Claims

1. A batch crystallization method for crystallizing an anti-hTNFalpha antibody, comprising the steps of:
   (a) providing an aqueous solution of said antibody in admixture with an inorganic phosphate salt as crystallization agent; and
   (b) incubating said aqueous crystallization mixture until crystals of said antibody are formed.

2. The crystallization method according to one of the preceding claims, wherein the pH of said aqueous crystallization mixture is in the range of about pH 3 to 5.

3. The crystallization method according to one of the preceding claims, wherein said aqueous crystallization mixture contains a buffer.

4. The crystallization method according to claim 3, wherein said buffer comprises an acetate buffer.

5. The crystallization method according to claim 4, wherein the buffer comprises sodium acetate.

6. The crystallization method according to one of the claim 3 to 5, wherein the buffer concentration in said aqueous crystallization mixture is 0 M to 0.5 M.

7. The crystallization method according to one of the preceding claims, wherein the phosphate salt is a hydrogenphosphate salt.

8. The crystallization method according to claim 7, wherein the phosphate salt is an alkali metal salt, or a mixture of at least two different alkali metal salts.

9. The crystallization method according to one of the preceding claims, wherein the phosphate salt concentration in the crystallization mixture is in the range of about 1 to 6 M.

10. The crystallization method according to claim 9, wherein the salt concentration in the crystallization mixture is in the range of about 1.0 to 3.0 M.

11. The crystallization method of one of the preceding claims, wherein at least one of the following additional crystallization conditions are met:

a) incubation is performed for between about 1 hour to about 60 days;

b) incubation is performed at a temperature between about 4 ^{O}C and about 37 ^{O}C;

c) the antibody concentration is in the range of about 0.5 to about 100 mg/ml.

12. The crystallization method according to one of the preceding claims, further comprising the step of drying said crystals.

13. The crystallization method according to one of the preceding claims, further comprising the step of exchanging the crystallization mother liquor with a different buffer.

14. The crystallization method according to one of the preceding claims, wherein the batch volume is in the range of about 1 ml to 20.000 l.

15. A crystal of an anti-hTNFalpha antibody, obtainable by a crystallization method as defined in anyone of the preceding claims.

16. A crystal of an anti-hTNFalpha antibody, with the proviso that said antibody is not INFLIXIMAB.

17. The crystal of anyone of the claims 15 and 16 having a needle-like morphology with a maximum length l of about 2 – 500 µm and an l/d ratio of about 3 to 30.

18. The crystal according to anyone of claims 15 to 17, wherein said antibody is a polyclonal antibody or a monoclonal antibody.

19. The crystal according to claim 18, wherein said antibody is selected from the group consisting of: chimeric antibodies, humanized antibodies, non-glycosylated antibodies, human antibodies and mouse antibodies.

20. The crystal according to anyone of claims 17 or 18, wherein said antibody is an IgG antibody.

21. The crystal according to claim 19, wherein said antibody is selected from the group consisting of: IgG1, IgG2, IgG3 and IgG4, antibodies.

22. The crystal according to claim 21, wherein said antibody is an anti-humanTNFalpha antibody of the group IgG1.

23. The crystal according to claim 22, prepared from an isolated human antibody, that dissociates from hTNFalpha with a Kd of 1 $x10^{-8}$ M or less and a Ka rate constant of 1 x 10^{-3} s^{-1} or less, both determined by surface plasmon resonance, and neutralizes hTNFalpha cytotoxicity in a standard in vitro L929 assay with an IC_{50} of 1 $x10^{-7}$ M or less.

24. The crystal according to claim 22, prepared from an isolated human antibody with the following characteristics: a) dissociates from human TNFalpha with a K_{off} rate constant of 1 x 10^{-3} s^{-1} or less, as determined by surface plasmon resonance; b) has a light chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 3, or modified from SEQ ID NO: 3 by a single alanine substitution at position 1, 4, 5, 7 or 8 or by one to five conservative amino acid substitutions at positions 1, 3, 4, 6, 7, 8 and/or 9; c) has a heavy chain CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4, or modified from SEQ ID NO: 4 by a single alanine substitution at position 2, 3, 4, 5, 6, 8, 9, 10 or 11 or by one to five conservative amino acid substitutions at positions 2, 3, 4, 5, 6, 8, 9, 10, 11 and/or 12.

25. The crystal according to claim 22, prepared from an isolated human antibody with a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 1 and a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 2.

26. The crystal according to claim 25, prepared from the antibody D2E7.

27. A pharmaceutical composition comprising: (a) crystals of an anti-hTNFalpha antibody as defined in anyone of claims 15 to 26, and (b) at least one pharmaceutical excipient; which composition is provided in as solid, semisolid or liquid formulation, each formulation containing said antibody in crystalline form.

28. A pharmaceutical composition comprising: (a) crystals of an anti-hTNFalpha antibody as defined in anyone of claims 15 to 26, and (b) at least one pharmaceutical excipient, which embeds or encapsulates crystals of said antibody.

29. The composition of claim 26, wherein said composition has an antibody concentration greater than about 1 mg/ml.

30. The composition of claim 27, wherein said composition has an antibody concentration greater than about 200 mg/ml.

31. The composition according to anyone of the claims 26 to 28, wherein said excipient comprises at least one polymeric optionally biodegradable carrier or at least one oil or lipid carrier.

32. The composition according to claim 29, wherein said polymeric carrier is a polymer selected from one or more of the group consisting of: poly (acrylic acid), poly (cyanoacrylates), poly (amino acids), poly (anhydrides), poly (depsipeptide), poly (esters), poly (lactic acid), poly (lactic-co-glycolic acid) or PLGA, poly (ß-hydroxybutryate), poly (caprolactone), poly (dioxanone); poly (ethylene glycol), poly (hydroxypropyl) methacrylamide, poly[(organo) phosphazene, poly (ortho esters), poly (vinyl alcohol), poly (vinylpyrrolidone), maleic anhydride alkyl vinyl ether copolymers, pluronic polyols, albumin, alginate, cellulose and cellulose derivatives, collagen, fibrin, gelatin, hyaluronic acid, oligosaccharides, glycaminoglycans, sulfated polysaccharides, blends and copolymers thereof.

33. An injectable liquid composition comprising anti-hTNFalpha antibody crystals as defined in anyone of claims 15 to 26 and having an antibody concentration in the range of about 10 to 400 mg/ml.

34. A crystal slurry comprising anti-hTNFalpha antibody crystals as defined in anyone of claims 15 to 26, having a antibody concentration greater than about 100 mg/ml.

35. A method for treating a mammal comprising the step of administering to the mammal an effective amount of antibody anti-hTNFalpha crystals as defined in anyone of claims 15 to 26.

36. A method for treating a mammal comprising the step of administering to the mammal an effective amount of the composition according to anyone of the claims 27 to 32.

37. The method according to anyone of the claims 34 and 35, wherein the composition is administered by parenteral route, oral route, or by injection.

38. A method of treating a TNFalpha-related disorder in a subject, which method comprises administering a therapeutically effective amount of antibody crystals as defined in anyone of claims 15 to 26.

39. The method of claim 37, wherein said TNFalpha- related disorder is selected from the group consisting of

an autoimmune disease, in particular rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis and gouty arthritis, an allergy, multiple sclerosis, autoimmune diabetes, autoimmune uveitis and nephrotic syndrome; an infectious disease, transplant rejection or graft-versus-host disease, malignancy, pulmonary disorder, intestinal disorder, cardiac disorder, inflammatory bone disorders, bone resorption disease, alcoholic hepatitis, viral hepatitis, fulminant hepatitis, coagulation disturbances, burns, reperfusion injury, keloid formation, scar tissue formation, pyrexia, periodontal disease, obesity and radiation toxicity; a spondyloarthropathy, a pulmonary disorder, a coronary disorder, a metabolic disorder, anemia, pain, a hepatic disorder, a skin disorder, a nail disorder, or vasculitis, Behcet's disease, ankylosing spondylitis, asthma, chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis(IPF), restenosis, diabetes, anemia, pain, a Crohn's disease-related disorder, juvenile rheumatoid arthritis (JRA), a hepatitis C virus infection, psoriasis, psoriatic arthritis, and chronic plaque psoriasis, age-related cachexia, Alzheimer's disease, brain edema, inflammatory brain injury, chronic fatigue syndrome, dermatomyositis, drug reactions, edema in and/or around the spinal cord, familial periodic fevers, Felty's syndrome, fibrosis,glomerulonephritides (e.g. post-streptococcal glomerulonephritis or IgA nephropathy), loosening of prostheses, microscopic polyangiitis, mixed connective tissue disorder, multiple myeloma, cancer and cachexia, multiple organ disorder, myelo dysplastic syndrome, orchitism osteolysis, pancreatitis, including acute, chronic, and pancreatic abscess, periodontal disease polymyositis, progressive renal failure, pseudogout, pyoderma gangrenosum, relapsing polychondritis, rheumatic heart disease, sarcoidosis, sclerosing cholangitis, stroke, thoracoabdominal aortic aneurysm repair(TAAA), TNF receptor associated periodic syndrome (TRAPS), symptoms related to Yellow Fever vaccination, inflammatory diseases associated with the ear, chronic ear inflammation, or pediatric ear inflammation, uveitis, sciatica, prostatitis, endometriosis, choroidal neovascularization, lupus, Sjogren's syndrome, and wet macular degeneration.

40. The use of anti-hTNFalpha antibody crystals as defined in anyone of claims 15 to 26 for preparing a pharmaceutical composition for treating a TNFalpha-related disease as defined in claim 38.

41. Anti-hTNFalpha antibody crystals as defined in anyone of claims 15 to 26 for use in medicine.

CLAIMS:

1. A crystal of an IgG human anti-hTNFalpha antibody comprising two heavy chains each with a molecular weight of about 50 kDa and comprising a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 1, and a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 2, wherein said crystal has a needle morphology with a length of about 2-500 µm and an l/d ratio of about 3 to 30.

2. The crystal according to claim 1, wherein said antibody is selected from the group consisting of: IgG1, IgG2, IgG3 and IgG4 antibodies.

3. The crystal according to claim 1, wherein said antibody is an IgG1 antibody.

4. The crystal according to claim 1, wherein the antibody is adalimumab (D2E7).

5. A pharmaceutical composition comprising: (a) crystals of an anti-hTNFalpha antibody according to claim 1, and (b) at least one pharmaceutical excipient; wherein the composition is provided as a solid, semisolid or liquid formulation, each formulation containing said antibody in crystalline form.

6. A pharmaceutical composition comprising: (a) crystals of an anti-hTNFalpha antibody according to claim 1, and (b) at least one pharmaceutical excipient, which embeds or encapsulates crystals of said antibody.

7. The composition according to claim 5, wherein said composition has an antibody concentration greater than about 1 mg/ml.

8. The composition according to claim 5, wherein said composition has an antibody concentration greater than about 200 mg/ml.

9. The composition according to claim 5, wherein said excipient comprises at least one polymeric optionally biodegradable carrier or at least one oil or lipid carrier.

10. The composition according to claim 9, wherein said polymeric carrier is a polymer selected from one or more of the group consisting of: poly (acrylic acid), poly (cyanoacrylates), poly (amino acids), poly (anhydrides), poly (depsipeptide), poly (esters), poly (lactic acid), poly (lactic-co-glycolic acid) or PLGA, poly (β-hydroxybutryate), poly (caprolactone), poly (dioxanone); poly (ethylene glycol), poly (hydroxypropyl) methacrylamide, poly (organo) phosphazene, poly (ortho esters), poly (vinyl alcohol), poly (vinylpyrrolidone), maleic anhydride alkyl vinyl ether copolymers, pluronic polyols, albumin, alginate, cellulose and cellulose derivatives, collagen, fibrin, gelatin, hyaluronic acid, oligosaccharides, glycaminoglycans, sulfated polysaccharides, blends and copolymers thereof.

11. An injectable liquid composition comprising anti-hTNFalpha antibody crystals according to claim 1 and having an antibody concentration in the range of about 10 to 400 mg/ml.

12. A crystal slurry comprising anti-hTNFalpha antibody crystals according to claim 1, having an antibody concentration greater than about 100 mg/ml.

13. A batch crystallization method for crystallizing an anti-hTNFalpha antibody, comprising the steps of:

(a) providing an aqueous solution of said antibody in admixture with an inorganic phosphate salt as a crystallization agent to obtain an aqueous crystallization mixture, wherein the aqueous crystallization mixture has a pH of about 3 to about 5; and

(b) incubating said aqueous crystallization mixture until crystals of said antibody are formed,

wherein the anti-hTNFalpha antibody is an IgG human anti-hTNFalpha antibody comprising a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 1, and a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 2, and wherein said crystal has a needle morphology with a length of about 2-500 µm and an l/d ratio of about 3 to 30.

14. The crystallization method according to claim 13, wherein said aqueous crystallization mixture contains a buffer.

15. The crystallization method according to claim 14, wherein said buffer comprises an acetate buffer.

16. The crystallization method according to claim 15, wherein the buffer comprises sodium acetate.

17. The crystallization method according to claim 14, wherein the buffer concentration in said aqueous crystallization mixture is about 0 M to about 0.5 M.

18. The crystallization method according to claim 15, wherein the buffer concentration in said aqueous crystallization mixture is about 0 M to about 0.5 M.

19. The crystallization method according to claim 16, wherein the buffer concentration in said aqueous crystallization mixture is about 0 M to about 0.5 M.

20. The crystallization method according to claim 13, wherein the phosphate salt is a hydrogenphosphate salt.

21. The crystallization method according to claim 13, wherein the phosphate salt is an alkali metal salt, or a mixture of at least two different alkali metal salts.

22. The crystallization method according to claim 13, wherein the phosphate salt concentration in the crystallization mixture is in the range of about 1 to 6 M.

23. The crystallization method according to claim 22, wherein the salt concentration in the crystallization mixture is in the range of about 1.0 to 3.0 M.

24. The crystallization method according to claim 13, wherein at least one of the following additional crystallization conditions are met:
a) incubation is performed for between about 1 hour to about 60 days;
b) incubation is performed at a temperature between about 4°C. and about 37°C;
c) the antibody concentration is in the range of about 0.5 to about 100 mg/ml.

25. The crystallization method according to claim 24, further comprising the step of drying said crystals.

26. The crystallization method according to claim 24, further comprising the step of exchanging a crystallization mother liquor with a different buffer.

27. The crystallization method according to claim 24, wherein the batch comprises a batch volume in the range of about 1 ml to about 20,000 liters.