization of anti-huIL-23p19 mAb 13B8-b at pH 7.5 was performed as follows. A solution of anti-huIL-23p19 mAb 13B8-b was prepared at 60 mg/ml in 10 mM sodium citrate (pH 5.5), and dialyzed in a DispoDialyzer® (8K MW cutoff) membrane (Spectrum Laboratories, Inc., Rancho Dominguez, Calif., USA) against 10 ml of 10% 1,2 propanediol; 0.1 M HEPES (pH 7.5); and 20% PEG 400 for 18 hours at room temperature (22°C). A photomicrograph of the resulting crystals is provided at FIG. 7. The crystalline suspension was recovered as described in Example 5 at 50% yield.

[00118] Bulk dialysis crystallization of anti-huIL-23p19 mAb 13B8-b was also performed at pH 4.5, as follows. A solution of anti-huIL-23p19 mAb 13B8-b was prepared at 60 mg/ml in 10 mM sodium citrate (pH 5.5), and dialyzed in a DispoDialyzer® (8K MW cutoff) membrane against 10 ml of 14% PEG 300; 0.1 M sodium acetate (pH 4.5); and 0.2 M NaCl for 18 hours at room temperature (22°C). A photomicrograph of the resulting crystals is provided at FIG. 8. The crystalline suspension was recovered as described in Example 5 at 33% yield.

Example 5

Harvest of Crystalline Suspensions by Centrifugation

[00119] Crystalline anti-huIL-23p19 mAb 13B8-b is harvested from crystal suspensions as follows. The suspension is centrifuged in a Fisher microfuge at 5000 rpm for 5 minutes at room temperature. The mother liquor is removed by aspiration and the pellet is re-suspended in stabilizing solution (100 mM sodium citrate, pH 4.8, 25% PEG 400, 200 mM NaCl, 0.1% methyl paraben).

[00120] The crystalline suspension is again centrifuged for 5 minutes at 5,000 rpm, the supernatant (wash) is removed by aspiration and the pellet is resuspended in stabilizing solution. This wash step is repeated two more times. The resulting pellet is re-suspended in sterile stabilizing solution and stored at room temperature.

Example 6

Characterization of Crystalline Anti-huIL-23p19 mAb 13B8-b

[00121] Re-dissolved crystalline anti-huIL-23p19 mAb 13B8-b was characterized by SDS PAGE, dynamic light scattering (DLS), and ELISA, as follows.

[00122] A suspension of crystalline anti-huIL-23p19 mAb 13B8-b was centrifuged in a Fisher microfuge at 5000 rpm for 5 minutes at room temperature. The mother liquor was removed by aspiration and the pellet was re-suspended in stabilizing solution (100 mM sodium citrate, pH 4.8, 25% PEG 400, 200 mM NaCl, 0.1% methyl paraben). The suspension was again centrifuged for 5 minutes at 5,000 rpm, the supernatant (wash) is removed by aspiration and the pellet was resuspended in 10 mM sodium citrate (pH 5.5). The resulting solution was clarified by centrifugation, and the re-dissolved crystalline solution was used for characterization studies.

[00123] Re-dissolved crystalline anti-huIL-23p19 mAb 13B8-b was compared to the original solution antibody by reducing SDS-PAGE, as illustrated by the gel photograph at FIG. 10. As used herein, "solution" anti-huIL-23p19 mAb 13B8-b refers to the same antibody as in the crystalline form of the invention (and is in fact its starting material), but it has never been crystallized. The re-dissolved crystalline anti-huIL-23p19 mAb 13B8-b, loaded in lanes 6 and 7 (20 and 10 μg, respectively), looks the same as the original solution form of the antibody, loaded in lanes 3 and 4 (10 and 20 μg, respectively).

[00124] Re-dissolved crystalline anti-huIL-23p19 mAb 13B8-b was also compared to the original solution antibody by dynamic light scattering. DLS can provide information about the distribution of species in solution, including the number of resolvable species, their size, and level of homogeneity from a single measurement. A DynaPro light scattering instrument (Wyatt Technologies, Santa Barbara, Calif., USA) was used to measure the aggregation state of anti-huIL-23p19 mAb 13B8-b in solution. The experimentally derived molecular weight for the re-dissolved crystal solution was judged to be monodisperse, and comparable to the anti IL-23p19 mAb starting material. *See* Table 3.

Table 3

Anti-Hu-IL 23 mAB 13B8-b	Theoretical MW (kDa)	Experimental MW (kDa)	Polydispersity Index	Prediction
Solution	150	90	16	Monodisperse
Re-dissolved crystals	150	45	15	Monodisperse

[00125] Re-dissolved crystalline anti-huIL-23p19 mAb 13B8-b was also compared to the original solution antibody by ELISA. Two different lots of each of solution and re-dissolved crystalline anti-huIL-23p19 mAb 13B8-b were tested for their ability to bind to IL-23. Human IL-23 was immobilized onto an ELISA plate, and serial dilutions of anti-huIL-23p19 mAb 13B8-b (solution or re-dissolved crystalline) were added to the wells. After the plates were washed, bound antibody was detected using an HRP-labeled anti-IgG antibody and a colorimetric substrate. Plates were read in a plate reader and the dose response curves were analyzed using a four-parameter logistic fit. At equilibrium, the EC50 of a saturation binding curve reflects the equilibrium binding dissociation constant (K_D), where a higher value represents lower affinity, and a lower value represents higher affinity, of the antibody for the ligand. The potency of the test sample is assessed by comparing binding curves of the test samples to a reference material by the ratio of the EC50's. Potency is expressed as percent of reference material, in this case, selected to be lot #1 of the solution form of anti-huIL-23p19 mAb 13B8-b. Results, provided at Table 4, indicate that re-dissolved crystalline anti-huIL-23p19 mAb 13B8-b exhibits comparable binding affinity to the original solution material.

Table 4

Sample	EC50 (pM)	(std dev)	Relative potency (% Solution #1)
Solution #1	71	± 15	[100]
Solution #2	106	± 50	67
Re-dissolved Crystals #1	73	± 10	97
Re-Dissolved Crystals #2	116	± 52	61

Example 7

X-ray Diffraction Analysis of Anti-huIL-23p19 mAb 13B8-b Crystals

[00126] Anti-huIL-23p19 mAb 13B8-b crystals were characterized by X-ray diffraction as follows. Crystals were grown from a 60 mg/ml solution of anti-huIL-23p19 mAb 13B8-b in 10 mM sodium citrate (pH 5.5) by adding an equal volume of 20% PEG 400, 200 mM Li_2SO_4, 100 mM cacodylate (pH 6.4) as the precipitant solution. Crystallization was setup at 22°C using a hanging drop technique. Crystals were harvested and cryo-protected using and 30% PEG 400, 200 mM Li_2SO_4, 100 mM cacodylate (pH 6.4). X-ray diffraction data were collected using synchrotron radiation at ID-17 (Argonne National Laboratory, Argonne, Ill., USA), and then processed and scaled using the HKL-2000 macromolecular crystallography software package (HKL Research, Inc., Charlottesville, Virginia, USA). A complete dataset was collected with a maximum resolution of 3.5Å. Anti-huIL-23p19 mAb 13B8-b crystals belong to the I4 system with a = b = 191.8 Å, c = 105.9 Å (rounding to a = b = 192 Å and c = 106 Å), and alpha = beta = gamma = 90 degrees.

Example 8

Use of Seeding in Crystallization of Anti-huIL-23p19 mAb 13B8-b

[00127] Crystals of anti-huIL-23p19 mAb 13B8-b were prepared using a seeding protocol, as follows. A micro-bridge was inserted in each well of a VDX crystallization plate (Hampton Research, Aliso Viejo, Calif. USA). On each micro-bridge, 25 µl of a 60 mg/ml solution of anti-huIL-23p19 mAb 13B8-b in 10 mM sodium citrate (pH 5.5) was mixed with

an equal volume of 27% PEG 400, 0.1 M sodium citrate (pH 4.83), 0.2 M NaCl at 22°C. The mixture was then incubated for 18 hours at room temperature (22° C). After five days each experiment was streaked with a pipette tip. A photomicrograph of a representative drop after streaking is provided at FIG. 11A, and a photomicrograph of the final observed crystalline suspension before harvesting is provided at FIG. 11B. The crystalline suspension was harvested as described in Example 5 in 70% yield.

Example 9

Determining the Equilibrium Dissociation Constant (K_D) for Anti-human IL-23p19 mAbs Using KinExA Technology

[00128] Experiments to confirm that re-dissolved crystalline anti-huIL-23p19 mAb of the present invention retains binding affinity for human IL-23 may be performed as follows. The equilibrium dissociation constant (K_D) for re-dissolved crystalline anti human IL-23p19 antibodies, as well as solution anti-huIL-23p19 mAb (the starting material for making the crystalline antibody of the present invention), are determined using the KinExA 3000 instrument. Sapidyne Instruments Inc., Boise Idaho, USA. KinExA uses the principle of the Kinetic Exclusion Assay method based on measuring the concentration of uncomplexed antibody in a mixture of antibody, antigen and antibody-antigen complex. The concentration of free antibody is measured by exposing the mixture to a solid-phase immobilized antigen for a very brief period of time. In practice, this is accomplished by flowing the solution phase antigen-antibody mixture past antigen-coated particles trapped in a flow cell. Data generated by the instrument are analyzed using custom software. Equilibrium constants are calculated using a mathematical theory based on the following assumptions:

[00129] 1. The binding follows the reversible binding equation for equilibrium:

$$k_{on}\,[Ab]\,[Ag] = k_{off}[AbAg]$$

2. Antibody and antigen bind 1:1 and total antibody equals antigen-antibody complex plus free antibody.

3. Instrument signal is linearly related to free antibody concentration.

[00130] 98 micron PMMA particles (Sapidyne, Cat No. 440198) are coated with biotinylated IL-23 according to Sapidyne "Protocol for coating PMMA particles with biotinylated ligands having short or nonexistent linker arms". In one embodiment of the experiment, biotinylated IL-23 comprises a complex of mouse IL-12p40 and human IL-23p19. EZ-link TFP PEO-biotin (Pierce, Cat. No. 21219) is used to make biotinylated IL-23 according to manufacturer's recommendations (Pierce bulletin 0874). All experimental procedures are done according to the KinExA 3000 manual.

[00131] Binding of re-dissolved crystalline anti-huIL-23p19 antibodies is assessed in a competition binding assay, in which antibodies are pre-incubated with non-linked (native) human IL-23 comprising two disulfide-linked chains, human p19 (SEQ ID NO: 13) and human p40 (GenBank Accession No. P29460), at a series of concentrations. The resulting samples, comprising a mixture of unbound antibodies and IL-23-bound antibodies, are then flowed over the rhIL-23 ("elastikine") PMMA particles described in the preceding paragraph. The amount of antibody captured by the PMMA particles is then detected using a fluorescently labeled secondary antibody.

[00132] The resulting K_Ds for the starting material and the re-dissolved crystalline antibody are compared to determine whether the re-dissolved crystalline antibody retains sufficient binding affinity for human IL-23.

Example 10

Determining the Equilibrium Dissociation Constant (K_D) for Anti-human IL-23p19 mAbs Using BIAcore Technology

[00133] Additional experiments to confirm that re-dissolved crystalline anti-huIL-23p19 mAb of the present invention retains binding affinity for human IL-23 may be performed as follows. The equilibrium dissociation constant (K_D) for re-dissolved crystalline anti human IL-23 antibodies, as well as solution anti-huIL-23p19 mAb (the starting material for making the crystalline antibody of the present invention), are determined using surface plasmon resonance (SPR) methods, *e.g.* BIAcore technology. BIAcore Life Sciences, GE Healthcare Biosciences, Pittsburgh, Penna., USA. BIAcore determinations are performed essentially as described at Example 4 of commonly assigned U.S. Patent Application Publication No. 2007/0048315. Briefly, ligands (anti-huIL-23p19 mAbs) are immobilized on a BIAcore CM5 sensor chip using standard amine-coupling procedure. IL-23 is diluted in

PBS to produce various concentrations. Kinetic constants for the various interactions are determined using BIAevaluation software 3.1. The K_D is determined using the calculated dissociation and association rate constants.

[00134] The resulting K_Ds for the starting material and the re-dissolved crystalline antibody are compared to determine whether the re-dissolved crystalline antibody retains sufficient binding affinity for human IL-23.

Example 11

Proliferation Bioassays for the Assessment of Neutralizing Anti-huIL-23p19 Antibodies

[00135] Experiments to confirm that re-dissolved crystalline anti-huIL-23p19 mAb of the present invention retains the ability to neutralize human IL-23 may be performed by the application of short-term proliferation bioassays that employ cells that express recombinant IL-23 receptors, as follows. The IL-23R transfectant cell line (Ba/F3-2.2lo-hIL-23R) expresses both hIL-23R and hIL-12Rβ1, and is responsive to both human IL-23 and cynomolgus monkey IL-23. The transfectant Ba/F3-2.2lo cells proliferate in response to human IL-23 and the response can be inhibited by a neutralizing anti-huIL-23p19 antibody. An antibody is titrated against a concentration of IL-23 chosen within the linear region of the dose-response curve, near plateau and above EC50. Proliferation, or lack thereof, is measured by colorimetric means using Alamar Blue, a growth indicator dye based on detection of metabolic activity. The ability of an antibody to neutralize IL-23 is assessed by its IC50 value, *i.e.* the concentration of antibody that induces half-maximal inhibition of IL-23 proliferation.

[00136] Ba/F3 transfectants are maintained in RPMI-1640 medium, 10% fetal calf serum, 50 μM 2-mercaptoethanol, 2 mM L-Glutamine, 50 μg/ml penicillin-streptomycin, and 10 ng/ml mouse IL-3. Ba/F3 proliferation bioassays are performed in RPMI-1640 medium, 10% fetal calf serum, 50 μM 2-mercaptoethanol, 2 mM L-Glutamine, and 50 μg/ml penicillin-streptomycin.

Procedure

[00137] Assays are performed in 96-well flat bottom plates (Falcon 3072 or similar) in 150 μL per well. Anti-huIL-23p19 antibodies are pre-incubated with IL-23 for 30-60 min, followed by addition of cells and incubation for 40-48 hours. Alamar Blue (Biosource Cat #DAL1100) is added and allowed to develop for 5-12 hours. Absorbance is then read at 570

nm and 600 nm (VERSAmax Microplate Reader, Molecular Probes, Eugene, Oregon, USA), and an $OD_{570-600}$ is obtained.

[00138] Cells are used in a healthy growth state, generally at densities of 3-8 x 10^5/ml. Cells are counted, pelleted, washed twice in bioassay medium, and suspended to the appropriate density for plating. An IL-23 dose response is performed using serial 1:3 dilutions (25:50 µL in bioassay medium) of human IL-23. An IL-23 concentration of 3 ng/ml (50 pM) is selected for use in antibody assays. A neutralizing antibody dose response is also performed using serial 1:3 dilutions (25:50 µL in bioassay medium).

[00139] IC50 values are determined using GraphPad Prism® 3.0 software (Graphpad Software Inc., San Diego, California, USA), in which absorbance is plotted against cytokine or antibody concentration and IC50 values are determined using non-linear regression (curve fit) of sigmoidal dose-response.

[00140] The resulting IC50s for the starting material and the re-dissolved crystalline antibody are compared to determine whether the re-dissolved crystalline antibody retains the ability to neutralize human IL-23 at an acceptable level.

Example 12

Mouse Splenocyte Assay for IL-23 Based on IL-17 Production

[00141] The biological activity (neutralizing ability) of re-dissolved crystalline anti-huIL-23p19 antibodies of the present invention may also be assessed using the splenocyte assay essentially as described in Aggarwal *et al.* (2003) *J. Biol. Chem.* 278:1910 and Stumhofer *et al.* (2006) *Nature Immunol.* 7:937. The mouse splenocyte assay measures the activity of human IL-23 in a sample as a level of IL-17 production by murine splenocytes. The inhibitory activity of anti-huIL-23p19 antibodies is then assessed by determining the concentration of antibody necessary to reduce the IL-23 activity in a given sample by 50% (the IC50). The IC50 as measured by this assay is greater than or equal to the equilibrium dissociation binding constant (K_D), *i.e.* the K_D may be equal to or lower than the IC50. As always, lower IC50 and K_D values reflect higher activities and affinities.

[00142] Briefly, spleens are obtained from 8-12 wk old female C57BL/6J mice (Jackson Laboratories, Bar Harbor, Maine, USA). Spleens are ground, pelleted twice, and filtered through a cell strainer (70 µm nylon). The recovered cells are cultured in 96-well plates (4 X 10^5 cells/well) in the presence of human IL-23 (10 ng/ml, ~170 pM) and mouse-

anti-CD3e antibodies (1 μg/ml) (BD Pharmingen, Franklin Lakes, New Jersey, USA), with or without the anti-huIL-23p19 antibody to be assayed. Anti-huIL-23p19 antibodies (re-dissolved from crystals or from solution) are added at 10 μg/ml and at a series of 3-fold dilutions. Cells are cultured for 72 hours, pelleted, and the supernatant is assayed for IL-17 levels by sandwich ELISA.

[00143] IL-17 ELISA is performed as follows. Plates are coated with a capture anti-IL-17 antibody (100 ng/well) overnight at 4°C, washed and blocked. Samples and standards are added and incubated for two hours at room temperature with shaking. Plates are washed, and a biotinylated anti-IL-17 detection antibody (100 ng/well) is added and incubated for one hour at room temperature with shaking. The capture and detection antibodies are different antibodies that both bind to mouse IL-17 but do not cross-block. Plates are washed, and bound detection antibody is detected using streptavidin-HRP (horseradish peroxidase) and TMB (3,3',5,5'-tetramethylbenzidine). The plate is then read at 450-650 nm and the concentration of IL-17 in samples is calculated by comparison with standards. IC50s are calculated from the IL-17 levels.

[00144] The resulting IC50s for the starting material and the re-dissolved crystalline antibody are compared to determine whether the re-dissolved crystalline antibody retains the ability to neutralize human IL-23 at an acceptable level.

[00145] Table 5 provides a brief description of the sequences in the sequence listing.

Table 5

Sequence Identifiers

SEQ ID NO:	Description
1	13B8 HC-a
2	13B8 HC-b
3	13B8 HC-c
4	13B8 LC
5	13B8 CDRH1
6	13B8 CDRH2-a
7	13B8 CDRH2-b
8	13B8 CDRH2-c

SEQ ID NO:	Description
9	13B8 CDRH3
10	13B8 CDRL1
11	13B8 CDRL2
12	13B8 CDRL3
13	human IL-23p19
14	13B8-b HC DNA
15	13B8 LC DNA
16	ustekinumab CDRH1
17	ustekinumab CDRH2
18	ustekinumab CDRH3
19	ustekinumab CDRL1
20	ustekinumab CDRL2
21	ustekinumab CDRL3
22	ustekinumab V_H
23	ustekinumab V_L
24	ustekinumab HC
25	ustekinumab LC
26	briakinumab CDRH1
27	briakinumab CDRH2
28	briakinumab CDRH3
29	briakinumab CDRL1
30	briakinumab CDRL2
31	briakinumab CDRL3
32	briakinumab V_H
33	briakinumab V_L
34	briakinumab HC
35	briakinumab LC
36	guselkumab CDRH1
37	guselkumab CDRH2
38	guselkumab CDRH3

SEQ ID NO:	Description
39	guselkumab CDRL1
40	guselkumab CDRL2
41	guselkumab CDRL3
42	guselkumab V_H
43	guselkumab V_L
44	guselkumab HC
45	guselkumab LC

WHAT IS CLAIMED IS:

1. A crystalline anti-human IL-23 antibody.

2. The crystalline anti-human IL-23 antibody of Claim 1 wherein the antibody binds to the p19 subunit of IL-23.

3. The crystalline anti-human IL-23 antibody of either of the preceding claims comprising crystalline particles with an average particle size between five and 200 microns.

4. The crystalline anti-human IL-23 antibody of any of the preceding claims characterized by unit cell dimensions a = b = 192 Å, c = 106 Å, $\alpha = \beta = \gamma = 90°$ and in space group I4.

5. A suspension of the crystalline anti-human IL-23 antibody of Claim 1 or Claim 2 in which the antibody is at a concentration of at least 150 mg/ml.

6. The suspension of Claim 5 in which the viscosity of the suspension is less than about half the viscosity of a solution formulation of the same antibody at the same concentration.

7. The crystalline anti-human IL-23p19 antibody of Claim 2, comprising:

a) an antibody light chain variable domain, or antigen binding fragment thereof, comprising CDRL1, CDRL2 and CDRL3, wherein:

i) CDRL1 comprises the sequence of SEQ ID NO: 10;

ii) CDRL2 comprises the sequence of SEQ ID NO: 11; and

iii) CDRL3 comprises the sequence of SEQ ID NO: 12;

and

b) an antibody heavy chain variable domain, or antigen binding fragment thereof, comprising CDRH1, CDRH2 and CDRH3, wherein:

i) CDRH1 comprises the sequence of SEQ ID NO: 5;

ii) CDRH2 comprises a sequence selected from the group consisting of SEQ ID NOs: 6 – 8; and

iii) CDRH3 comprises the sequence of SEQ ID NO: 9.

8. The crystalline anti-human IL-23p19 antibody of Claim 7 wherein CDRH2 comprises the sequence of SEQ ID NO: 7.

9. The crystalline anti-human IL-23p19 antibody of Claim 7 comprising:

a) an antibody light chain variable domain comprising residues 1 – 108 of SEQ ID NO: 4; and

b) an antibody heavy chain variable domain comprising residues 1 – 116 of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3.

10. The crystalline anti-human IL-23p19 antibody of Claim 9 comprising:

a) an antibody light chain variable domain comprising residues 1 – 108 of SEQ ID NO: 4; and

b) an antibody heavy chain variable domain comprising residues 1 – 116 of SEQ ID NO: 2.

11. The crystalline anti-human IL-23p19 antibody of Claim 9 comprising an antibody light chain and an antibody heavy chain, wherein:

a) the antibody light chain comprises the sequence of SEQ ID NO: 4; and

b) the antibody heavy chain comprises the sequence of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3.

12. The crystalline anti-human IL-23p19 antibody of Claim 11 comprising an antibody light chain and an antibody heavy chain, wherein:

a) the antibody light chain comprises the sequence of SEQ ID NO: 4; and

b) the antibody heavy chain comprises the sequence of SEQ ID NO: 2.

13. The crystalline anti-human IL-23p19 antibody of Claim 2, comprising:

a) an antibody light chain variable domain, or antigen binding fragment thereof, comprising CDRL1, CDRL2 and CDRL3, wherein:

i) CDRL1 comprises the sequence of SEQ ID NO: 39;

ii) CDRL2 comprises the sequence of SEQ ID NO: 40; and

iii) CDRL3 comprises the sequence of SEQ ID NO: 41;

and

b) an antibody heavy chain variable domain, or antigen binding fragment thereof, comprising CDRH1, CDRH2 and CDRH3, wherein:

i) CDRH1 comprises the sequence of SEQ ID NO: 36;

ii) CDRH2 comprises the sequence of SEQ ID NO: 37; and

iii) CDRH3 comprises the sequence of SEQ ID NO: 38.

14. The crystalline anti-human IL-23 antibody of Claim 1, comprising:

a) an antibody light chain variable domain, or antigen binding fragment thereof, comprising CDRL1, CDRL2 and CDRL3, wherein:

i) CDRL1 comprises the sequence of SEQ ID NO: 19;

ii) CDRL2 comprises the sequence of SEQ ID NO: 20; and

iii) CDRL3 comprises the sequence of SEQ ID NO: 21;

and

b) an antibody heavy chain variable domain, or antigen binding fragment thereof, comprising CDRH1, CDRH2 and CDRH3, wherein:

i) CDRH1 comprises the sequence of SEQ ID NO: 16;

ii) CDRH2 comprises the sequence of SEQ ID NO: 17; and

iii) CDRH3 comprises the sequence of SEQ ID NO: 18.

15. The crystalline anti-human IL-23p19 antibody of Claim 7, Claim 13 or Claim 14, further comprising a heavy chain constant region comprising a γ1 human heavy chain constant region.

16. The crystalline anti-human IL-23p19 antibody of Claim 7, Claim 13 or Claim 14, further comprising a heavy chain constant region comprising a γ4 human heavy chain constant region.

17. A pharmaceutical composition comprising the crystalline anti-human IL-23 antibody of Claim 1 in combination with a pharmaceutically acceptable carrier or diluent.

18. A pharmaceutical composition of Claim 17 formulated for:

a) oral delivery;

b) pulmonary administration; or

c) needle-free injection.

19. Use of the crystalline anti-human IL-23 antibody of Claim 1 in the manufacture of a medicament.

20. The use of Claim 19 wherein the medicament is for treatment of an autoimmune or proliferative disorder.

21. The crystalline anti-human IL-23 antibody of Claim 1 for use as a medicament.

22. The crystalline anti-human IL-23 antibody of Claim 1 for use in the treatment of an inflammatory, autoimmune or proliferative disorder.

23. A method for treatment of a disorder in a subject, comprising administering to said subject an effective amount of the crystalline anti-human IL-23 antibody of Claim 1.

24. The use of Claim 19, crystalline antibody of Claim 21, or method of treatment of Claim 23, wherein the disorder is selected from the group consisting of arthritis, psoriasis, psoriatic arthritis, inflammatory bowel disease, ankylosing spondylitis and graft-versus-host disease.

25. The use of Claim 19, crystalline antibody of Claim 21, or method of treatment of Claim 23, wherein the disorder is selected from the group consisting of multiple sclerosis, systemic lupus erythematosus and diabetes.

26. A method of producing a crystalline anti-huIL-23 antibody, said method comprising:

a) mixing a solution of anti-human IL-23 antibody and a precipitant solution to obtain a crystallization solution;

b) incubating that crystallization solution for a period of time sufficient for crystal formation; and

c) harvesting the crystals from the crystallization solution.

27. The method of Claim 26 wherein the anti-human IL-23 antibody is at a concentration of 5 – 70 mg/ml prior to addition of the precipitant solution.

28. The method of Claim 26 wherein the anti-human IL-23 antibody is at a concentration of about 100 mg/ml prior to addition of the precipitant solution.

29. The method Claim 26 in which the crystallization solution comprises:

a) an anti-human IL-23p19 antibody;

b) a buffer (pH 4.0 – 8.0);

c) 5 – 70% (v/v) 1, 2 propanediol; and

d) PEG 300 or 400.

30. The method of Claim 29 wherein the buffer is citric acid or acetic acid (pH 4.0 – 7.0).

31. The method of Claim 29 wherein the buffer is HEPES or BisTris (pH 5.8 – 8.0).

32. The method of Claim 29 wherein the incubation is performed at 10 – 40° C.

33. The method Claim 26 in which the crystallization solution comprises:

a) an anti-human IL-23p19 antibody;

b) a buffer (pH 4.0 – 6.0);

c) 2 – 5% (wt/v) sucrose;

c) 0.1 – 0.5% (v/v) polysorbate 80; and

d) PEG 300 or 400.

34. The method of Claim 33 wherein the buffer is sodium citrate at pH 4.8 – 5.1.

35. The method of Claim 33 wherein the sucrose is present at about 3.5% in the crystallization solution.

36. The method of Claim 33 wherein the polysorbate 80 is present at about 0.125% (wt/v) in the crystallization solution.

37. The method of Claim 33 wherein the PEG 300 or PEG 400 is present at about 25 - 30% in the crystallization solution.

38. The method of Claim 33 wherein the incubation is performed at about 22° C.

39. A method of producing a crystalline anti-huIL-23 antibody, said method comprising:

a) dialyzing a solution of anti-human IL-23 antibody against a dialysis solution for a period of time sufficient for crystal formation; and

b) harvesting the crystals from the retentate.

40. The method of Claim 39 further comprising seeding the solution with a crystal of anti-human IL-23p19 antibody during step (a).

41. The method of Claim 39 further comprising agitating the solution during step (b).

42. The method of Claim 39 further comprising ramping the temperature of the solution from 10° C to 40° C during step (a).

43. A method of purifying an anti-human IL-23 antibody comprising crystallizing by the method of any of Claims 26 – 42, and re-dissolving the crystalline anti-huIL-23 antibody prior to use.

44. A crystalline anti-human IL-23 antibody prepared by the method of any of Claims 26 – 42.

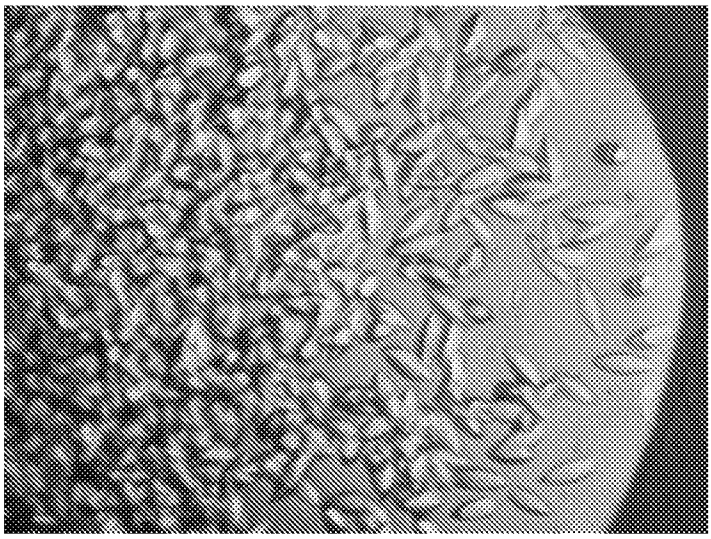


FIG. 1

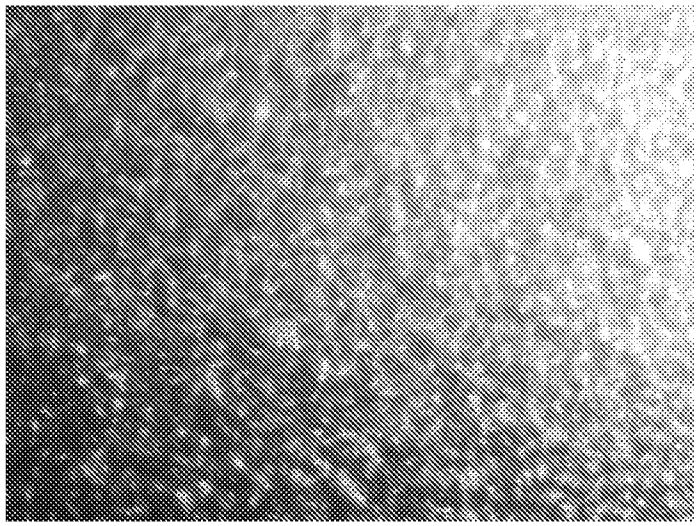


FIG. 2

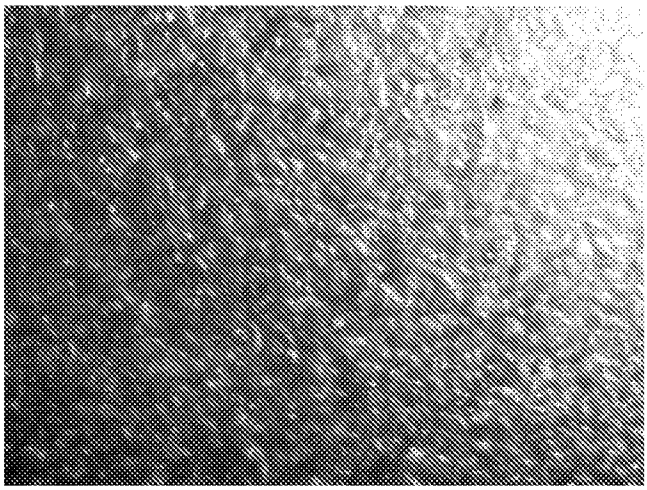


FIG. 3

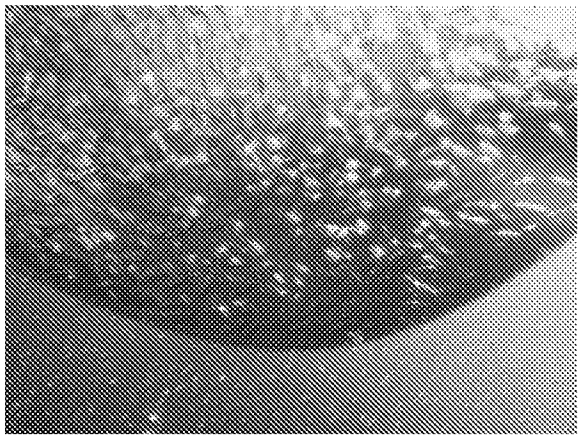


FIG. 4

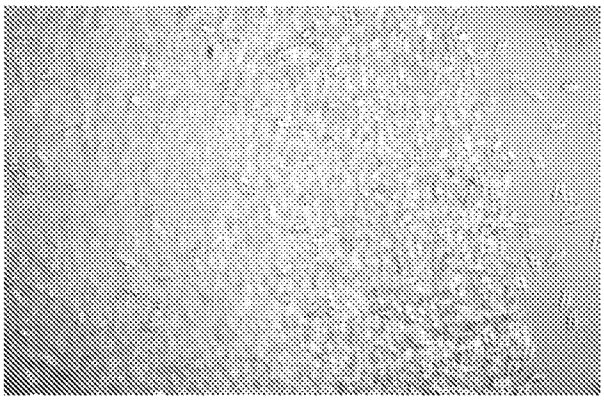


FIG. 5

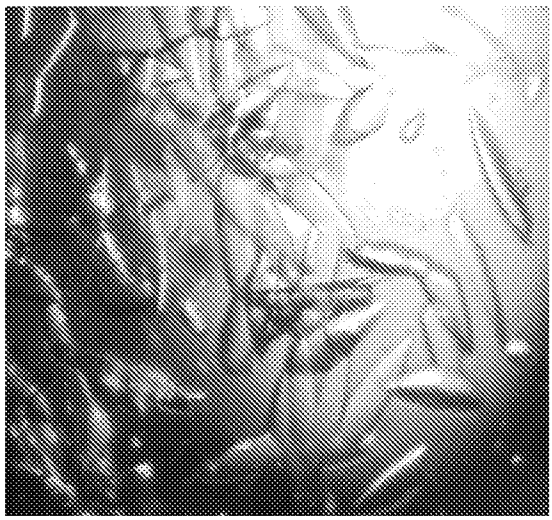


FIG. 6

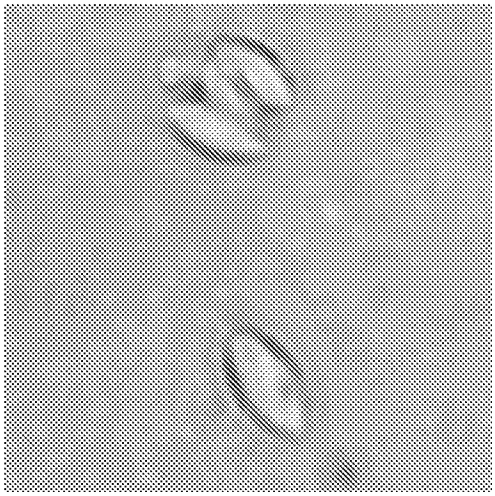


FIG. 7

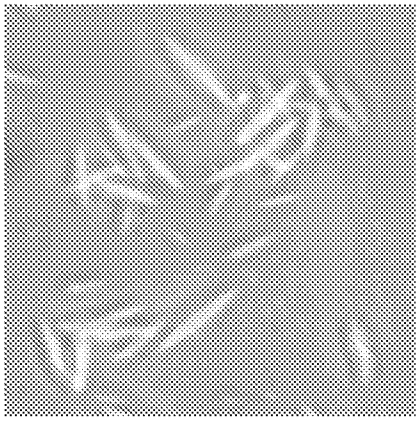


FIG. 8

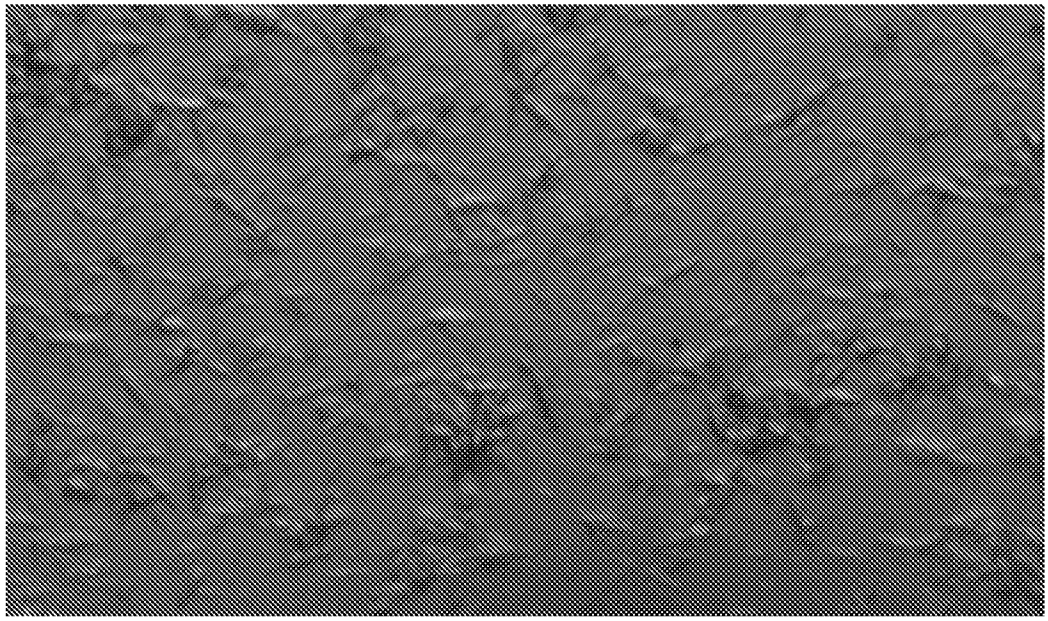
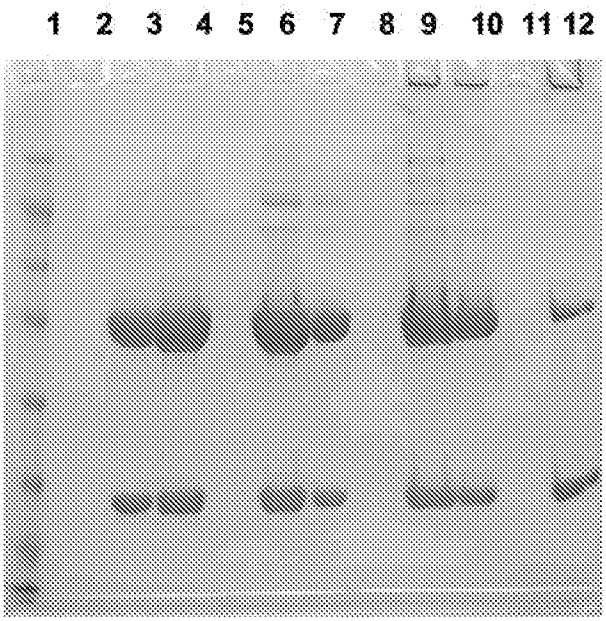


FIG. 9



Legend

well	sample (load)
1	Std
3	Anti-IL23 mAB 13B8 (10 ug)
4	Anti-IL23 mAB 13B8 (20 ug)
6	Re-dissolved crystals (20 ug)
7	Re-dissolved crystals (10 ug)
9	Mother liquor (20 ug)
10	Mother liquor (10 ug)
12	Wash

FIG. 10

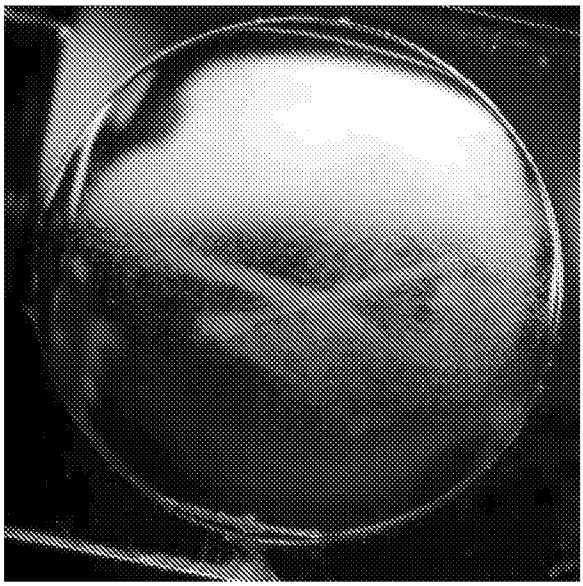


FIG. 11A

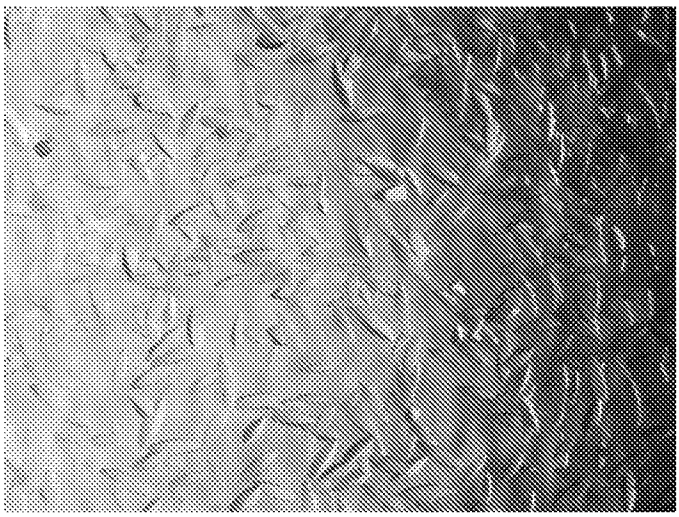


FIG. 11B