Title: ANTIBODIES AGAINST INTERLEUKIN-10 LIKE CYTOKINES AND USES THEREOF

Abstract: Specific binding fragments, antibodies, and antigen-binding fragments thereof that bind IL-10-like cytokines, including human interleukin-22 (IL-22) are provided. Methods of making and using such binding fragments, antibodies, and antigen-binding fragments are also provided. Kits containing such binding fragments, antibodies, and antigen-binding fragments are also provided.

FIG. 1
ANTIBODIES AGAINST INTERLEUKIN-10-LIKE CYTOKINES
AND USES THEREFOR

CROSS-REFERENCE TO RELATED APPLICATIONS

[001] This application claims the benefit of, and relies on the filing date of, U.S. provisional patent application number 61/064,718, filed 21 March 2008, the entire disclosure of which is hereby incorporated herein by reference.

FIELD

[002] Specific binding fragments, antibodies, and antigen-binding fragments thereof that bind interleukin-22 (IL-22) are provided. The specific binding fragments, antibodies, and antigen-binding fragments disclosed herein are useful in diagnosing, preventing, and/or treating IL-22-associated disorders, including, e.g., autoimmune disorders. Methods for determining which amino acids of the human IL-22 protein sequence are important in the binding of IL-22 to its cell surface receptor complex and to its binding protein are also provided, along with the resulting epitope defined by the method.

BACKGROUND

[003] Interleukin 22 (IL-22) is a member of the Interleukin 10 (IL-10) -like subgroup of type II cytokines. (Renauld, J.-C. Nature Reviews Immunology 3, 667-76 (2003)). The members of this subgroup (i.e., IL-10, IL-19, IL-20, IL-22, IL-24, and IL-26) are proposed to have a conserved six α-helical structural and functional unit that is also shared with the interferons (Renauld et al. Nature Reviews Immunology 3, 667-76 (2003) and Langer et al. Cytokine & Growth Factor Reviews 15, 33-48 (2004)). IL-22 is produced by activated T helper (Th) 17 CD4+ lymphocytes, as well as monocytes, and its expression is highly dependent on IL-23 (Liang, S.C. et al. Journal of Experimental Medicine 203, 2271-9 (2006) and Zheng, Y. et al. Nature 445, 648-51 (2007)). IL-22 is known to regulate local tissue inflammation while acting only on non-immune cells, and plays a critical role in mucosal immunity as well as dysregulated inflammation observed in autoimmune disease. (Wolk, K. et al. Immunity 21, 241-54 (2004); Wolk et al., Cytokine & Growth Factor Reviews 17, 367-80 (2006); Wolk et al. Journal of Immunology 168, 5397-402 (2002); Pan et al. Cellular & Molecular Immunology 1, 43-9

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Recent clinical and pre-clinical studies strongly implicate Th17 cell and IL-22 activities in the progression of psoriasis, a human autoimmune disease of the skin (Zheng et al. *Nature* 445, 648-51 (2007); Nickoloff et al. *Nature Medicine* 13, 242-244 (2007); Zaba et al. *Journal of Experimental Medicine* 204, 3183-94 (2007); Ma et al. *Journal of Clinical Investigation* in press(2008); Lowes et al. *Nature* 445, 866-73 (2007); and Wolk et al. *European Journal of Immunology* 36, 1309-23 (2006). Administration of IL-22 has been shown to induce the hyperproliferation of skin keratinocytes and resultant thickening of the epidermis, both characteristics of psoriatic lesions (Boniface et al. *Journal of Immunology* 174, 3695-702 (2005)). In addition, the administration of IL-22 has been shown to induce gene expression from keratinocytes that appear to be involved in the recruitment of immune cells and the maintenance of psoriatic tissue inflammation (Wolk et al. *European Journal of Immunology* 36, 1309-23 (2006); Boniface et al. *Journal of Immunology* 174, 3695-702 (2005); and Sa et al. *Journal of Immunology* 178, 2229-40 (2007) [erratum appears in J Immunol. 2007 Jun 1;178(1 1):7487]).

[004] Expression of IL-22 is up-regulated in T cells by IL-9 or ConA (Dumoutier L. et al. (2000) *Proc Nail Acad Sci USA* 97(18):10144-9). Further studies have shown that expression of IL-22 mRNA is induced *in vivo* in response to LPS administration, and that IL-22 modulates parameters indicative of an acute phase response (Dumoutier L. et al. (2000) supra; Pittman D. et al. (2001) *Genes and Immunity* 2:172). Taken together, these observations indicate that IL-22 plays a critical role in inflammation (Kotenko S.V. (2002) *Cytokine & Growth Factor Reviews* 13(3):223-40).

[005] At low concentrations, IL-22 exits as a monomer in solution, sharing a six α-helical structural and functional monomeric unit with the intercalated IL-10 dimer, as well as other IL-10-like cytokines and IFN-γ (Nagem et al. *Vitamins & Hormones* 74, 77-103 (2006); Logsdon et al. *Journal of Interferon & Cytokine Research* 22, 1099-112 (2002); Nagem et al. *Structure* 10, 1051-62 (2002); and Chang et al. *Journal of Biological Chemistry* 278, 3308-13 (2003)). The six defined helices of IL-22 are known as helices A, B, C, D, E and F. The portion of the IL-22 protein that connects helix A with helix B is known as loop AB. Glycosylated IL-22, expressed from insect cells, can
exist and is proposed to function as a monomer (Logsdon, NJ., Jones, B.C., Josephson, K., Cook, J. & Walter, M.R. *Journal of Interferon & Cytokine Research* 22, 1099-112 (2002). A recent report indicates that E co/δ-derived IL-22 dimerizes at high concentrations, via a proposed interaction between its DE loops, and is able to associate with the IL-22R receptor subunit (de Oliveira Neto, M. et al *Biophys J.* 94, 1754-65 (2008)). The resultant low resolution quaternary structure is similar to that observed between intercalated IL-10 dimers and it's high affinity receptor, IL-10R1 (Josephson, K., Logsdon, NJ. & Walter, M.R. *Immunity* 15, 35-46 (2001)).

[006] The cell surface receptor for IL-22 is believed to be a receptor complex consisting of an IL-22 receptor (IL-22R) and an IL-22 receptor 2 (IL-10R2) subunit, each of which is a member of the type II cytokine receptor family (CRF2) (Xie M.H. et al. (2000) *J Biol Chem* 275(40):1335-9; Kotenko S.V. et al. (2001) *J Biol Chem* 276(4):2725-32). CRF2 members are receptors for IFNα/β, IFNγ, coagulation factor Vila, IL-10 and the IL-10 related proteins IL-19, IL-20, IL-22, IL-24, IL-26, as well as the recently identified IFN-like cytokines, IL-28 and IL-29 (Kotenko S.V. (2002) *Cytokine & Growth Factor Reviews* 13(3):223-40; Kotenko, S.V. et al. (2000) *Oncogene* 19(21):2557-65; Sheppard, P. et al. (2003) *Nature Immunology* 4(l):63-8; Kotenko, S.V. et al. (2003) *Nature Immunology* 4(l):69-77). Each of the subunits, or chains, of the IL-22 receptor complex presents on epithelial cells and some fibroblasts within various tissues (Wolk et al. *Journal of Immunology* 168, 5397-402 (2002); Xie et al. *Journal of Biological Chemistry* 275, 31335-9 (2000); Kotenko et al. *Journal of Biological Chemistry* 276, 2725-32 (2001); Ikeuchi et al. *Arthritis & Rheumatism* 52, 1037-46 (2005); Andoh et al. *Gastroenterology* 129, 969-84 (2005)). Both chains of the IL-22 receptor complex are also expressed constitutively in a number of organs, and epithelial cell lines derived from these organs have been shown to be responsive to IL-22 in vitro (Kotenko S.V. (2002) *Cytokine & Growth Factor Reviews* 13(3):223-40.

[007] While the IL-22R and IL-10R2 subunits individually contribute to the formation of different receptor complexes for other type II cytokines, together the subunits form a single receptor complex that is specific for IL-22. IL-22 is believed to first bind to the extracellular domain (ECD) of IL-22R (Logsdon et al. *Journal of Interferon & Cytokine Research* 22, 1099-112 (2002) and Li et al. *International

The interaction between IL-22R and IL-10R2 has been characterized in an ELISA based format using biotinylated cytokine and receptor extracellular domain (ECD) Fc fusion dimers. See, e.g., U.S. Published Patent Application No. 2005-0042220. IL-22 was shown to have measurable affinity for the ECD of IL-22R and no detectable affinity for IL-10R2 alone. IL-22 was also shown to have a substantially greater affinity for IL-22R/IL-10R2 ECD presented as Fc heterodimers. IL-10R2 appears to bind to a surface created by the association between IL-22 and IL-22R, suggesting that IL-10R2 ECD further stabilizes the association of IL-22 within its cytokine receptor complex. See, e.g., U.S. Published Patent Application No. 2005-0042220.

In addition to binding to the IL-22 receptor complex, IL-22 also binds to an IL-22 binding protein (IL-22BP), which is a secreted ‘receptor’ specific for IL-22 and has 33% primary sequence identity to the extracellular domain (ECD) of IL-22R (Dumoutier, L., Lejeune, D., Colau, D. & Renauld, J.C. Cloning and characterization of IL-22 binding protein, a natural antagonist of IL-10-related T cell-derived inducible factor/IL-22. Journal of Immunology 166, 7090-5 (2001)). While a cell surface form of IL-22BP has not been specifically identified, in vitro, IL-22BP has been shown to act as a decoy receptor and block IL-22 signaling into the cell (Dumoutier et al. Journal of...
Neutralizing anti-IL-22 antibodies have been generated and characterized in terms of their binding specificity, affinity and IL-22 neutralizing activity. See, e.g., U.S. Published Patent Application No. 2005-0042220. Administration of IL-22 in vivo has been shown to induce parameters of an acute phase response, and the administration of a neutralizing anti-IL-22 antibody has been shown to reduce IL-22 activity and ameliorates inflammatory symptoms in a mouse collagen-induced arthritis (CIA) model. See, e.g., U.S. Published Patent Application No. 2005-0042220. In addition, the expression of IL-22 mRNA has been shown to be upregulated within inflamed areas. Accordingly, IL-22 antagonists, such as, e.g., neutralizing anti-IL-22 antibodies and fragments thereof, can be used to induce immune suppression in vivo and they provide a promising approach to the treatment of various inflammatory and/or autoimmune disorders. Thus, to aid in generating and evaluating neutralizing anti-IL-22 antibodies, it would be beneficial to understand which IL-22 amino acids play an important role in the interaction between the IL-22 protein and its receptor complex and/or between the IL-22 protein and its binding protein.

One focused mutagenesis study performed by Logsdon et al. demonstrated that six amino acids in IL-22 helices A and D, including a glycosylated asparagine, are involved in binding to IL-10R2 (Logsdon et al. *Journal of Molecular Biology* 342, 503-14 (2004)). The relevance of helices A and D peptides for IL-10R2 binding was subsequently substantiated (Wolk et al. *Genes & Immunity* 6, 8-18 (2005)). Residues within helices A and F, and loop AB of IL-22 have been proposed to be important for binding to a receptor based on superimposition of IL-22 structure to cytokine within IL-10/IL-10R1E Fv and IFN-γ/IFN-γRIECD co-crystal structures, as well as inferences from an IL-22/IL-22RIECD model (Logsdon et al. *Journal of Interferon & Cytokine Research* 22, 1099-112 (2002) and Nagem et al. *Structure* 10, 1051-62 (2002)). However prior to the current invention, the IL-22 side chains that are important for binding to IL-22R and IL-22BP had not been determined. The present invention provides for systematic mutagenesis of human IL-22 to reveal specific amino acids that are involved in binding of IL-22 to IL-22R, IL-10R2, and IL-22BP, and provides methods for
designing and making inhibitors of IL-22 activity, as well as inhibitors of IL-19, IL-20, IL-24 and IL-26 activity.

**SUMMARY**

[012] In certain embodiments, an IL-22 specific binding agent that binds to the wild-type human IL-22 but fails to bind to a mutant IL-22 is provided. In certain embodiments, the mutant IL-22 comprises one or more of the following changes relative to wild-type human IL-22: a) the amino acid at position 34 of the mutant IL-22 is alanine; b) the amino acid at position 52 of the mutant IL-22 is alanine; c) the amino acid at position 61 of the mutant IL-22 is alanine; d) the amino acid at position 83 of the mutant IL-22 is alanine; e) the amino acid at position 88 of the mutant IL-22 is alanine; f) the amino acid at position 113 of the mutant IL-22 is alanine; i) the amino acid at position 121 of the mutant IL-22 is alanine; j) the amino acid at position 122 of the mutant IL-22 is alanine; k) the amino acid at position 125 of the mutant IL-22 is alanine; and l) the amino acid at position 172 of the mutant IL-22 is alanine. In certain embodiments, the mutant IL-22 further comprises one or more of the following changes relative to wild-type human IL-22: a) the amino acid at position 51 of the mutant IL-22 is alanine; b) the amino acid at position 54 of the mutant IL-22 is alanine; c) the amino acid at position 55 of the mutant IL-22 is alanine; d) the amino acid at position 114 of the mutant IL-22 is alanine; or e) the amino acid at position 117 of the mutant IL-22 is alanine. In certain embodiments, the mutant IL-22 further comprises one or more of the following changes relative to wild-type human IL-22: a) the amino acid at position 57 of the mutant IL-22 is alanine; b) the amino acid at position 59 of the mutant IL-22 is alanine; c) the amino acid at position 67 of the mutant IL-22 is alanine; d) the amino acid at position 72 of the mutant IL-22 is alanine; e) the amino acid at position 159 of the mutant IL-22 is alanine; f) the amino acid at position 161 of the mutant IL-22 is alanine; g) the amino acid at position 162 of the mutant IL-22 is alanine; or h) the amino acid at position 169 of the mutant IL-22 is alanine. In certain embodiments, the mutant IL-22 further comprises one or more of the following changes relative to wild-type human IL-22: a) the amino acid at position 70 of the mutant IL-22 is alanine; b) the amino acid at position 71 of the mutant IL-22 is alanine; c) the amino acid...
at position 73 of the mutant IL-22 is alanine; or d) the amino acid at position 165 of the mutant IL-22 is alanine. In certain embodiments, the IL-22 specific binding agent is an antibody.

[013] In certain embodiments, an IL-22 specific binding agent that binds to the wild-type human IL-22 but fails to bind to a mutant IL-22 is provided. In certain embodiments, the mutant IL-22 comprises one or more of the following changes relative to wild-type human IL-22: a) the amino acid at position 57 of the mutant IL-22 is alanine; b) the amino acid at position 59 of the mutant IL-22 is alanine; c) the amino acid at position 67 of the mutant IL-22 is alanine; d) the amino acid at position 72 of the mutant IL-22 is alanine; e) the amino acid at position 159 of the mutant IL-22 is alanine; f) the amino acid at position 161 of the mutant IL-22 is alanine; g) the amino acid at position 162 of the mutant IL-22 is alanine; or h) the amino acid at position 169 of the mutant IL-22 is alanine. In certain embodiments, the mutant IL-22 further comprises one or more of the following changes relative to wild-type human IL-22: a) the amino acid at position 70 of the mutant IL-22 is alanine; b) the amino acid at position 71 of the mutant IL-22 is alanine; c) the amino acid at position 73 of the mutant IL-22 is alanine; or d) the amino acid at position 165 of the mutant IL-22 is alanine. In certain embodiments, the IL-22 specific binding agent is an antibody.

[014] In certain embodiments, an IL-22 specific binding agent that binds to the wild-type human IL-22 but fails to bind to a mutant IL-22 is provided. In certain embodiments, the mutant IL-22 comprises one or more of the following changes relative to wild-type human IL-22: a) the amino acid at position 67 of the mutant IL-22 is alanine; b) the amino acid at position 73 of the mutant IL-22 is alanine; c) the amino acid at position 83 of the mutant IL-22 is alanine; or d) the amino acid at position 162 of the mutant IL-22 is alanine. In certain embodiments, the IL-22 specific binding agent is an antibody.

[015] In certain embodiments, a method of selecting a specific binding agent to an IL-22 polypeptide is provided. In certain embodiments, the specific binding agent binds to at least a portion of an epitope on an IL-22 polypeptide. In certain embodiments, an IL-22 polypeptide is contacted with an agent. In certain embodiments, the affinity of the agent for the IL-22 polypeptide is determined. In certain embodiments, a mutant IL-
22 polypeptide is contacted with the agent, wherein the mutant IL-22 polypeptide comprises at least one point mutation at at least one amino acid position selected from A34, 152, T56, K61, A66, V83, R88, P1 13, F121, L122, L125, or M172. In certain embodiments, the affinity of the agent for the mutant IL-22 polypeptide is determined. In certain embodiments, the agent is selected if the affinity for the IL-22 polypeptide is greater than the affinity for the mutant IL-22 polypeptide. In certain embodiments, the mutant IL-22 polypeptide is the same as the IL-22 polypeptide except for the at least one mutation. In certain embodiments, the agent is selected from a protein, peptide, nucleic acid, carbohydrate, lipid, and small molecule compound. In certain embodiments, the agent is an antibody. In certain embodiments, the agent is a small molecule compound. In certain embodiments, the amount of binding to the IL-22 polypeptide is at least 2-fold greater than the amount of binding to the mutant IL-22 polypeptide. In certain embodiments, the affinity for the IL-22 polypeptide is at least 5-fold greater than the affinity for the mutant IL-22 polypeptide. In certain embodiments, the affinity for the IL-22 polypeptide is at least 10-fold greater than the affinity for the mutant IL-22 polypeptide. In certain embodiments, the mutant IL-22 polypeptide comprises at least one point mutation selected from Y51, N54, R55, Y1 14, or E117. In certain embodiments, the mutant IL-22 polypeptide comprises at least one point mutation selected from F57, L59, D67, T70, D71, V72, R73, G159, 1161, K162, G165, or L169. In certain embodiments, the mutant IL-22 polypeptide comprises at least one point mutation selected from D67, R73, and K162. In certain embodiments, the IL-22 specific binding agent is an antibody.

[016] In certain embodiments, a method of selecting a specific binding agent to an IL-22 polypeptide is provided. In certain embodiments, the specific binding agent binds to at least a portion of an epitope on an IL-22 polypeptide. In certain embodiments, an IL-22 polypeptide is contacted with an agent. In certain embodiments, the affinity of the agent for the IL-22 polypeptide is determined. In certain embodiments, a mutant IL-22 polypeptide is contacted with the agent, wherein the mutant IL-22 polypeptide comprises at least one point mutation at at least one amino acid position selected from F57, L59, D67, V72, G159, 1161, K162, or L169. In certain embodiments, the affinity of the agent for the mutant IL-22 polypeptide is determined. In certain embodiments, the
agent is selected if the affinity for the IL-22 polypeptide is greater than the affinity for the mutant IL-22 polypeptide. In certain embodiments, the mutant IL-22 polypeptide is the same as the IL-22 polypeptide except for the at least one mutation. In certain embodiments, the agent is selected from a protein, peptide, nucleic acid, carbohydrate, lipid, and small molecule compound. In certain embodiments, the agent is an antibody. In certain embodiments, the agent is a small molecule compound. In certain embodiments, the amount of binding to the IL-22 polypeptide is at least 2-fold greater than the amount of binding to the mutant IL-22 polypeptide. In certain embodiments, the affinity for the IL-22 polypeptide is at least 5-fold greater than the affinity for the mutant IL-22 polypeptide. In certain embodiments, the affinity for the IL-22 polypeptide is at least 10-fold greater than the affinity for the mutant IL-22 polypeptide. In certain embodiments, the mutant IL-22 polypeptide comprises at least one point mutation selected from T70, D71, R73, or G165. In certain embodiments, the mutant IL-22 polypeptide comprises at least one point mutation selected from A34, Y51, 152, N54, R55, T56, K61, A66, V83, R88, P113, Y114, E117, F121, L122, L125, or M172. In certain embodiments, the mutant IL-22 polypeptide comprises at least one point mutation selected from R73 or V83.

[017] In certain embodiments, a method of selecting a specific binding agent to an IL-22 polypeptide is provided. In certain embodiments, the specific binding agent binds to at least a portion of an epitope on an IL-22 polypeptide. In certain embodiments, an IL-22 polypeptide is contacted with an agent. In certain embodiments, the affinity of the agent for the IL-22 polypeptide is determined. In certain embodiments, a mutant IL-22 polypeptide is contacted with the agent, wherein the mutant IL-22 polypeptide comprises at least one point mutation at least one amino acid position selected from D67, R73, V83, or K162. In certain embodiments, the affinity of the agent for the mutant IL-22 polypeptide is determined. In certain embodiments, the agent is selected if the affinity for the IL-22 polypeptide is greater than the affinity for the mutant IL-22 polypeptide. In certain embodiments, the mutant IL-22 polypeptide is the same as the IL-22 polypeptide except for the at least one mutation. In certain embodiments, the agent is selected from a protein, peptide, nucleic acid, carbohydrate, lipid, and small molecule compound. In certain embodiments, the agent is an antibody. In certain embodiments,
the agent is a small molecule compound. In certain embodiments, the amount of binding to the IL-22 polypeptide is at least 2-fold greater than the amount of binding to the mutant IL-22 polypeptide. In certain embodiments, the affinity for the IL-22 polypeptide is at least 5-fold greater than the affinity for the mutant IL-22 polypeptide. In certain embodiments, the affinity for the IL-22 polypeptide is at least 10-fold greater than the affinity for the mutant IL-22 polypeptide. In certain embodiments, the mutant IL-22 polypeptide comprises at least one point mutation selected from A34, Y51, 152, N54, R55, T56, K61, A66, R88, P113, Y114, E117, F121, L122, L125, or M172. In certain embodiments, the mutant IL-22 polypeptide comprises at least one point mutation selected from F57, L59, T70, D71, V72, G159, 1161, G165, or L169.

[018] In certain embodiments, a pharmaceutical composition comprising an IL-22 specific binding agent is provided. In certain embodiments, the pharmaceutical composition further comprising a cytokine inhibitor, a growth factor inhibitor, an immunosuppressant, an anti-inflammatory agent, a metabolic inhibitor, an enzyme inhibitor, a cytotoxic agent, or a cytostatic agent. In certain embodiments, the pharmaceutical composition further comprises at least one of a TNF antagonist, an IL-12 antagonist, an IL-15 antagonist, an IL-17 antagonist, an IL-18 antagonist, an IL-21R antagonist, a T cell depleting agent, a B cell depleting agent, methotrexate, leflunomide, sirolimus (rapamycin) or an analog thereof, a Cox-2 inhibitor, a cPLA2 inhibitor, an NSAID, or ap38 inhibitor.

[019] In certain embodiments, a method of treating or preventing an IL22-associated disorder, in a subject is provided, comprising, administering to the subject an IL-22 specific binding agent, in an amount sufficient to treat or prevent the IL22-associated disorder. In certain embodiments, the IL22-associated disorder is an autoimmune disorder, a respiratory disorder, or an inflammatory condition. In certain embodiments, the IL22-associated disorder is rheumatoid arthritis, osteoarthritis, multiple sclerosis, myasthenia gravis, Crohn's disease, inflammatory bowel disease, lupus, diabetes, psoriasis, asthma, chronic obstructive pulmonary disease (COPD), cardiovascular inflammation, pancreatitis, hepatitis or nephritis. In certain embodiments, the method of treating or preventing an IL-22 associated disorder further comprises administering to the subject a cytokine inhibitor, a growth factor inhibitor, an
immunosuppressant, an anti-inflammatory agent, a metabolic inhibitor, an enzyme inhibitor, a cytotoxic agent, or a cytostatic agent. In certain embodiments, the method of treating or preventing an IL-22 associated disorder further comprises administering to the subject at least one of a TNF antagonist, an IL-12 antagonist, an IL-15 antagonist, an IL-17 antagonist, an IL-18 antagonist, an IL-21R antagonist, a T cell depleting agent, a B cell depleting agent, methotrexate, leflunomide, sirolimus (rapamycin) or an analog thereof, a Cox-2 inhibitor, a cPLA2 inhibitor, an NSAID, or a p38 inhibitor.

[020] In certain embodiments, an IL-19 specific binding agent that binds to the wild-type human IL-19 but fails to bind to a mutant IL-19 is provided. In certain embodiments, the mutant IL-19 comprises one or more of the following changes relative to wild-type human IL-19: a) the amino acid at position 36 of the mutant IL-19 is alanine; b) the amino acid at position 37 of the mutant IL-19 is alanine; c) the amino acid at position 39 of the mutant IL-19 is alanine; d) the amino acid at position 40 of the mutant IL-19 is alanine; e) the amino acid at position 41 of the mutant IL-19 is alanine; f) the amino acid at position 42 of the mutant IL-19 is alanine; g) the amino acid at position 44 of the mutant IL-19 is alanine; h) the amino acid at position 46 of the mutant IL-19 is alanine; i) the amino acid at position 51 of the mutant IL-19 is alanine; j) the amino acid at position 52 of the mutant IL-19 is alanine; k) the amino acid at position 55 of the mutant IL-19 is alanine; l) the amino acid at position 56 of the mutant IL-19 is alanine; m) the amino acid at position 57 of the mutant IL-19 is alanine; n) the amino acid at position 58 of the mutant IL-19 is alanine; o) the amino acid at position 68 of the mutant IL-19 is alanine; p) the amino acid at position 74 of the mutant IL-19 is alanine; q) the amino acid at position 102 of the mutant IL-19 is alanine; r) the amino acid at position 103 of the mutant IL-19 is alanine; s) the amino acid at position 106 of the mutant IL-19 is alanine; t) the amino acid at position 110 of the mutant IL-19 is alanine; u) the amino acid at position 111 of the mutant IL-19 is alanine; v) the amino acid at position 114 of the mutant IL-19 is alanine; w) the amino acid at position 152 of the mutant IL-19 is alanine; x) the amino acid at position 154 of the mutant IL-19 is alanine; y) the amino acid at position 155 of the mutant IL-19 is alanine; z) the amino acid at position 158 of the mutant IL-19 is alanine; aa) the amino acid at position 162 of the mutant IL-19 is alanine;
or bb) the amino acid at position 165 of the mutant IL-19 is alanine. In certain embodiments, the IL-19 specific binding agent is an antibody.

[021] In certain embodiments, a method of selecting a specific binding agent to an IL-19 polypeptide is provided. In certain embodiments, the specific binding agent binds to at least a portion of an epitope on an IL-19 polypeptide. In certain embodiments, an IL-19 polypeptide is contacted with an agent. In certain embodiments, determining the affinity of the agent for the IL-19 polypeptide is determined. In certain embodiments, a mutant IL-19 polypeptide is contacted with the agent. In certain embodiments, the mutant IL-19 polypeptide comprises at least one point mutation at at least one amino acid position selected from H36, 137, E39, S40, F41, Q42, 144, R46, K51, D52, P55, N56, V57, T58, 168, V74, R102, K103, S106, S110, F111, M114, A152, 1541, 155K, G158, V162, or A165. In certain embodiments, the affinity of the agent for the mutant IL-19 polypeptide is determined. In certain embodiments, the agent is selected if the affinity for the IL-19 polypeptide is greater than the affinity for the mutant IL-19 polypeptide. In certain embodiments, the mutant IL-19 polypeptide is the same as the IL-19 polypeptide except for the at least one mutation. In certain embodiments, the agent is selected from a protein, peptide, nucleic acid, carbohydrate, lipid, and small molecule compound. In certain embodiments, the agent is an antibody. In certain embodiments, the agent is a small molecule compound. In certain embodiments, the amount of binding to the IL-19 polypeptide is at least 2-fold greater than the amount of binding to the mutant IL-19 polypeptide. In certain embodiments, the affinity for the IL-19 polypeptide is at least 5-fold greater than the affinity for the mutant IL-19 polypeptide. In certain embodiments, the affinity for the IL-19 polypeptide is at least 10-fold greater than the affinity for the mutant IL-19 polypeptide.

[022] In certain embodiments, a pharmaceutical composition comprising an IL-19 specific binding agent is provided. In certain embodiments, a method of treating or preventing an IL-19-associated disorder, in a subject is provided, comprising, administering to the subject an IL-19 specific binding agent, in an amount sufficient to treat or prevent the IL-19-associated disorder.

[023] In certain embodiments, an IL-20 specific binding agent that binds to the wild-type human IL-20 but fails to bind to a mutant IL-20 is provided. In certain
embodiments, the mutant IL-20 comprises one or more of the following changes relative
to wild-type human IL-20: a) the amino acid at position 41 of the mutant IL-20 is alanine;
b) the amino acid at position 42 of the mutant IL-20 is alanine; c) the amino acid at
position 44 of the mutant IL-20 is alanine; d) the amino acid at position 45 of the mutant
IL-20 is alanine; e) the amino acid at position 46 of the mutant IL-20 is alanine; f) the
amino acid at position 47 of the mutant IL-20 is alanine; g) the amino acid at position 49
of the mutant IL-20 is alanine; h) the amino acid at position 51 of the mutant IL-20 is
alanine; i) the amino acid at position 56 of the mutant IL-20 is alanine; j) the amino acid
at position 57 of the mutant IL-20 is alanine; k) the amino acid at position 60 of the
mutant IL-20 is alanine; l) the amino acid at position 61 of the mutant IL-20 is alanine m)
the amino acid at position 62 of the mutant IL-20 is alanine; n) the amino acid at position
63 of the mutant IL-20 is alanine; o) the amino acid at position 73 of the mutant IL-20 is
alanine; p) the amino acid at position 79 of the mutant IL-20 is alanine; q) the amino acid
at position 107 of the mutant IL-20 is alanine; r) the amino acid at position 108 of the
mutant IL-20 is alanine; s) the amino acid at position 111 of the mutant IL-20 is alanine;
t) the amino acid at position 115 of the mutant IL-20 is alanine; u) the amino acid at
position 116 of the mutant IL-20 is alanine; v) the amino acid at position 119 of the
mutant IL-20 is alanine; w) the amino acid at position 157 of the mutant IL-20 is alanine;
x) the amino acid at position 159 of the mutant IL-20 is alanine; y) the amino acid at
position 160 of the mutant IL-20 is alanine; z) the amino acid at position 163 of the
mutant IL-20 is alanine; aa) the amino acid at position 170 of the mutant IL-20 is alanine;
or bb) the amino acid at position 173 of the mutant IL-20 is alanine. In certain
embodiments, the IL-20 specific binding agent is an antibody.

[024] In certain embodiments, a method of selecting a specific binding agent to
an IL-20 polypeptide is provided. In certain embodiments, the specific binding agent
binds to at least a portion of an epitope on an IL-20 polypeptide. In certain embodiments,
an IL-20 polypeptide is contacted with an agent. In certain embodiments, determining
the affinity of the agent for the IL-20 polypeptide is determined. In certain embodiments,
a mutant IL-20 polypeptide is contacted with the agent. In certain embodiments, the
mutant IL-20 polypeptide comprises at least one point mutation at at least one amino acid
position selected from E41, 142, N44, G45, F46, S47, 149, G51, D57, 160, D61, 162, R63,
164. L65, D73, R79, R107, K108, S111, S115, F116, 1119, A157, V159, K160, G163, 1170, or Q173. In certain embodiments, the affinity of the agent for the mutant IL-20 polypeptide is determined. In certain embodiments, the agent is selected if the affinity for the IL-20 polypeptide is greater than the affinity for the mutant IL-20 polypeptide. In certain embodiments, the mutant IL-20 polypeptide is the same as the IL-20 polypeptide except for the at least one mutation. In certain embodiments, the agent is selected from a protein, peptide, nucleic acid, carbohydrate, lipid, and small molecule compound. In certain embodiments, the agent is an antibody. In certain embodiments, the agent is a small molecule compound. In certain embodiments, the amount of binding to the IL-20 polypeptide is at least 2-fold greater than the amount of binding to the mutant IL-20 polypeptide. In certain embodiments, the affinity for the IL-20 polypeptide is at least 5-fold greater than the affinity for the mutant IL-20 polypeptide. In certain embodiments, the affinity for the IL-20 polypeptide is at least 10-fold greater than the affinity for the mutant IL-20 polypeptide.

[025] In certain embodiments, a pharmaceutical composition comprising an IL-20 specific binding agent is provided. In certain embodiments, a method of treating or preventing an IL-20-associated disorder, in a subject is provided, comprising, administering to the subject an IL-20 specific binding agent, in an amount sufficient to treat or prevent the IL-20-associated disorder.

[026] In certain embodiments, an IL-24 specific binding agent that binds to the wild-type human IL-24 but fails to bind to a mutant IL-24 is provided. In certain embodiments, the mutant IL-24 comprises one or more of the following changes relative to wild-type human IL-24: a) the amino acid at position 68 of the mutant IL-24 is alanine; b) the amino acid at position 69 of the mutant IL-24 is alanine; c) the amino acid at position 71 of the mutant IL-24 is alanine; d) the amino acid at position 72 of the mutant IL-24 is alanine; e) the amino acid at position 73 of the mutant IL-24 is alanine; f) the amino acid at position 74 of the mutant IL-24 is alanine; g) the amino acid at position 76 of the mutant IL-24 is alanine; h) the amino acid at position 78 of the mutant IL-24 is alanine; i) the amino acid at position 83 of the mutant IL-24 is alanine; j) the amino acid at position 84 of the mutant IL-24 is alanine; k) the amino acid at position 87 of the mutant IL-24 is alanine; l) the amino acid at position 88 of the mutant IL-24 is alanine;
m) the amino acid at position 89 of the mutant IL-24 is alanine; n) the amino acid at position 90 of the mutant IL-24 is alanine; o) the amino acid at position 100 of the mutant IL-24 is alanine; p) the amino acid at position 105 of the mutant IL-24 is alanine; q) the amino acid at position 135 of the mutant IL-24 is alanine; r) the amino acid at position 136 of the mutant IL-24 is alanine; s) the amino acid at position 139 of the mutant IL-24 is alanine; t) the amino acid at position 143 of the mutant IL-24 is alanine; u) the amino acid at position 144 of the mutant IL-24 is alanine; v) the amino acid at position 147 of the mutant IL-24 is alanine; w) the amino acid at position 185 of the mutant IL-24 is alanine; x) the amino acid at position 187 of the mutant IL-24 is alanine; y) the amino acid at position 188 of the mutant IL-24 is alanine; z) the amino acid at position 191 of the mutant IL-24 is alanine; aa) the amino acid at position 195 of the mutant IL-24 is alanine; or bb) the amino acid at position 198 of the mutant IL-24 is alanine. In certain embodiments, the IL-24 specific binding agent is an antibody.

[027] In certain embodiments, a method of selecting a specific binding agent to an IL-24 polypeptide is provided. In certain embodiments, the specific binding agent binds to at least a portion of an epitope on an IL-24 polypeptide. In certain embodiments, an IL-24 polypeptide is contacted with an agent. In certain embodiments, determining the affinity of the agent for the IL-24 polypeptide is determined. In certain embodiments, a mutant IL-24 polypeptide is contacted with the agent. In certain embodiments, the mutant IL-24 polypeptide comprises at least one point mutation at at least one amino acid position selected from K68, L69, E71, A72, F73, W74, V76, D78, Q83, D84, T87, S88, A89, R90, VIOO, S105, K135, S136, T139, N143, F144, 1147, A185, T187, K188, G191, 1195, or T198. In certain embodiments, the affinity of the agent for the mutant IL-24 polypeptide is determined. In certain embodiments, the agent is selected if the affinity for the IL-24 polypeptide is greater than the affinity for the mutant IL-24 polypeptide. In certain embodiments, the mutant IL-24 polypeptide is the same as the IL-24 polypeptide except for the at least one mutation. In certain embodiments, the agent is selected from a protein, peptide, nucleic acid, carbohydrate, lipid, and small molecule compound. In certain embodiments, the agent is an antibody. In certain embodiments, the agent is a small molecule compound. In certain embodiments, the amount of binding to the IL-24 polypeptide is at least 2-fold greater than the amount of binding to the mutant IL-24
polypeptide. In certain embodiments, the affinity for the IL-24 polypeptide is at least 5-fold greater than the affinity for the mutant IL-24 polypeptide. In certain embodiments, the affinity for the IL-24 polypeptide is at least 10-fold greater than the affinity for the mutant IL-24 polypeptide.

[028] In certain embodiments, a pharmaceutical composition comprising an IL-24 specific binding agent is provided. In certain embodiments, a method of treating or preventing an IL-24-associated disorder, in a subject is provided, comprising, administering to the subject an IL-24 specific binding agent, in an amount sufficient to treat or prevent the IL-24-associated disorder.

[029] In certain embodiments, an IL-26 specific binding agent that binds to the wild-type human IL-26 but fails to bind to a mutant IL-26 is provided. In certain embodiments, the mutant IL-26 comprises one or more of the following changes relative to wild-type human IL-26: a) the amino acid at position 40 of the mutant IL-26 is alanine; b) the amino acid at position 41 of the mutant IL-26 is alanine; c) the amino acid at position 43 of the mutant IL-26 is alanine; d) the amino acid at position 44 of the mutant IL-26 is alanine; e) the amino acid at position 45 of the mutant IL-26 is alanine; f) the amino acid at position 46 of the mutant IL-26 is alanine; g) the amino acid at position 48 of the mutant IL-26 is alanine; h) the amino acid at position 50 of the mutant IL-26 is alanine; i) the amino acid at position 55 of the mutant IL-26 is alanine; j) the amino acid at position 59 of the mutant IL-26 is alanine; k) the amino acid at position 61 of the mutant IL-26 is alanine; l) the amino acid at position 62 of the mutant IL-26 is alanine; m) the amino acid at position 63 of the mutant IL-26 is alanine; n) the amino acid at position 64 of the mutant IL-26 is alanine; o) the amino acid at position 75 of the mutant IL-26 is alanine; p) the amino acid at position 78 of the mutant IL-26 is alanine; q) the amino acid at position 106 of the mutant IL-26 is alanine; r) the amino acid at position 107 of the mutant IL-26 is alanine; s) the amino acid at position 110 of the mutant IL-26 is alanine; t) the amino acid at position 114 of the mutant IL-26 is alanine; u) the amino acid at position 115 of the mutant IL-26 is alanine; v) the amino acid at position 118 of the mutant IL-26 is alanine; w) the amino acid at position 148 of the mutant IL-26 is alanine; x) the amino acid at position 150 of the mutant IL-26 is alanine; y) the amino acid at position 151 of the mutant IL-26 is alanine; z) the amino acid at position 154 of
the mutant IL-26 is alanine; aa) the amino acid at position 158 of the mutant IL-26 is alanine; or bb) the amino acid at position 161 of the mutant IL-26 is alanine. In certain embodiments, the IL-26 specific binding agent is an antibody.

[030] In certain embodiments, a method of selecting a specific binding agent to an IL-26 polypeptide is provided. In certain embodiments, the specific binding agent binds to at least a portion of an epitope on an IL-26 polypeptide. In certain embodiments, an IL-26 polypeptide is contacted with an agent. In certain embodiments, determining the affinity of the agent for the IL-26 polypeptide is determined. In certain embodiments, a mutant IL-26 polypeptide is contacted with the agent. In certain embodiments, the mutant IL-26 polypeptide comprises at least one point mutation at at least one amino acid position selected from Q40, A41, D43, A44, L45, Y46, K48, A50, T55, D59, 161, K62, N63, 164, F75, N78, R106, F107, D110, L114, R115, L118, G148, Y150, K151, S154, 1158, or S161. In certain embodiments, the affinity of the agent for the mutant IL-26 polypeptide is determined. In certain embodiments, the agent is selected if the affinity for the IL-26 polypeptide is greater than the affinity for the mutant IL-26 polypeptide. In certain embodiments, the mutant IL-26 polypeptide is the same as the IL-26 polypeptide except for the at least one mutation. In certain embodiments, the agent is selected from a protein, peptide, nucleic acid, carbohydrate, lipid, and small molecule compound. In certain embodiments, the agent is an antibody. In certain embodiments, the agent is a small molecule compound. In certain embodiments, the amount of binding to the IL-26 polypeptide is at least 2-fold greater than the amount of binding to the mutant IL-26 polypeptide. In certain embodiments, the affinity for the IL-26 polypeptide is at least 5-fold greater than the affinity for the mutant IL-26 polypeptide. In certain embodiments, the affinity for the IL-26 polypeptide is at least 10-fold greater than the affinity for the mutant IL-26 polypeptide.

[031] In certain embodiments, a pharmaceutical composition comprising an IL-26 specific binding agent is provided. In certain embodiments, a method of treating or preventing an IL-26-associated disorder, in a subject is provided, comprising, administering to the subject an IL-26 specific binding agent, in an amount sufficient to treat or prevent the IL-26-associated disorder.
In certain embodiments, a method of systematic mutagenesis of a target protein is provided. In certain embodiments, a series often or more different nucleic acid molecules is generated. In certain embodiments, each different nucleic acid molecule comprises a nucleic acid sequence that encodes a polypeptide comprising a different mutant of the target protein. In certain embodiments, each polypeptide comprising a different mutant further comprises a secretory sequence; a first tag, and a second tag. In certain embodiments, each of the ten or more different nucleic acid molecules is introduced into a different group of cells, wherein each different group of cells is in a separate well comprising liquid media. In certain embodiments, ten or more different mutants of the target protein are expressed in the separate wells; wherein the ten or more different mutants of the target protein are secreted into the liquid media of the separate wells. In certain embodiments, the ten or more different mutants of the target protein are quantitated in the liquid media in the separate wells using the first tag and the second tag. In certain embodiments, the ten or more different mutants of the target protein are subjected to at least one assay. In certain embodiments, the cells are eukaryotic cells. In certain embodiments, the introducing each of the ten or more different nucleic acid molecules into a different group of cells comprises transfecting each of the ten or more different nucleic acid molecules into a different group of cells. In certain embodiments, the cells are bacterial cells. In certain embodiments, each amino acid of the target protein is mutated in a different mutant target protein. In certain embodiments, each amino acid of the target protein that is not alanine is mutated to alanine in a different mutant target protein. In certain embodiments, the first tag is a six histidine tag and the second tag is a FLAG tag.

In certain embodiments, a peptidomimetic that mimics an epitope comprising two or more of the following amino acids of IL-22: A34, 152, T56, K61, A66, V83, R88, P113, F121, L122, L125, or M172, is provided. In certain embodiments, the peptidomimetic mimics an epitope further comprising one or more of the following amino acids of IL-22: Y51, N54, R55, Y114, or E117. In certain embodiments, the peptidomimetic mimics an epitope further comprising one or more of the following amino acids of IL-22: F57, L59, D67, T70, D71, V72, R73, G159, 1161, K162, G165, or
L169. In certain embodiments, the peptidomimetic mimics an epitope further comprising one or more of the following amino acids of IL-22: D67, R73, or K162.

[034] In certain embodiments, a peptidomimetic that mimics an epitope comprising two or more of the following amino acids of IL-22: F57, L59, D67, V72, G159, 1161, K162, or L169, is provided. In certain embodiments, the peptidomimetic mimics an epitope further comprising one or more of the following amino acids of IL-22: T70, D71, R73, or G165. In certain embodiments, the peptidomimetic mimics an epitope further comprising one or more of the following amino acids of IL-22: A34, Y51, 152, N54, R55, T56, K61, A66, V83, R13, Y14, E17, F121, L122, L125, or M172. In certain embodiments, the peptidomimetic mimics an epitope further comprising one or more of the following amino acids of IL-22: R73 or V83.

[035] In certain embodiments, a peptidomimetic that mimics an epitope comprising two or more of the following amino acids of IL-22: D67, R73, V83, or K162, is provided. In certain embodiments, the peptidomimetic mimics an epitope further comprising one or more of the following amino acids of IL-22: A34, Y51, 152, N54, R55, T56, K61, A66, V83, R13, Y14, E17, F121, L122, L125, or M172. In certain embodiments, the peptidomimetic mimics an epitope further comprising one or more of the following amino acids of IL-22: F57, L59, T70, D71, V72, G159, 1161, G165, or L169.

[036] In certain embodiments, a peptidomimetic that mimics an epitope comprising two or more of the following amino acids of IL-19: H36, 137, E39, S40, F41, Q42, 144, R46, K51, D52, P55, N56, V57, T58, 168, V74, R102, K103, S106, S110, F111, M114, A152, 1541, 155K, G158, V162, or A165, is provided.

[037] In certain embodiments, a peptidomimetic that mimics an epitope comprising two or more of the following amino acids of IL-20: E41, 142, N44, G45, F46, S47, 149, G51, D57, 160, D61, 162, R63, 164, L65, D73, R79, R107, K108, S111, S115, F116, 1119, 157A, V159, K160, G163, 1170, or Q173 is provided.

[038] In certain embodiments, a peptidomimetic that mimics an epitope comprising two or more of the following amino acids of IL-24: K68, L69, E71, A72, F73, W74, V76, D78, Q83, D84, T87, S88, A89, R90, V100, S105, K135, S136, T139, N143, F144, 1147, A185, T187, K188, G191, 1195, or T198, is provided.
In certain embodiments, a peptidomimetic that mimics an epitope comprising two or more of the following amino acids of IL-26: Q40, A41, D43, A44, L45, Y46, K48, A50, T55, D59, 161, K62, N63, 164, F75, N78, R106, F107, D110, L114, R115, L118, G148, Y150, K151, S154, 1158, or S161, is provided.

In certain embodiments, a method for increasing antagonistic activity of an antagonist of IL-22 comprising the step of altering a structure of the antagonist such that the antagonist binds to one or more of the following amino acids of IL-22: F57, L59, D67, T70, D71, V72, R73, G159, 1161, K162, G165, or L169, is provided. In certain embodiments, the step of altering the structure is performed by computer modeling. In certain embodiments, the structure is an amino acid sequence. In certain embodiments, the antagonist is an antibody. In certain embodiments, the structure is a crystal structure. In certain embodiments, the antagonist is a small molecule.

In certain embodiments, a method for increasing antagonistic activity of an antagonist of IL-22 comprising the step of altering a structure of the antagonist such that the antagonist binds to one or more of the following amino acids of IL-22: A34, 152, T56, K61, A66, V83, R88, P113, F121, L122, L125, or M172, is provided. In certain embodiments, the step of altering the structure is performed by computer modeling. In certain embodiments, the structure is an amino acid sequence. In certain embodiments, the antagonist is an antibody. In certain embodiments, the structure is a crystal structure. In certain embodiments, the antagonist is a small molecule.

In certain embodiments, a method for increasing antagonistic activity of an antagonist of IL-22 comprising the step of altering a structure of the antagonist such that the antagonist binds to one or more of the following amino acids of IL-22: D67, R73, V83, or K162, is provided. In certain embodiments, the step of altering the structure is performed by computer modeling. In certain embodiments, the structure is an amino acid sequence. In certain embodiments, the antagonist is an antibody. In certain embodiments, the structure is a crystal structure. In certain embodiments, the antagonist is a small molecule.

In certain embodiments, a method for increasing antagonistic activity of an antagonist of IL-19 comprising the step of altering a structure of the antagonist such that the antagonist binds to one or more of the following amino acids of IL-19: H36, 137,
E39, S40, F41, Q42, 144, R46, K51, D52, P55, N56, V57, T58, 168, V74, R102, K103, S106, S110, F111, M114, A152, 1541, 155K, G158, V162, or A165, is provided. In certain embodiments, the step of altering the structure is performed by computer modeling. In certain embodiments, the structure is an amino acid sequence. In certain embodiments, the antagonist is an antibody. In certain embodiments, the structure is a crystal structure. In certain embodiments, the antagonist is a small molecule.

[044] In certain embodiments, a method for increasing antagonistic activity of an antagonist of IL-20 comprising the step of altering a structure of the antagonist such that the antagonist binds to one or more of the following amino acids of IL-20: E41, 142, N44, G45, F46, S47, 149, G51, D57, 160, D61, 162, R63, 164, L65, D73, R79, R107, K108, Si11, S115, F116, 1119, 157A, V159, K160, G163, 1170, or Q173, is provided. In certain embodiments, the step of altering the structure is performed by computer modeling. In certain embodiments, the structure is an amino acid sequence. In certain embodiments, the antagonist is an antibody. In certain embodiments, the structure is a crystal structure. In certain embodiments, the antagonist is a small molecule.

[045] In certain embodiments, a method for increasing antagonistic activity of an antagonist of IL-24 comprising the step of altering a structure of the antagonist such that the antagonist binds to one or more of the following amino acids of IL-24: K68, L69, E71, A72, F73, W74, V76, D78, Q83, D84, T87, S88, A89, R90, V900, S105, K135, S136, T139, N143, F144, 1147, A185, T187, K188, G191, 1195, or T198, is provided. In certain embodiments, the step of altering the structure is performed by computer modeling. In certain embodiments, the structure is an amino acid sequence. In certain embodiments, the antagonist is an antibody. In certain embodiments, the structure is a crystal structure. In certain embodiments, the antagonist is a small molecule.

[046] In certain embodiments, a method for increasing antagonistic activity of an antagonist of IL-26 comprising the step of altering a structure of the antagonist such that the antagonist binds to one or more of the following amino acids of IL-26: Q40, A41, D43, A44, L45, Y46, K48, A50, T55, D59, 161, K62, N63, 164, F75, N78, R106, F107, D110, L114, R115, L118, G148, Y150, K151, S154, 1158, or S161, is provided. In certain embodiments, the step of altering the structure is performed by computer modeling. In certain embodiments, the structure is an amino acid sequence. In certain
embodiments, the antagonist is an antibody. In certain embodiments, the structure is a crystal structure. In certain embodiments, the antagonist is a small molecule.

**BRIEF DESCRIPTION OF THE DRAWINGS**

[047] Figure 1 shows the results of systematic mutagenesis of human IL-22. Systematic mutagenesis of human IL-22 revealed specific amino acids that are involved in the binding of IL-22 to IL-22R, IL-10R2, and IL-22BP. Figure 1(a) shows the mature primary sequence of IL-22 with its numbering beginning at the N terminus of the secretory leader. The dashed lines indicate intramolecular disulfide bonds while the stick models indicate glycosylation of asparagines at 54, 68, and 97. Figure 1(b) shows a ribbon rendering of the IL-22 tertiary backbone with helices A-F annotated. Amino acids defined as being involved in the binding of IL-22 to IL-22R (F57, L59, D67, T70, D71, V72, R73, G159, **1161**, **K162**, G165, and **L169**), IL-10R2 (A34, Y51, 152, N54, R55, T56, K61, A66, V83, R88, P113, Y114, E117, **F121**, L122, L125, and M172), and IL-22BP (D67, R73, V83, and K162) are highlighted.

[048] Figure 2 shows certain IL-22 point substitutions in helices A, D, and F and loop AB that bind more weakly than wild-type IL-22 to IL-22BP, IL-22R, and/or a complex of IL22R/IL-10R2. Purified H/F-IL-22 mutants were evaluated for binding to plates coated indirectly with (a) IL-22R-Fc, (b) IL-22R-Fc/IL-10R2-Fc, or (c) IL-22BP-Fc using His-Probe-HRP to detect the HIS-tag at the N terminus of the cytokine. Nine IL-22 substitutions that exhibited weak binding to IL-22BP (D67A, R73A, and K162A), IL-22R (D67A, V72A, R73A, **1161A**, K162A, and L169A), or **IL-10R2** (Y51A, R55A, and E117A) in the systematic high-throughput assays were evaluated as were two substitutions that had an adverse effect in all five binding assays (L100A and C132A) and two substitutions that had normal binding characteristics in all five assays (S86A and Q94A). The binding of wild-type IL-22, with no mutations, is shown as a dashed line. Data are representative of at least two experiments.

[049] Figure 3 shows IL-22 point substitutions in helices A, D, and F, and loop AB that reduce the ability of IL-22 to induce the proliferation of cells. IL-22-dependent proliferation of BaF3 cells expressing both receptor subunits was evaluated after 72 hours by ^3^H-thymidine incorporation. Nine IL-22 substitutions were evaluated that exhibited relatively poor binding to IL-22R (D67A, V72A, R73A, **1161A**, K162A, and L169A) or...
IL-22R/IL-10R2 (Y51A, R55A, and E117A) in the ELISA-based receptor binding assays. In addition, two IL-22 mutants were evaluated that had an adverse effect in all five assays (L10OA and C132A) as well as two IL-22 mutants that had normal binding characteristics in all five assays (S86A and Q94A). The binding of IL-22 with no mutations, is shown as a dashed line. Data are representative of at least two experiments.

Figure 4 shows that the IL-22 amino acids that are involved in binding to IL-22R, IL-10R2, and IL-22BP contribute to adjacent and overlapping binding sites on the surface of IL-22. Figures (a)-(d) are sequential 90° rotations to the right, around the vertical axis, of a CPK rendering of IL-22. Shown are amino acid side chains that were defined as involved in binding to IL-22R (F57, D67, T70, D71, V72, R73, 1161, K162, and L169), IL-10R2 (Y51, 152, N54, R55, K61, A66, V83, R88, P113, Y114, E117, F121, L125, and M172), and IL-22BP-Fc (D67, R73, V83, and K162). Figures (e)-(h) are comparable sequential rotations to those in (a)-(d), respectively. The shading of the IL-22 CPK renderings in (e)-(h) delineates the various helices and loops in the IL-22 structure. Renderings (a) and (e) can be compared, as can the other horizontal pairs of renderings, to visualize the contribution of a given amino acid (e.g., F57 in (a) from a given secondary structure (e.g., helix A in (e) to a given binding site (e.g., IL-22R in (a)).

Figure 5 shows a structure-based alignment of IL-22 and IL-10 sequences and receptor binding sites. The IL-22 sequence in Figure 5 corresponds to amino acids 44-179 of SEQ ID NO:2. The IL-10 sequence in Figure 5 corresponds to SEQ ID NO:9. This alignment of IL-22 and IL-10 sequences was derived from the superimposition of IL-10, IL-19, and IL-22 monomeric structures. The first line of sequence is IL-22 with amino acids highlighted that are involved in binding to IL-22R (F57, L59, D67, T70, D71, V72, R73, G159, 1161, K162, G165, and L169), IL-10R2 (Y51, 152, N54, R55, T56, K61, A66, V83, R88, P113, Y114, E117, F121, L122, L125, and M172), and IL-22BP (D67, R73, V83, and K162). The second line of sequence is IL-10 with the short bars underneath corresponding to certain amino acids that have been previously demonstrated to be important for binding to IL-10R1 (upper bars) and IL-10R2 (lower bars) (Josephson et al. Immunity 15, 35-46 (2001) and Yoon et al. Journal of Biological Chemistry 281, 35088-96 (2006)). The cylinders show the positions of the IL-22 and IL-10 helices in relation to their respective above sequences. The dashed bars
below the helices indicate the regions that contribute to IL-22’s IL-22R binding site, IL-22’s IL-10R2 binding site, IL-10’s IL-10R1 binding site, and IL-22’s IL-10R2 binding site.

[052] Figure 6 shows side-chain atoms within helix A, loop AB and helix F of IL-22 and IL-10 that define the cytokine binding interfaces for the respective high affinity receptor subunits. Figure 6(a) shows a solvent accessible surface (1.4 angstrom probe radius) rendering of a portion of IL-22’s tertiary structure. The residues that contribute to the IL-22R binding site are F57, L59, D67, T70, D71, V72, R73, G159, 1161, K162, G165, and L169. K161’s solvent accessible surface cannot be seen from this perspective; residues L59 and G159 are completely buried. Residues K61, S64, N68, E166, and D168 were previously proposed, based on modeling, to participate in recognition of IL-22R (Logsdon et al. *Journal of Interferon & Cytokine Research* 22, 1099-112 (2002)); however, point substitution to alanine had no impact on binding to IL-22R in our study. Figure 6(b) shows the same orientation as in (a) of helices A, and F loop AB of IL-22 superimposed with the corresponding IL-10 side chains, as aligned in Figure 5. The light ribbon represents the IL-22 backbone with the annotated ball and stick models representing side chains that are involved in binding to IL-22R and includes those that are also involved in binding to IL-22BP (corresponding to F57, L59, D67, T70, D71, V72, R73, G159, 1161, K162, G165, and L169 residues in Figures 1 and 5). The orientation of the helices is comparable to those shown in Figures 1(b), 4(a) and (e), and 8. G159 of IL-22 cannot be seen well from this perspective since it is immediately behind K162. The darker ribbon represents the superimposed IL-10 backbone with the remaining annotated models indicating side chains that are involved in IL-10R1 binding (R42, R45, Q56, Q60, D62, K156, S159, E160, D162, and E169), based on the analysis of structure from IL-10/IL-10R1 ECD co-crystals (Josephson et al. *Immunity* 15, 35-46 (2001) and Yoon et al. *Journal of Biological Chemistry* 281, 35088-96 (2006)). The alignment, numbering and annotation are as shown in Figure 5. Figure 5(e) shows a solvent accessible surface (1.4 angstrom probe radius) rendering of IL-22, with residues D67, R73, V83, and K162 that contribute to the IL-22BP binding site.

[053] Figure 7 shows side-chain atoms mostly within helix A and D of IL-22 and IL-10 that define the low affinity receptor binding sites for IL-10R2. Figure 7(a)
shows a solvent accessible surface (1.4 angstrom probe radius) rendering of a portion of IL-22's structure. The residues that contribute to the IL-22/IL-10R2 binding interface are Y51, 152, N54, R55, T56, L59, K61, A66, R88, P113, Y114, E117, F121, L122, L125, G159, and M172. T56 and L122 have very low solvent accessible surface and cannot be seen from this perspective. Residues L59 and G159 are completely buried. Figure 7(b) shows the light ribbon that represents the IL-22 backbone for helix A and D secondary structure with the annotated ball and stick models indicating side chains that are involved in binding to IL-10R2 in the presence of IL-22R (Y51, 152, N54, R55, T56, L59, K61, A66, R88, P113, Y114, E117, F121, L122, L125, G159, and M172). The orientation of the helices is similar to those shown in Figures 4(d) and (h). The dark ribbon represents the superimposed IL-10 backbone with the remaining annotated models indicating side chains that are proposed to contribute to IL-10R2 binding (N39, M40, R42, S49, R50, H108, and S111) (Yoon et al. Journal of Biological Chemistry 281, 35088-96 (2006)). The alignment, numbering, and annotation are the same as in Figure 5.

[054] Figure 8 shows putative receptor binding sites for IL-19, IL-20, IL-24, and IL-26 based on the elucidated IL-22 receptor binding sites and the proposed conservation of the structure-function relationship between the IL-10-like cytokines. The ribbon representations of IL-19 and IL-22 backbones are from crystal structures, while those for IL-20, IL-24, and IL-26 are from models generated as described in the Examples. The IL-22 amino acid side chains that were defined as involved in binding to IL-22R (F57, L59, T70, D71, V72, G159, 1161, G165, and L169), IL-10R2 (A34, Y51, 152, N54, R55, T56, K61, A66, R88, P113, Y114, E117, F121, L122, L125, and M172), and IL-22BP (D67, R73, V83, and K162) are highlighted as ball and stick models, and correspond to the same highlighted residues as in Figures 1 and 4(a)-(d). Based on a structural alignment of the IL-10-like cytokines, shown at the primary sequence level in Figure 10, the above IL-22 binding sites were transposed to the equivalent positions in the IL-19, IL-20, IL-24, and IL-26 sequences and to the tertiary structures shown here. These transpositions may be predictive of the cognate high affinity and low affinity receptor subunit binding sites for these cytokines.

[055] Figure 9 shows certain IL-22 point mutants that do not bind as well as control cytokine to IL-22R, IL-22R/IL-10R2 and IL-22BP. A systematic collection of
146 IL-22 point mutants were expressed in mammalian cells from linear expression cassettes and evaluated, relative to silent substitution mutants, at a fixed and limiting concentration for binding to receptors. Figure 9(a) shows data for the twenty-nine IL-22 substitutions that bound weakly (shown in solid symbols) relative to control IL-22 (silent substitutions; shown in open symbols) in the high-throughput receptor binding assays. Data were normalized to binding of purified IL-22 with no mutations, the value of this sample was set to '1' in each assay. Figure 9(b) shows a solvent accessible surface (1.4 angstrom probe radius) rendering of IL-22 structure with those amino acids highlighted that had statistically weaker than normal binding in the binding assays. Most of these 30 residues have low solvent accessibility and are proposed to be required for maintaining IL-22 secondary or tertiary structure.

[056] Figure 10 shows a structure-based alignment of IL-10-like cytokines. The first line of sequence is IL-22 with amino acids highlighted that are involved in binding to IL-22R (F57, L59, D67, T70, D71, V72, R73, G159, 1161,K162, G165, and L169), IL-10R2 (Y51, 152, N54, R55, T56, K61, A66, V83, R88, P113, Y114, E117, F121, L122, L125, and M172), and IL-22BP (D67, R73, V83, and K162). The IL-22 sequence repeated in the first and second lines in Figure 10 corresponds to amino acids 44-179 of SEQ ID NO:2. The IL-10-like cytokine primary sequence alignment is derived from the structural superimposition and modeling described in the Examples. The IL-10 sequence in Figure 10 corresponds to SEQ ID NO:9. The IL-19 sequence in Figure 10 corresponds to amino acid residues 31 to 177 in SEQ ID NO:5. The IL-20 sequence in Figure 10 corresponds to amino acid residues 36 to 176 in SEQ ID NO:6. The IL-24 sequence in Figure 10 corresponds to amino acid residues 63 to 206 in SEQ ID NO:7. The IL-26 sequence in Figure 10 corresponds to amino acid residues 36 to 171 in SEQ ID NO:8. Shared physicochemical properties of amino acid groups are indicated in the visual legend. The short bars underneath the IL-10 sequence correspond to certain amino acids that have been previously demonstrated to be important for binding to IL-10R1 (upper bars) and IL-10R2 (lower bars) (Josephson et al, Immunity 15, 35-46 (2001) and Yoon et al. Journal of Biological Chemistry 281, 35088-96 (2006)). The cylinders show the positions of the IL-22, IL-10 and IL-19 helices in relation to the above sequence, with dashed bars below indicating the regions that contribute to IL-22’s IL-22R binding site.
and IL-22's IL-10R2 binding site and in relation to IL-10's IL-10R1 binding site and IL-10's IL-10R2 binding site.

[057] Figure 11 shows IL-22 point substitutions that impact binding to at least one of IL-22BP, IL-22R, and IL-10R2 as described in the Examples.

[058] Figure 12 shows the effect of IL-22 point substitutions on binding assays as described in the Examples.

[059] Figure 13 shows the solvent accessibility of certain IL-22 amino acids as described in the Examples.

[060] Figure 14 shows the solvent accessibility of certain IL-22 amino acids identified as being involved in binding at least one of IL-22BP, IL-22R, and IL-10R2 as described in the Examples.

[061] Figure 15 shows the human IL-22 amino acid and nucleotide sequences.

[062] Figure 16 shows the mouse IL-22 amino acid and nucleotide sequences.

[063] Figure 17(a) shows the human IL-19 amino acid sequence. Figure 17(b) shows the human IL-20 amino acid sequence.

[064] Figure 18(a) shows the human IL-24 amino acid sequence. Figure 18(b) shows the human IL-26 amino acid sequence.

**DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS**

[065] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the claims, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

[066] In order that the present invention may be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the detailed description. Unless specific definitions are provided, the nomenclatures utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques may be
used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, delivery, and treatment of patients.

[067] In this application, the use of the singular includes the plural unless specifically stated otherwise. In this application, the use of "or" means "and/or" unless stated otherwise. In the context of a multiple dependent claim, the use of "or" refers back to more than one preceding independent or dependent claim in the alternative only. Furthermore, the use of the term "including", as well as other forms, such as "includes" and "included", is not limiting. Also, terms such as "element" or "component" encompass both elements and components comprising one unit and elements and components that comprise more than one subunit unless specifically stated otherwise.

[068] Other features and advantages will be apparent from the following detailed description and claims.

[069] The present application provides for, at least in part, antibodies and antigen-binding fragments thereof that bind to IL-22, in particular, human IL-22, with high affinity and specificity. In certain embodiments, the anti-IL-22 antibody or fragment thereof reduces, inhibits or antagonizes at least one IL-22-associated activity. For example, the anti-IL-22 antibody or fragment thereof can bind to IL-22, e.g., an epitope of IL-22, and interfere with an interaction, e.g., binding, between IL-22 and an IL-22 receptor complex, e.g., a complex comprising IL-22 receptor ("IL-22R") and interleukin-10 receptor 2 ("IL-10R2"), or a subunit thereof (e.g., IL-22R or IL-10R2, individually). Thus, in certain embodiments, the antibodies and fragments thereof can be used to interfere with (e.g., inhibit, block or otherwise reduce) an interaction, e.g., binding, between IL-22 and an IL-22 receptor complex, or a subunit thereof. In certain embodiments, the anti-IL-22 antibodies or fragments thereof can be used to diagnose, treat or prevent IL-22-associated disorders, e.g., autoimmune disorders, e.g., arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis, lupus-associated arthritis or ankylosing spondylitis), scleroderma, systemic lupus erythematosus, HIV, Sjogren's syndrome, vasculitis, multiple sclerosis, autoimmune thyroiditis, dermatitis (including atopic dermatitis and eczematous dermatitis), myasthenia gravis, inflammatory bowel disease (IBD), Crohn's disease, colitis, diabetes mellitus (type I); inflammatory conditions of, e.g., the skin (e.g.,
psoriasis), cardiovascular system (e.g., atherosclerosis), nervous system (e.g., Alzheimer's disease), liver (e.g., hepatitis), kidney (e.g., nephritis) and pancreas (e.g., pancreatitis); cardiovascular disorders, e.g., cholesterol metabolic disorders, oxygen free radical injury, ischemia; disorders associated with wound healing; respiratory disorders, e.g., asthma and COPD (e.g., cystic fibrosis); acute inflammatory conditions (e.g., endotoxemia, sepsis and septicaemia, toxic shock syndrome and infectious disease); transplant rejection and allergy. In one embodiment, the IL-22-associated disorder is, an arthritic disorder, e.g., a disorder chosen from one or more of rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis, or ankylosing spondylitis; a respiratory disorder (e.g., asthma, chronic obstructive pulmonary disease (COPD)); or an inflammatory condition of, e.g., the skin (e.g., psoriasis), cardiovascular system (e.g., atherosclerosis), nervous system (e.g., Alzheimer's disease), liver (e.g., hepatitis), kidney (e.g., nephritis), pancreas (e.g., pancreatitis), and gastrointestinal organs, e.g., colitis, Crohn's disease and IBD.

[070] The term "interleukin-22" or "IL-22" refers to a class II cytokine (which may be mammalian) capable of binding to IL-22R and/or a receptor complex of IL-22R and IL-10R2, and has at least one of the following features: (1) an amino acid sequence of a naturally occurring mammalian IL-22 polypeptide (full length or mature form) or a fragment thereof, e.g., an amino acid sequence shown as SEQ ID NO:1 (human) or SEQ ID NO:3 (murine) or a fragment thereof; (2) an amino acid sequence substantially identical to, e.g., at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to, an amino acid sequence shown as SEQ ID NO:1 or amino acids 34-179 thereof (human) or SEQ ID NO:3 (murine) or a fragment thereof; (3) an amino acid sequence which is encoded by a naturally occurring mammalian IL-22 nucleotide sequence or a fragment thereof (e.g., SEQ ID NO:2 or nucleotides 71 to 610 (human) or SEQ ID NO:4 (murine) or a fragment thereof); (4) an amino acid sequence encoded by a nucleotide sequence which is substantially identical to, e.g., at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to, a nucleotide sequence shown as SEQ ID NO:2 or nucleotides 71 to 610 thereof (human) or SEQ ID NO:4 (murine) or a fragment thereof; (5) an amino acid sequence encoded by a nucleotide sequence degenerate to a naturally occurring IL-22 nucleotide sequence or a fragment
thereof, e.g., SEQ ID NO:2 (human) or SEQ ID NO:4 (murine) or a fragment thereof; or (6) a nucleotide sequence that hybridizes to one of the foregoing nucleotide sequences under stringent conditions, e.g., highly stringent conditions. The IL-22 may bind to IL-22R and/or a receptor complex of IL-22R and IL-10R2 of mammalian origin, e.g., human or mouse.


[072] The human IL-22 cDNA was deposited with the American Type Culture Collection (10801 University Boulevard, Manassas, Virginia, U.S.A. 201 10-2209) on April 28, 1999 as an original deposit under the Budapest Treaty and was given the accession number ATCC 20723 1.

[073] The phrase "an IL-22 activity" or "IL-22 associated activity" refers to one or more of the biological activities of an IL-22 polypeptide, e.g., a mature IL-22 polypeptide (e.g., a mammalian, e.g., human or murine IL-22 having an amino acid sequence as shown in SEQ ID NO:2 and 4, respectively), including, but not limited to, (1) interacting with, e.g., binding to, an IL-22 receptor (e.g., an IL-22R or IL-10R2 or a complex thereof, preferably of mammalian, e.g., murine or human origin); (2) associating with one or more signal transduction molecules; (3) stimulating phosphorylation and/or activation of a protein kinase, e.g., JAK/STAT3, ERK, and MAPK; (4) modulating, e.g., stimulating or decreasing, proliferation, differentiation, effector cell function, cytolytic activity, cytokine or chemokine secretion, and/or survival of an IL-22 responsive cell, e.g., an epithelial cell from, e.g., kidney, liver, colon, small intestine, thyroid gland, pancreas, skin); (5) modulating at least one parameter of an acute phase response, e.g., a metabolic, hepatic, hematopoietic (e.g., anemia, platelet increase) or neuroendocrine change, or a change (e.g., increase or decrease in an acute phase protein, e.g., an increase in fibrinogen and/or serum amyloid A, or a decrease in albumin); and/or (6) modulating at least one parameter of an inflammatory state, e.g., modulating cytokine-mediated proinflammatory actions (e.g., fever, and/or prostaglandin synthesis, for example PGE₂
synthesis), modulating cellular immune responses, modulating cytokine, chemokine (e.g., GRO1), or lymphokine production and/or secretion (e.g., production and/or secretion of a proinflammatory cytokine).

[074] As used herein, a "therapeutically effective amount" of an antagonist, e.g., an antibody or a fragment thereof, refers to an amount of an agent which is effective, upon single or multiple dose administration to a subject, e.g., a human patient, at treating, curing, reducing the severity of, or ameliorating one or more symptoms of a disorder, or in prolonging the survival of the subject beyond that expected in the absence of such treatment.

[075] As used herein, "a prophylactically effective amount" of an antagonist, e.g., antibody, refers to an amount of an agent which is effective, upon single- or multiple-dose administration to a subject, e.g., a human patient, in preventing or delaying the occurrence of the onset or recurrence of a disorder, e.g., a disorder as described herein.

[076] The term "induce", "reduce," "inhibit," "potentiate," "elevate," "increase," "decrease" or the like, e.g., which denote quantitative differences between two states, refer to at least statistically significant differences between the two states.

[077] As used herein, an "IL-22 antagonist" refers to an agent which reduces, inhibits or otherwise diminishes one or biological activities of an IL-22 polypeptide, e.g., a human IL-22 polypeptide, or fragment thereof. Preferably, the antagonist interacts with, e.g., binds to, an IL-22 polypeptide. Antagonism using an IL-22 antagonist does not necessarily indicate a total elimination of the IL-22-associated biological activity. IL-22 antagonists include without limitation antibodies directed to human IL-22 proteins; soluble forms of the receptor or other target to which human IL-22 is directed; antibodies directed to the receptor or other target to which human IL-22 is directed; and peptide and small molecule compounds that inhibit or interfere with the interaction of human IL-22 with its receptor or other target.

[078] As used herein, an "IL-22 agonist" refers to an agent which potentiates, induces or otherwise enhances one or biological activities of an IL-22 polypeptide.

[079] The terms "specific binding" or "specifically binds" refers to two molecules forming a complex that is relatively stable under physiologic conditions.
Specific binding is characterized by a high affinity and a low to moderate capacity as distinguished from nonspecific binding which usually has a low affinity with a moderate to high capacity. Typically, binding is considered specific when the association constant $K_A$ is higher than $10^6 \text{M}^{-1}$. If necessary, nonspecific binding can be reduced without substantially affecting specific binding by varying the binding conditions. The appropriate binding conditions, such as concentration of antibodies, ionic strength of the solution, temperature, time allowed for binding, concentration of a blocking agent (e.g., serum albumin, milk casein), etc., may be optimized by a skilled artisan using routine techniques.

[080] The term "specific binding agent" refers to a natural or non-natural molecule that specifically binds to a target. Examples of specific binding agents include, but are not limited to, proteins, peptides, nucleic acids, carbohydrates, lipids, and small molecule compounds. In certain embodiments, a specific binding agent is an antibody. In certain embodiments, a specific binding agent is an antigen binding region.

[081] The term "structure" encompasses both structures of biologies (for example and not limitation, antibodies and fragments thereof) and small molecules.

[082] The term "antibody" refers to an immunoglobulin or fragment thereof, such as Fab, Fab', F(ab')$_2$, Fc, Fd, Fd', Fv, single chain antibodies (scFv for example), single variable domain antibodies (Dab), diabodies (bivalent and bispecific), and chimeric (e.g., humanized) antibodies, which may be produced by the modification of whole antibodies or those synthesized de novo using recombinant DNA technologies. These functional antibody fragments retain the ability to selectively bind with their respective antigen or receptor. Antibodies and antibody fragments can be from any class of antibodies including, but not limited to, IgG, IgA, IgM, IgD, and IgE, and from any subclass (e.g., IgGl, IgG2, IgG3, and IgG4) of antibodies. The antibodies of the present invention can be monoclonal or polyclonal. The antibody can also be a monospecific, polyspecific, non-specific, humanized, human, single-chain, chimeric, synthetic, recombinant, hybrid, mutated, CDR-grafted, and/or in vitro generated antibody. The antibody can have a heavy chain constant region chosen from, e.g., IgGl, IgG2, IgG3, or IgG4. The antibody can also have a light chain chosen from, e.g., kappa or lambda. Constant regions of the antibodies can be altered, e.g., mutated, to modify the properties
of the antibody (e.g., to increase or decrease one or more of: Fc receptor binding, antibody glycosylation, the number of cysteine residues, effector cell function, or complement function). Typically, the antibody specifically binds to a predetermined antigen, e.g., an antigen associated with a disorder, e.g., a neurodegenerative, metabolic, inflammatory, autoimmune and/or a malignant disorder. Unless preceded by the word "intact", the term "antibody" includes, in addition to complete antibody molecules, antibody fragments such as Fab, F(ab')₂, Fv, scFv, Fd, dAb, and other antibody fragments that retain antigen-binding function. Typically, such fragments comprise an antigen-binding domain.

[083] The antibody can further include a heavy and light chain constant region, to thereby form a heavy and light immunoglobulin chain, respectively. In certain embodiments, the antibody is a tetramer of two heavy immunoglobulin chains and two light immunoglobulin chains, wherein the heavy and light immunoglobulin chains are inter-connected by, e.g., disulfide bonds. The heavy chain constant region is comprised of three domains, CH₁, CH₂ and CH₃. The light chain constant region is comprised of one domain, CL. The variable region of the heavy and light chains contains a binding domain that interacts with an antigen. The constant regions of the antibodies typically mediate the binding of the antibody to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement system.

[084] The terms "antigen-binding domain" and "antigen-binding fragment" refer to a part of an antibody molecule that comprises amino acids responsible for the specific binding between antibody and antigen. The part of the antigen that is specifically recognized and bound by the antibody is referred to as the "epitope." An antigen-binding domain may comprise an antibody light chain variable region (Vₐ) and an antibody heavy chain variable region (Vₕ); however, it does not have to comprise both. Fd fragments, for example, have two Vₕ regions and often retain some antigen-binding function of the intact antigen-binding domain. Examples of antigen-binding fragments of an antibody include (1) a Fab fragment, a monovalent fragment having the Vₐ, Vₕ, Cₐ and Cₕ regions; (2) a F(ab')₂ fragment, a bivalent fragment having two Fab fragments linked by a disulfide bridge at the hinge region; (3) a Fd fragment having the
two \( V_H \) and \( C_H \) domains; (4) a Fv fragment having the \( V_L \) and \( V_H \) domains of a single arm of an antibody, (5) a dAb fragment (Ward et al., (1989) Nature 341:544-546), which has a \( V_H \) domain; (6) an isolated complementarity determining region (CDR); and (7) a single chain Fv (scFv). Although the two domains of the Fv fragment, \( V_L \) and \( V_H \), are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the \( V_L \) and \( V_H \) regions pair to form monovalent molecules (known as single chain Fv (scFv)); see e.g., Bird et al. (1988) Science 242:423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883). These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are evaluated for function in the same manner as are intact antibodies.

[085] As used herein, the term "immunoglobulin" refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. The recognized human immunoglobulin genes include the kappa, lambda, alpha (IgA1 and IgA2), gamma (IgG1, IgG2, IgG3, IgG4), delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Full-length immunoglobulin "light chains" (about 25 Kd or 214 amino acids) are encoded by a variable region gene at the NH2-terminus (about 110 amino acids) and a kappa or lambda constant region gene at the COOH—terminus. Full-length immunoglobulin "heavy chains" (about 50 Kd or 446 amino acids), are similarly encoded by a variable region gene (about 116 amino acids) and one of the other aforementioned constant region genes, e.g., gamma (encoding about 330 amino acids).

[086] As used herein, "isotype" refers to the antibody class (e.g., IgM or IgG1) that is encoded by heavy chain constant region genes.

[087] The term "human antibody" includes antibodies having variable and constant regions corresponding substantially to human germline immunoglobulin sequences known in the art, including, for example, those described by Kabat et al. (See Kabat, et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). In certain embodiments, human antibodies may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-
specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs, and in particular, CDR3. The human antibody can have at least one, two, three, four, five, or more positions replaced with an amino acid residue that is not encoded by the human germline immunoglobulin sequence.

[088] The phrase "inhibit" or "antagonize" IL-22 activity and its cognates refer to a reduction, inhibition, or otherwise diminution of at least one activity of IL-22 due to binding an anti-IL-22 antibody, wherein the reduction is relative to the activity of IL-22 in the absence of the same antibody. The activity can be measured using any technique known in the art. Inhibition or antagonism does not necessarily indicate a total elimination of the IL-22 polypeptide biological activity. A reduction in activity may be about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or more.

[089] The term "IL-22 activity" or "IL-22-associated activity" refers to at least one cellular process initiated or interrupted as a result of IL-22 binding to a receptor complex consisting of IL-22R and IL-10R2 on the cell. IL-22 activities include at least one of, but are not limited to: (1) binding IL-22R or a receptor complex of IL-22R and IL-10R2 (e.g., human IL-22R with or without human IL-10R2); (2) associating with signal transduction molecules (e.g., JAK-I); (3) stimulating phosphorylation of STAT proteins (e.g., STAT5, STAT3, or combination thereof); (4) activating STAT proteins; and (5) modulating (e.g., increasing or decreasing) proliferation, differentiation, effector cell function, cytolytic activity, cytokine secretion, survival, or combinations thereof, of epithelial cells, fibroblasts, or immune cells. Epithelial cells include, but are not limited to, cells of the skin, gut, liver, and kidney, as well as endothelial cells. Fibroblasts include, but are not limited to, synovial fibroblasts. Immune cells may include CD8+ and CD4+ T cells, NK cells, B cells, macrophages, and megakaryocytes. IL-22 activity can be determined *in vitro*, for example, using an IL-22 receptor inhibition assay, a GROα secretion assay, or a BAF3 proliferation assay. IL-22 activity can also be determined *in vivo*, for example, by scoring progression of an immune response or disorder.

[090] The term "isolated" refers to a molecule that is substantially free of its natural environment. For instance, an isolated protein is substantially free of cellular material or other proteins from the cell or tissue source from which it was derived. The term also refers to preparations where the isolated protein is sufficiently pure for
pharmaceutical compositions; or at least 70-80% (w/w) pure; or at least 80-90% (w/w) pure; or at least 90-95% pure; or at least 95%, 96%, 97%, 98%, 99%, or 100% (w/w) pure.

[091] The term "therapeutic agent" is a substance that treats or assists in treating a medical disorder. Therapeutic agents may include, but are not limited to, substances that modulate immune cells or immune responses in a manner that complements the IL-22 activity of anti-IL-22 antibodies. Non-limiting examples and uses of therapeutic agents are described herein.

[092] The term "treatment" refers to a therapeutic or preventative measure. The treatment may be administered to a subject having a medical disorder or who ultimately may acquire the disorder, in order to prevent, cure, delay, reduce the severity of, and/or ameliorate one or more symptoms of a disorder or recurring disorder, or in order to prolong the survival of a subject beyond that expected in the absence of such treatment.

[093] The term "effective amount" refers to a dosage or amount that is sufficient to regulate an activity to ameliorate clinical symptoms or achieve a desired biological outcome, e.g., decreased T cell and/or B cell activity, suppression of autoimmunity, suppression of transplant rejection, etc.

[094] The term "in combination" in the context of treatment with two agents means that the agents are given substantially contemporaneously, either simultaneously or sequentially. If given sequentially, at the onset of administration of the second compound, the first of the two compounds is preferably still detectable at effective concentrations at the site of treatment.

[095] The phrase "percent identical" or "percent identity" refers to the similarity between at least two different sequences. This percent identity can be determined by standard alignment algorithms, for example, the Basic Local Alignment Tool (BLAST) described by Altshul et al. ((1990) J. Mol. Biol., 215: 403-410); the algorithm of Needleman et al. ((1970) J. Mol. Biol., 48: 444-453); or the algorithm of Meyers et al. ((1988) Comput. Appl. Biosci., 4: 11-17). A set of parameters may be the Blosum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5. The percent identity between two amino acid or nucleotide
sequences can also be determined using the algorithm of E. Meyers and W. Miller (1989) CABIOS, 4:11-17) which has been incorporated into the ALIGN program (version 2.0), using a PAM 120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. The percent identity is usually calculated by comparing sequences of similar length.

[096] In certain embodiments, sequences similar or homologous (e.g., at least about 85% sequence identity) to the sequences disclosed are provided. In certain embodiments, the sequence identity can be about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or higher. Alternatively, substantial identity exists when the nucleic acid segments will hybridize under selective hybridization conditions (e.g., highly stringent hybridization conditions), to the complement of the strand. The nucleic acids may be present in whole cells, in a cell lysate, or in a partially purified or substantially pure form.

[097] Isolated polynucleotides may be used as hybridization probes and primers to identify and isolate nucleic acids having sequences identical to or similar to those encoding the disclosed polynucleotides. Polynucleotides isolated in this fashion may be used, for example and not limitation, to produce antibodies against IL-22 or other IL-10-like cytokines or to identify cells expressing such antibodies. Hybridization methods for identifying and isolating nucleic acids include Southern hybridizations, in situ hybridization and Northern hybridization, and are well known to those skilled in the art.

[098] Hybridization reactions can be performed under conditions of different stringencies. Preferably, each hybridizing polynucleotide hybridizes to its corresponding polynucleotide under reduced stringency conditions, more preferably stringent conditions, and most preferably highly stringent conditions. Examples of stringency conditions are shown in Table 1 below: highly stringent conditions are those that are at least as stringent as, for example, conditions A-F; stringent conditions are at least as stringent as, for example, conditions G-L; and reduced stringency conditions are at least as stringent as, for example, conditions M-R.

**Table 1**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Hybrid</th>
<th>Hybrid Length (bp)</th>
<th>Hybridization Temperature and Buffer</th>
<th>Wash Temperature and Buffer</th>
</tr>
</thead>
</table>
The hybrid length is that anticipated for the hybridized region(s) of the hybridizing polynucleotides. When hybridizing a polynucleotide to a target polynucleotide of unknown sequence, the hybrid length is assumed to be that of the hybridizing polynucleotide. When polynucleotides of known sequence are hybridized, the hybrid length can be determined by aligning the sequences of the polynucleotides and identifying the region or regions of optimal sequence complementarity.

SSPE (IxSSPE is 0.15M NaCl, 10mM NaH₂PO₄, and 1.25mM EDTA, pH 7.4) can be substituted for SSC (IxSSC is 0.15M NaCl and 15mM sodium citrate) in the

<table>
<thead>
<tr>
<th>Condition</th>
<th>Hybrid</th>
<th>Hybrid Length (bp)¹</th>
<th>Hybridization Temperature and Buffer²</th>
<th>Wash Temperature and Buffer²</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>DNA:DNA</td>
<td>&gt; 50</td>
<td>65°C; 1X SSC -or- 42°C; 1X SSC, 50% formamide</td>
<td>65°C; 0.3X SSC</td>
</tr>
<tr>
<td>B</td>
<td>DNA:DNA</td>
<td>≤50</td>
<td>Tₛ*: 1X SSC</td>
<td>Tₛ*: 1X SSC</td>
</tr>
<tr>
<td>C</td>
<td>DNA:RNA</td>
<td>&gt; 50</td>
<td>67°C; 1X SSC -or- 45°C; 1X SSC, 50% formamide</td>
<td>67°C; 0.3X SSC</td>
</tr>
<tr>
<td>D</td>
<td>DNA:RNA</td>
<td>≤50</td>
<td>Tₛ*: 1X SSC</td>
<td>Tₛ*: 1X SSC</td>
</tr>
<tr>
<td>E</td>
<td>RNA:RNA</td>
<td>&gt; 50</td>
<td>70°C; 1X SSC -or- 50°C; 1X SSC, 50% formamide</td>
<td>70°C; 0.3X SSC</td>
</tr>
<tr>
<td>F</td>
<td>RNA:RNA</td>
<td>≤50</td>
<td>Tₛ*: 1X SSC</td>
<td>Tₛ*: 1X SSC</td>
</tr>
<tr>
<td>G</td>
<td>DNA:DNA</td>
<td>&gt; 50</td>
<td>65°C; 4X SSC -or- 42°C; 4X SSC, 50% formamide</td>
<td>65°C; 1X SSC</td>
</tr>
<tr>
<td>H</td>
<td>DNA:DNA</td>
<td>≤50</td>
<td>Tₛ*: 4X SSC</td>
<td>Tₛ*: 4X SSC</td>
</tr>
<tr>
<td>I</td>
<td>DNA:RNA</td>
<td>&gt; 50</td>
<td>67°C; 4X SSC -or- 45°C; 4X SSC, 50% formamide</td>
<td>67°C; 1X SSC</td>
</tr>
<tr>
<td>J</td>
<td>DNA:RNA</td>
<td>≤50</td>
<td>Tₛ*: 4X SSC</td>
<td>Tₛ*: 4X SSC</td>
</tr>
<tr>
<td>K</td>
<td>RNA:RNA</td>
<td>&gt; 50</td>
<td>70°C; 4X SSC -or- 50°C; 4X SSC, 50% formamide</td>
<td>67°C; 1X SSC</td>
</tr>
<tr>
<td>L</td>
<td>RNA:RNA</td>
<td>≤50</td>
<td>Tₛ*: 2X SSC</td>
<td>Tₛ*: 2X SSC</td>
</tr>
<tr>
<td>M</td>
<td>DNA:DNA</td>
<td>&gt; 50</td>
<td>50°C; 4X SSC -or- 40°C; 6X SSC, 50% formamide</td>
<td>50°C; 2X SSC</td>
</tr>
<tr>
<td>N</td>
<td>DNA:DNA</td>
<td>≤50</td>
<td>Tₛ*: 6X SSC</td>
<td>Tₛ*: 6X SSC</td>
</tr>
<tr>
<td>O</td>
<td>DNA:RNA</td>
<td>&gt; 50</td>
<td>55°C; 4X SSC -or- 42°C; 6X SSC, 50% formamide</td>
<td>55°C; 2X SSC</td>
</tr>
<tr>
<td>P</td>
<td>DNA:RNA</td>
<td>≤50</td>
<td>Tₛ*: 6X SSC</td>
<td>Tₛ*: 6X SSC</td>
</tr>
<tr>
<td>Q</td>
<td>RNA:RNA</td>
<td>&gt; 50</td>
<td>60°C; 4X SSC -or- 45°C; 6X SSC, 50% formamide</td>
<td>60°C; 2X SSC</td>
</tr>
<tr>
<td>R</td>
<td>RNA:RNA</td>
<td>≤50</td>
<td>Tₛ*: 4X SSC</td>
<td>Tₛ*: 4X SSC</td>
</tr>
</tbody>
</table>

¹ The hybrid length is that anticipated for the hybridized region(s) of the hybridizing polynucleotides.
² SSPE (IxSSPE is 0.15M NaCl, 10mM NaH₂PO₄, and 1.25mM EDTA, pH 7.4) can be substituted for SSC (IxSSC is 0.15M NaCl and 15mM sodium citrate) in the
hybridization and wash buffers; washes are performed for 15 minutes after hybridization is complete.

\[ T_B^* - T_R^* : \] The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature \( T_m \) of the hybrid, where \( T_m \) is determined according to the following equations. For hybrids less than 18 base pairs in length, \( T_m(°C) = 2(\# \text{ of } A + T \text{ bases}) + 4(\# \text{ of } G + C \text{ bases}) \). For hybrids between 18 and 49 base pairs in length, \( T_m(°C) = 81.5 + 16.6(\log_{10} \text{Na}^+) + 0.41(%G + C) - (600/N) \), where \( N \) is the number of bases in the hybrid, and \( \text{Na}^+ \) is the concentration of sodium ions in the hybridization buffer (\( \text{Na}^+ \) for IX SSC = 0.165 M).


[099] Isolated polynucleotides may be used as hybridization probes and primers to identify and isolate DNAs having sequences encoding allelic variants of the disclosed polynucleotides. Allelic variants are naturally occurring alternative forms of the disclosed polynucleotides that encode polypeptides that are identical to or have significant similarity to the polypeptides encoded by the disclosed polynucleotides. Preferably, allelic variants have at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity with the disclosed polynucleotides.

[0100] Isolated polynucleotides may also be used as hybridization probes and primers to identify and isolate DNAs having sequences encoding polypeptides homologous to the disclosed polynucleotides. These homologs are polynucleotides and polypeptides isolated from a different species than that of the disclosed polypeptides and polynucleotides, or within the same species, but with significant sequence similarity to the disclosed polynucleotides and polypeptides. In certain embodiments, polynucleotide homologs have at least 50%, 75%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity with the disclosed polynucleotides, whereas polypeptide homologs have at least 30%, 45%, or 60% identity with the disclosed antibodies/polypeptides. In certain
embodiments, homologs of the disclosed polynucleotides and polypeptides are those isolated from mammalian species.

[0101] Isolated polynucleotides may also be used as hybridization probes and primers to identify cells and tissues that express proteins, including antibodies, and the conditions under which they are expressed.

[0102] It is understood that the IL-22 polypeptides and antagonists, e.g., antibodies, may have additional conservative or non-essential amino acid substitutions, which do not have a substantial effect on their functions. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

**IL-22 Proteins, Fragments and Polynucleotides Encoding the Same**

[0103] IL-22 nucleotide and amino acid sequences are described in US Patent 7,307,161 and provided below. The nucleotide sequence of each clone can also be determined by sequencing of the deposited clone in accordance with known methods. As used herein, a "secreted" protein is one which, when expressed in a suitable host cell, is transported across or through a membrane, including transport as a result of signal sequences in its amino acid sequence. "Secreted" proteins include without limitation proteins secreted wholly (e.g., soluble proteins) or partially (e.g., receptors) from the cell in which they are expressed. "Secreted" proteins also include without limitation proteins that are transported across the membrane of the endoplasmic reticulum.

[0104] The nucleotide sequence of human IL-22 is provided in SEQ ID NO: 1, and includes a poly(A) tail. The disclosed nucleotide sequence includes an open reading frame and the amino acid sequence of full-length IL-22 protein corresponding to the
foregoing nucleotide sequence is reported in SEQ ID NO:2. The amino acid sequence of mature IL-22 corresponds to about amino acids 34-179 of SEQ ID NO:2.

[0105] Nucleotide sequences encoding murine IL-22, and the sequence of the encoded polypeptide, are provided in SEQ. ID. NO. 3 and 4, respectively.

[0106] Any form of IL-22 proteins less than full length can be used in the methods and compositions of the present claims. IL-22 fragments, e.g., IL-22 proteins of less than full length, can be produced by expressing a corresponding fragment of the polynucleotide encoding the full-length IL-22 protein in a host cell. Modified polynucleotides as described above may be made by standard molecular biology techniques, including construction of appropriate desired deletion mutants, site-directed mutagenesis methods, or by the polymerase chain reaction using appropriate oligonucleotide primers.

[0107] Fragments of the protein can be in linear form, or they can be cyclized using known methods, for example, as described in H.U. Saragovi, et al., Bio/Technology 10, 773-778 (1992) and in R.S. McDowell, et al., J. Amer. Chem. Soc. 114, 9245-9253 (1992), both of which are incorporated herein by reference. Such fragments can be fused to carrier molecules such as immunoglobulins for many purposes, including increasing the valency of protein binding sites. For example, fragments of the protein can be fused through "linker" sequences to the Fc portion of an immunoglobulin. For a bivalent form of the protein, the fusion can be to the Fc portion of an IgG molecule. Other immunoglobulin isotypes may be used to generate such fusions. For example, a protein- IgM fusion can be used to generate a decavalent form of the protein.

[0108] IL-22 proteins and fragments thereof include proteins with amino acid sequence lengths that are at least 25% (more preferably at least 50%, and most preferably at least 75%) of the length of a disclosed protein and have at least 60% sequence identity (more preferably, at least 75% identity; most preferably at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along the length of the fragment) with that disclosed protein, where sequence identity is determined by comparing the amino acid sequences of the proteins when aligned so as to maximize overlap and identity while minimizing sequence gaps. In certain embodiments, proteins and protein fragments contain a segment comprising 8 or more (more preferably 20 or more, most
preferably 30 or more) contiguous amino acids that shares at least 75% sequence identity (more preferably, at least 85% identity; most preferably at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity) with any such segment of any of the disclosed proteins.

[0109] In certain embodiments, proteins, protein fragments, and recombinant proteins include those that can be identified based on the presence of at least one “IL-22 receptor-binding motif.” As used herein, the term “IL-22 receptor-binding motif” includes amino acid sequences or residues that are important for binding of IL-22 to its receptor. In certain embodiments, an IL-22 protein contains an IL-22 receptor-binding motif comprising amino acids 50-60 of SEQ ID NO:2. In one embodiment, an IL-22 protein contains an IL-22 receptor-binding motif comprising amino acids 63-81 of SEQ ID NO:2. In certain embodiments, an IL-22 protein contains a IL-22 receptor-binding motif comprising amino acids 168-177 of SEQ II) NO:2. In another embodiment, an IL-22 protein contains an IL-22 receptor-binding motif comprising at least one of amino acids 50-60, amino acids 63-81, and/or amino acids 168-177 of SEQ ID NO:2. In certain embodiments, an IL-22 protein contains an IL-22 receptor-binding motif comprising amino acids 20-90, 30-80, 40-70, and/or 50-60 of SEQ ID NO: 2. In certain embodiments, the IL-22 protein comprises an IL-22 receptor binding motif comprising amino acids 33-111, 43-101, 53-91, and/or 63-81 of SEQ ID NO: 2. In certain embodiments, the IL-22 protein comprises an IL-22 receptor binding motif comprising amino acids 138-179, 148-179, 158-179, and/or 168-177 of SEQ ID NO: 2.

[0110] In certain embodiments, an IL-22 receptor-binding motif is bound by an antibody or binding fragment that binds to IL-22. In certain embodiments, an IL-22 antibody or binding fragment binds to at least part of a receptor-binding motif comprising amino acids 20-90, 30-80, 40-70, and/or 50-60 of SEQ ID NO: 2. In certain embodiments, an IL-22 antibody or binding fragment binds to at least part of a receptor-binding motif comprising amino acids 33-111, 43-101, 53-91, and/or 63-81 of Seq Id 2. In certain embodiments, an IL-22 antibody or binding fragment binds to at least part of a receptor-binding motif comprising amino acids 138-179, 148-179, 158-179, and/or 168-177 of SEQ ID NO: 2. In certain embodiments, binding of an IL-22 antibody or binding fragment to an IL-22 receptor-binding motif interferes with IL-22 binding to IL-22R.
1OR, and/or IL-22BP. In certain such embodiments, an IL-22 antibody or binding fragment reduces an IL-22 associated activity and/or treats or prevents an IL-22 associated disorder.

[01 11] In certain embodiments, an IL-22 receptor binding motif has an amino acid sequence at least 95%, 96%, 97%, 98%, 99%, or more identical to an amino acid sequence selected from the group consisting of amino acids 50-60 of SEQ ID NO:2, amino acids 63-81 of SEQ ID NO:2, and amino acids 168-177 of SEQ ID NO:2. In other embodiments, proteins, protein fragments, and/or recombinant proteins include those which can be identified based on the presence of at least one, two, three, four or more sites for N-linked glycosylation. Length can be determined by aligning the sequences of the polynucleotides and identifying the region or regions of optimal sequence complementarity.

Vectors and Host Cells

[01 12] Recombinant polynucleotides can be operably linked to an expression control sequence such as, for example and not limitation, the pMT2 or pED expression vectors disclosed in Kaufman et al., Nucleic Acids Res. 19, 4485-4490 (1991), to produce the protein recombinantly. Many suitable expression control sequences are known in the art. General methods of expressing recombinant proteins are also known and are exemplified in R. Kaufman (1990) Methods in Enzymology 185, 537-566. As defined herein "operably linked" means that the isolated polynucleotide and an expression control sequence are situated within a vector or cell in such a way that the protein is expressed by a host cell which has been transformed (transfected) with the ligated polynucleotide/expression control sequence.

[01 13] The term "vector", as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into
the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids.

[01 14] The term "regulatory sequence" as used herein includes promoters, enhancers and other expression control elements (e.g., polyadenylation signals) that control the transcription or translation of the antibody chain genes. Such regulatory sequences are described, for example, in Goeddel; Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990). It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. Regulatory sequences for mammalian host cell expression include, but are not limited to, viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from FF-1a promoter and BGH poly A, cytomegalovirus (CMV) (such as the CMV promoter/enhancer), Simian Virus 40 (SV4O) (such as the SV4O promoter/enhancer), adenovirus, (e.g., the adenovirus major late promoter (AdMLP)) and polyoma. For further description of viral regulatory elements, and sequences thereof, see e.g., U.S. Patent No. 5,168,062 by Stinski, U.S. Patent No. 4,510,245 by Bell et al. and U.S. Patent No. 4,968,615 by Schaffner et al.

[01 15] In certain embodiments, recombinant expression vectors may carry additional sequences, such as sequences that regulate replication of the vector in host cells (e.g., origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see e.g., U.S. Patents Nos. 4,399,216, 4,634,665 and 5,179,017, all by Axel et al). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in
dhfr host cells with methotrexate selection/amplification) and the neo gene (for G418 selection).

[01 16] A number of types of cells may act as suitable host cells for expression of the IL-22 protein or fusion protein thereof. Any cell type capable of expressing functional IL-22 protein may be used. Suitable mammalian host cells include, for example, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-I cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK, Rat2, BaF3, 32D, FDCP-I, PC12, Mix or C2C12 cells.

[01 17] In certain embodiments, an IL-22 protein or fusion protein thereof may also be produced by operably linking an isolated polynucleotide to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, e.g., Invitrogen, San Diego, Calif. U.S.A. (the MaxBac® kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. Soluble forms of the IL-22 protein may also be produced in insect cells using appropriate isolated polynucleotides as described above.

[01 18] Alternatively, the IL-22 protein or fusion protein thereof may be produced in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Suitable yeast strains include Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyces strains, Candida, or any yeast strain capable of expressing heterologous proteins. Suitable bacterial strains include Escherichia coli, Bacillus subtilis, Salmonella typhimurium, or any bacterial strain capable of expressing heterologous proteins.

[01 19] Expression in bacteria may result in formation of inclusion bodies incorporating the recombinant protein. Thus, refolding of the recombinant protein may be required in order to produce active or more active material. Several methods for obtaining correctly folded heterologous proteins from bacterial inclusion bodies are known in the art. These methods generally involve solubilizing the protein from the inclusion bodies, then denaturing the protein completely using a chaotropie agent. When
cysteine residues are present in the primary amino acid sequence of the protein, it is often necessary to accomplish the refolding in an environment which allows correct formation of disulfide bonds (a redox system). General methods of refolding are disclosed in Kohno, Meth. Enzym., 185:187-195 (1990). EP 0433225 and copending application U.S. Ser. No. 08/163,877 describe other appropriate methods.

[0120] An IL-22 protein or fusion protein thereof may also be expressed as a product of transgenic animals, e.g., as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a polynucleotide sequence encoding the IL-22 protein or fusion protein thereof.

[0121] The IL-22 protein or fusion protein thereof may be prepared by growing a culture transformed host cells under culture conditions necessary to express the desired protein. The resulting expressed protein may then be purified from the culture medium or cell extracts. Soluble forms of the IL-22 protein or fusion protein thereof can be purified from conditioned media. In certain embodiments, membrane-bound forms of IL-22 protein can be purified by preparing a total membrane fraction from the expressing cell and extracting the membranes with a non-ionic detergent such as Triton X-100.

[0122] In certain embodiments, the IL-22 protein can be purified using methods known to those skilled in the art. For example, and not limitation, the IL-22 protein can be concentrated using a commercially available protein concentration filter, including, but not limited to, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a purification matrix such as a gel filtration medium. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAB) or polyethyleneimine (PEI) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. In certain embodiments, sulfopropyl groups are preferred (e.g., S-Sepharose ® columns). The purification of the IL-22 protein or fusion protein from culture supernatant may also include one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl® or Cibacrom blue 3GA Sepharose®; or by hydrophobic interaction chromatography using such resins as phenyl
ether, butyl ether, or propyl ether; or by immunoaffinity chromatography. Finally, one or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify the IL-22 protein. Affinity columns including antibodies to the IL-22 protein can also be used in purification in accordance with known methods. Some or all of the foregoing purification steps, in various combinations or with other known methods, can also be employed to provide a substantially purified isolated recombinant protein. Preferably, the isolated IL-22 protein is purified so that it is substantially free of other mammalian proteins.

[0123] In certain embodiments, IL-22 proteins or fusion proteins may also be used to screen for agents (e.g., IL-22 antagonists, e.g., anti-IL-22 antibodies or fragments thereof) that are capable of binding to IL-22 and/or to various portions of IL-22. Binding assays using a desired binding protein, immobilized or not, are well known in the art and may be used for this purpose using the IL-22 protein. Purified cell based or protein based (cell free) screening assays may be used to identify such agents. For example, IL-22 protein may be immobilized in purified form on a carrier and binding or potential ligands to purified IL-22 protein may be measured.

[0124] IL-22 polypeptides may also be produced by known conventional chemical synthesis. Methods for constructing proteins by synthetic means are known to those skilled in the art. The synthetically-constructed protein sequences, by virtue of sharing primary, secondary or tertiary structural and/or conformational characteristics with proteins may possess biological properties in common therewith, including protein activity. Thus, they can be employed as biologically active or immunological substitutes for natural, purified proteins in screening of therapeutic compounds and in immunological processes for the development of antibodies.

**Antibodies and Antigen-Binding Fragments Thereof**

[0125] Antibodies, also known as immunoglobulins, are typically tetrameric glycosylated proteins composed of two light (L) chains of approximately 25 kDa each and two heavy (H) chains of approximately 50 kDa each. Two types of light chain, termed lambda and kappa, may be found in antibodies. Depending on the amino acid sequence of the constant domain of heavy chains, immunoglobulins can be assigned to
five major classes: A, D, E, G, and M, and several of these may be further divided into subclasses (isotypes), e.g., IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, and IgA₂. Each light chain includes an N-terminal variable (V) domain (VL) and a constant (C) domain (CL). Each heavy chain includes an N-terminal V domain (VH), three or four C domains (CHs), and a hinge region. The CH domain most proximal to VH is designated as CH₁. The VH and VL domains consist of four regions of relatively conserved sequences called framework regions (FR1, FR2, FR3, and FR4), which form a scaffold for three regions of hypervariable sequences (complementarity determining regions, CDRs). The CDRs contain most of the residues responsible for specific interactions of the antibody with the antigen. CDRs are referred to as CDR1, CDR2, and CDR3. Accordingly, CDR constituents on the heavy chain are referred to as H1, H2, and H3, while CDR constituents on the light chain are referred to as L1, L2, and L3.

[0126] CDR3 is typically the greatest source of molecular diversity within the antibody-binding site. H3, for example, can be as short as two amino acid residues or greater than 26 amino acids. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known in the art. For a review of the antibody structure, see Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, eds. Harlow et al., 1988. One of skill in the art will recognize that each subunit structure, e.g., a CH, VH, CL, VL, CDR, FR structure, comprises active fragments, e.g., the portion of the VH, VL, or CDR subunit the binds to the antigen, i.e., the antigen-binding fragment, or, e.g., the portion of the CH subunit that binds to and/or activates, e.g., an Fc receptor and/or complement. The CDRs typically refer to the Kabat CDRs, as described in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services (1991), eds. Kabat et al. Another standard for characterizing the antigen binding site is to refer to the hypervariable loops as described by Chothia. See, e.g., Chothia, D. et al. (1992; J Mol. Biol. 227:799-817; and Tomlinson et al. (1995) EMBO J. 14:4628-4638. Still another standard is the AbM definition used by Oxford Molecular's AbM antibody modeling software. See, generally, e.g., Protein Sequence and Structure Analysis of Antibody Variable Domains. In: Antibody Engineering Lab Manual (Ed.: Duebel, S. and Kontermann, R., Springer-Verlag, Heidelberg). Embodiments described with respect to Kabat CDRs can alternatively be
implemented using similar described relationships with respect to Chothia hypervariable loops or to the AbM-defined loops.

[0127] The Fab fragment (Fragment antigen-binding) consists OfVn-CnI and V_L-C_L domains covalently linked by a disulfide bond between the constant regions. The F_v fragment is smaller and consists of V_H and V_L domains non-covalently linked. To overcome the tendency of non-covalently linked domains to dissociate, a single chain F_v fragment (scF_v) can be constructed. The scF_v contains a flexible polypeptide that links (1) the C-terminus of V_H to the N-terminus of V_L, or (2) the C-terminus of V_L to the N-terminus of V_H- A 15-mer (GIy4Scr)3 peptide may be used as a linker, but other linkers are known in the art.

[0128] The sequence of antibody genes after assembly and somatic mutation is highly varied, and these varied genes are estimated to encode 10^10 different antibody molecules (Immunoglobulin Genes, 2nd ed., eds. Jonio et al., Academic Press, San Diego, CA, 1995).

[0129] Numerous methods known to those skilled in the art are available for obtaining antibodies or antigen-binding fragments thereof. For example, antibodies can be produced using recombinant DNA methods (U.S. Patent 4,816,567). Monoclonal antibodies may also be produced by generation of hybridomas (see e.g., Kohler and Milstein (1975) Nature, 256: 495-499) in accordance with known methods. Hybridomas formed in this manner are then screened using standard methods, such as enzyme-linked immunosorbent assay (ELISA) and surface plasmon resonance (BIACORE™) analysis, to identify one or more hybridomas that produce an antibody that specifically binds with a specified antigen. Any form of the specified antigen may be used as the immunogen, e.g., recombinant antigen, naturally occurring forms, any variants or fragments thereof, as well as antigenic peptide thereof.

In addition to the use of display libraries, the specified antigen can be used to immunize a non-human animal, e.g., including, but not limited to, mouse, hamster, rat, monkey, camel, llama, fish, shark, goat, rabbit, and bovine. In certain embodiments, the non-human animal includes at least a part of a human immunoglobulin gene. For example, it is possible to engineer mouse strains deficient in mouse antibody production with large fragments of the human Ig loci. Using the hybridoma technology, antigen-specific monoclonal antibodies derived from the genes with the desired specificity may be produced and selected. See, e.g., XENOMOUSE™, Green et al (1994) Nature Genetics 7:13-21, US 2003-0070185, WO 96/34096, published Oct. 31, 1996, and PCT Application No. PCT/US96/05928, filed Apr. 29, 1996.

In certain embodiments, a monoclonal antibody is obtained from a non-human animal, e.g., including, but not limited to, mouse, hamster, rat, monkey, camel, llama, fish, shark, goat, rabbit, and bovine and then modified, e.g., humanized or deimmunized. In certain embodiments chimeric antibodies may be produced using recombinant DNA techniques known in the art. A variety of approaches for making chimeric antibodies have been described. See e.g., Morrison et al, Proc. Natl. Acad. Sci. U.S.A. 81:6851, 1985; Takeda et al, Nature 314:452, 1985, Cabilly et al, U.S. Patent No. 4,816,567; Boss et al, U.S. Patent No. 4,816,397; Tanaguchi et al, European Patent Publication EP171496; European Patent Publication 0173494, United Kingdom Patent GB 2177096B. Humanized antibodies may also be produced, for example, using transgenic mice that express human heavy and light chain genes, but are incapable of expressing the endogenous mouse immunoglobulin heavy and light chain genes. Winter describes an exemplary CDR-grafting method that may be used to prepare the humanized antibodies described herein (U.S. Patent No. 5,225,539). All of the CDRs of a particular human antibody may be replaced with at least a portion of a non-human CDR, or only some of the CDRs may be replaced with non-human CDRs. It is only necessary to replace the number of CDRs required for binding of the humanized antibody to a predetermined antigen.

Humanized antibodies or fragments thereof can be generated by replacing sequences of the Fv variable domain that are not directly involved in antigen binding with equivalent sequences from human Fv variable domains. Exemplary
methods for generating humanized antibodies or fragments thereof are provided by Morrison (1985) Science 229:1202-1207; by Oi et al. (1986) BioTechniques 4:214; and by US 5,585,089; US 5,693,761; US 5,693,762; US 5,859,205; and US 6,407,213. Those methods include isolating, manipulating, and expressing the nucleic acid sequences that encode all or part of immunoglobulin Fv variable domains from at least one of a heavy or light chain. Such nucleic acids may be obtained from a hybridoma producing an antibody against a predetermined target, as described above, as well as from other sources. The recombinant DNA encoding the humanized antibody molecule can then be cloned into an appropriate expression vector.

[0134] In certain embodiments, a humanized antibody is optimized by the introduction of conservative substitutions, consensus sequence substitutions, germline substitutions and/or back mutations. Such altered immunoglobulin molecules can be made by any of several techniques known in the art, e.g., Teng et al., Proc. Natl. Acad. Sci. U.S.A., 80: 7308-7312, 1983; Kozbor et al., Immunology Today, 4: 7279, 1983; Olsson et al., Meth. Enzymol, 92: 3-16, 1982), and may be made according to the teachings of PCT Publication WO92/06193 or EP 0239400).

[0135] An antibody or fragment thereof may also be modified by specific deletion of human T cell epitopes or "deimmunization" by the methods disclosed in WO 98/52976 and WO 00/343 17. Briefly, the heavy and light chain variable domains of an antibody can be analyzed for peptides that bind to MHC Class II; these peptides represent potential T-cell epitopes (as defined in WO 98/52976 and WO 00/343 17). For detection of potential T-cell epitopes, a computer modeling approach termed "peptide threading" can be applied, and in addition a database of human MHC class II binding peptides can be searched for motifs present in the V_{H} and V_{L} sequences, as described in WO 98/52976 and WO 00/343 17. These motifs bind to any of the 18 major MHC class II DR allotypes, and thus constitute potential T cell epitopes. Potential T-cell epitopes detected can be eliminated by substituting small numbers of amino acid residues in the variable domains, or preferably, by single amino acid substitutions. Typically, conservative substitutions are made. Often, but not exclusively, an amino acid common to a position in human germline antibody sequences may be used. Human germline sequences, e.g., are disclosed in Tomlinson, et al. (1992) J. Mol. Biol. 227:776-798; Cook, G. P. et al. (1995)
Immuno. Today Vol. 16 (5): 237-242; Chothia, D. et al. (1992) J Mol. Biol. 227:799-817; and Tomlinson et al. (1995) EMBO J. 14:4628-4638. The V BASE directory provides a comprehensive directory of human immunoglobulin variable region sequences (compiled by Tomlinson, L.A. et al. MRC Centre for Protein Engineering, Cambridge, UK). These sequences can be used as a source of human sequence, e.g., for framework regions and CDRs. Consensus human framework regions can also be used, e.g., as described in U.S. Patent No. 6,300,064.

[0136] In certain embodiments, an antibody can contain an altered immunoglobulin constant or Fc region. For example, an antibody produced in accordance with the teachings herein may bind more strongly or with more specificity to effector molecules such as complement and/or Fc receptors, which can control several immune functions of the antibody such as effector cell activity, lysis, complement-mediated activity, antibody clearance, and antibody half-life. Typical Fc receptors that bind to an Fc region of an antibody (e.g., an IgG antibody) include, but are not limited to, receptors of the FcγRI, FcγRII, and FcγRIII and FcRn subclasses, including allelic variants and alternatively spliced forms of these receptors. Fc receptors are reviewed in Ravetch and Kinet, Annu. Rev. Immunol 9:457-92, 1991; Capel et al, Immunomethods 4:25-34,1994; and de Haas et al., J. Lab. Clin. Med. 126:330-41, 1995).


[0138] A bispecific or bifunctional antibody is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. See, e.g., Songsivilai & Lachmann, CHN. Exp. Immunol. 79:315-321 (1990); Kostelny et al. J. Immunol. 148, 1547-1553 (1992). In certain embodiments, the bispecific antibody comprises a first binding domain polypeptide, such as a Fab' fragment, linked via an immunoglobulin constant region to a second binding domain polypeptide.

[0139] Antibodies of the present invention can also be single domain antibodies. Single domain antibodies can include antibodies whose complementary determining regions are part of a single domain polypeptide. Examples include, but are not limited to,
heavy chain antibodies, antibodies naturally devoid of light chains, single domain antibodies derived from conventional 4-chain antibodies, engineered antibodies and single domain scaffolds other than those derived from antibodies. Single domain antibodies may be any of the art, or any future single domain antibodies. Single domain antibodies may be derived from any species including, but not limited to mouse, human, camel, llama, fish, shark, goat, rabbit, and bovine. In one aspect of the invention, a single domain antibody can be derived from a variable region of the immunoglobulin found in fish, such as, for example, that which is derived from the immunoglobulin isotype known as Novel Antigen Receptor (NAR) found in the serum of shark. Methods of producing single domain antibodies derived from a variable region of NAR ("IgNARs") are described in WO 03/014161 and Streltsov (2005) Protein Sci. 14:2901-2909.

[0140] According to another aspect of the invention, a single domain antibody is a naturally occurring single domain antibody known as heavy chain antibody devoid of light chains. Such single domain antibodies are disclosed in WO 9404678, for example. For clarity reasons, this variable domain derived from a heavy chain antibody naturally devoid of light chain is known herein as a VHH or nanobody to distinguish it from the conventional VH of four chain immunoglobulins. Such a VHH molecule can be derived from antibodies raised in Camelidae species, for example in camel, llama, dromedary, alpaca and guanaco. Other species besides Camelidae may produce heavy chain antibodies naturally devoid of light chain; such VHHs are within the scope of the invention.

[0141] The invention also contemplates the use of called Modular Immunopharmaceuticals ("SMIPs™") which typically refers to binding domain-immunoglobulin fusion proteins including a binding domain polypeptide that is fused or otherwise connected to an immunoglobulin hinge or hinge-acting region polypeptide, which in turn is fused or otherwise connected to a region comprising one or more native or engineered constant regions from an immunoglobulin heavy chain, other than CH1, for example, the CH2 and CH3 regions of IgG and IgA, or the CH3 and CH4 regions of IgE (see e.g., U.S. 2005/0136049 by Ledbetter, J. et al. for a more complete description). The binding domain-immunoglobulin fusion protein can further include a region that includes a native or engineered immunoglobulin heavy chain CH2 constant region polypeptide (or
CH3 in the case of a construct derived in whole or in part from IgE) that is fused or otherwise connected to the hinge region polypeptide and a native or engineered immunoglobulin heavy chain CH3 constant region polypeptide (or CH4 in the case of a construct derived in whole or in part from IgE) that is fused or otherwise connected to the CH2 constant region polypeptide (or CH3 in the case of a construct derived in whole or in part from IgE). Typically, such binding domain-immunoglobulin fusion proteins are capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity, complement fixation, and/or binding to a target, for example, a target antigen.

[0142] In certain embodiments, therapeutic proteins, i.e., a protein or peptide that has a biological effect on a region in the body on which it acts or on a region of the body on which it remotely acts via intermediates, and method of designing and making these therapeutic proteins, are provided. Therapeutic proteins of the current invention can include peptide mimetics. Mimetics are peptide-containing molecules that mimic elements of protein secondary structure. See, for example, Johnson et al., "Peptide Turn Mimetics" in BIOTECHNOLOGY AND PHARMACY, Pezzuto et al., Eds., Chapman and Hall, New York (1993), incorporated herein by reference. The underlying rationale behind the use of peptide mimetics is that the peptide backbone of proteins exists chiefly to orient amino acid side chains in such a way as to facilitate molecular interactions, such as those of antibody and antigen. A peptide mimic is expected to permit molecular interactions similar to the natural molecule. In conjunction with the information provided by the current invention, these principles may be used to engineer second generation molecules having many of the natural properties of the targeting peptides disclosed herein. These second generation molecules can also be altered and provide potentially improved characteristics. Using the current invention, both protein and small molecule therapeutics can be designed to interrupt the desired cytokine activity by, for example, being specifically designed to bind at the desired positions, i.e., at the amino acid positions demonstrated as being important for a binding complex, and therefore effectively reducing or inhibiting the activity associated with the cytokine and its receptor or receptor complex.
[0143] Other embodiments of therapeutic proteins include fusion proteins. These molecules generally have all or a substantial portion of a targeting peptide, for example, IL-22 or an anti-IL-22 antibody, linked at the N- or C-terminus, to all or a portion of a second polypeptide or protein. For example, fusions may employ leader sequences from other species to permit the recombinant expression of a protein in a heterologous host. Another useful fusion includes the addition of an immunologically active domain, such as an antibody epitope, to facilitate purification of the fusion protein. Inclusion of a cleavage site at or near the fusion junction will facilitate removal of the extraneous polypeptide after purification. Other useful fusions include linking of functional domains, such as active sites from enzymes, glycosylation domains, cellular targeting signals or transmembrane regions. Examples of proteins or peptides that may be incorporated into a fusion protein include cytostatic proteins, cytocidal proteins, pro-apoptosis agents, anti-angiogenic agents, hormones, cytokines, growth factors, peptide drugs, antibodies, Fab fragments of antibodies, antigens, receptor proteins, enzymes, lectins, MHC proteins, cell adhesion proteins and binding proteins. Methods of generating fusion proteins are well known to those of skill in the art. Such proteins can be produced, for example, by chemical attachment using bifunctional cross-linking reagents, by de novo synthesis of the complete fusion protein, or by attachment of a DNA sequence encoding the targeting peptide to a DNA sequence encoding the second peptide or protein, followed by expression of the intact fusion protein.

[0144] In certain embodiments, the targeting peptide, for example, IL-22 or an anti-IL-22 antibody, is fused with an immunoglobulin heavy chain constant region, such as an Fc fragment, which contains two constant region domains and a hinge region but lacks the variable region (See, U.S. Pat. Nos. 6,018,026 and 5,750,375, incorporated herein by reference). The Fc region may be a naturally occurring Fc region, or may be altered to improve certain qualities, such as therapeutic qualities, circulation time, reduced aggregation, etc. Peptides and proteins fused to an Fc region typically exhibit a greater half-life in vivo than the unfused counterpart. Also, a fusion to an Fc region permits dimerization/multimerization of the fusion polypeptide.

[0145] In certain embodiments, mutagenesis is used to make an antibody more similar to one or more germline sequences. This may be desirable when mutations are
introduced into the framework region of an antibody through somatic mutagenesis or through error prone PCR. Germline sequences for the \( V_H \) and \( V_L \) domains can be identified by performing amino acid and nucleic acid sequence alignments against the VBASE database (MRC Center for Protein Engineering, UK). VBASE is a comprehensive directory of all human germline variable region sequences compiled from over a thousand published sequences, including those in the current releases of the Genbank and EMBL data libraries. In some embodiments, the FR regions of the scFvs are mutated in conformity with the closest matches in the VBASE database and the CDR portions are kept intact.

[0146] Using recombinant DNA methodology, a disclosed CDR sequence may be introduced into a repertoire of \( V_H \) or \( V_L \) domains lacking the respective CDR (Marks et al. (BioTechnology (1992) 10: 779-783). For example, a primer adjacent to the 5' end of the variable domain and a primer to the third FR can be used to generate a repertoire of variable domain sequences lacking CDR3. This repertoire can be combined with a CDR3 of a disclosed antibody. Using analogous techniques, portions of a disclosed CDR sequence may be shuffled with portions of CDR sequences from other antibodies to provide a repertoire of antigen-binding fragments that bind IL-22. Either repertoire can be expressed in a host system such as phage display (described in WO 92/01047 and its corresponding U.S. Patent No. 5,969,108) so suitable antigen-binding fragments that bind to IL-22 can be selected.


[0149] A portion of a variable domain will comprise at least one CDR region substantially as set out herein and, optionally, intervening framework regions from the \( V_H \) or \( V_L \) domains as set out herein. The portion may include the C-terminal half of FR1 and/or the N-terminal half of FR4. Additional residues at the N-terminal or C-terminal
end of the variable domain may not be the same residues found in naturally occurring antibodies. For example, construction of antibodies by recombinant DNA techniques often introduces N- or C-terminal residues from its use of linkers. Some linkers may be used to join variable domains to other variable domains (e.g., diabodies), constant domains, or proteinaceous labels.

[0150] The disclosed antibodies can be modified to alter their glycosylation; that is, at least one carbohydrate moiety can be deleted or added to the antibody. Deletion or addition of glycosylation sites can be accomplished by changing amino acid sequence to delete or create glycosylation consensus sites, which are well known in the art. Another means of adding carbohydrate moieties is the chemical or enzymatic coupling of glycosides to amino acid residues of the antibody (see WO 87/05330 and Aplin et al. (1981) CRC Crit. Rev. Biochem., 22: 259-306). Removal of carbohydrate moieties can also be accomplished chemically or enzymatically (see Hakimuddin et al. (1987) Arch. Biochem. Biophys., 259: 52; Edge et al. (1981) Anal. Biochem., 118: 131; Thotakura et al. (1987) Meth. Enzymol, 138: 350).

[0151] Methods for altering an antibody constant region are known in the art. Antibodies with altered function (e.g., altered affinity for an effector ligand such as FcR on a cell or the C1 component of complement) can be produced by replacing at least one amino acid residue in the constant portion of the antibody with a different residue (see e.g., EP 388,151 Al, US 5,624,821 and US 5,648,260). Similar types of alterations could be described which if applied to a murine or other species antibody would reduce or eliminate similar functions.

[0152] For example, it is possible to alter the affinity of an Fc region of an antibody (e.g., an IgG, such as a human IgG) for FcR (e.g., Fc gamma RI) or Clq. The affinity may be altered by replacing at least one specified residue with at least one residue having an appropriate functionality on its side chain, or by introducing a charged functional group, such as glutamate or aspartate, or perhaps an aromatic non-polar residue such as phenylalanine, tyrosine, tryptophan or alanine (see e.g., US 5,624,821). 

[0153] In another example, replacing residue 297 (asparagine) with alanine in the IgG constant region significantly inhibits recruitment of effector cells, while only slightly reducing (about three fold weaker) affinity for Clq (see e.g., US 5,624,821). The
numbering of the residues in the heavy chain is that of the EU index (see Kabat et al., 1991 *supra*). This alteration destroys the glycosylation site and it is believed that the presence of carbohydrate is required for Fc receptor binding. Any other substitution at this site that destroys the glycosylation site is believed to cause a similar decrease in lytic activity. Other amino acid substitutions, e.g., changing any one of residues 318 (Glu), 320 (Lys) and 322 (Lys), to Ala, are also known to abolish Clq binding to the Fc region of IgG antibodies (see e.g., US 5,624,821).

[0154] Modified antibodies can be produced which have a reduced interaction with an Fc receptor. For example, it has been shown that in human IgG3, which binds to the human Fc gamma R1 receptor, changing Leu 235 to Glu destroys its interaction with the receptor. Mutations on adjacent or close sites in the hinge link region of an antibody (e.g., replacing residues 234, 236 or 237 with Ala) can also be used to affect antibody affinity for the Fc gamma R1 receptor. The numbering of the residues in the heavy chain is based in the EU index (see Kabat et al., 1991 *supra*).

[0155] Additional methods for altering the lytic activity of an antibody, for example, by altering at least one amino acid in the N-terminal region of the CH2 domain, are described in WO 94/29351 by Morgan et al. and US 5,624,821.

[0156] In certain embodiments, antibodies may be tagged with a detectable or functional label. These labels include radiolabels (e.g., 131I or 99Tc), enzymatic labels (e.g., horseradish peroxidase or alkaline phosphatase), and other chemical moieties (e.g., biotin).

[0157] In certain embodiments, the IL-22 antagonists are antibodies, or fragments thereof (e.g., antigen-binding fragments thereof), that bind to mammalian (e.g., human or murine) IL-22. In certain embodiments, the anti-IL22 antibody or fragment thereof (e.g., an Fab, F(ab')2, Fv or a single chain Fv fragment) is a monoclonal or single specificity antibody. The antibody or fragment thereof can also be a human, humanized, chimeric, or in vitro generated antibody against human IL-22.

[0158] The production of anti-IL-22 antibodies is described in more detail in U.S. Published Patent Application Nos. 2005-0042220 and 2007-0243589. One non-limiting example of an anti-IL22 antibody that interferes with IL-22 binding to IL-22R is referred to as "Ab-04" or "IL22-04" in US Published Patent Application No. 2005-
0042220. Ab-04 (also referred to herein as rat monoclonal antibody "P3/2") binds to human IL-22 and neutralizes human IL-22 activity. A hybridoma cell line producing Ab-04 has been deposited with the ATCC on June 5, 2003 and has been assigned ATCC accession number PTA-5255. Another non-limiting example of an anti-IL22 antibody that interferes with IL-22 binding to IL-10R2 is "Ab-02" or "IL22-02." Ab-02 (also referred to herein as rat monoclonal antibody "P3/3") binds to mouse and human IL-22 and neutralizes the activity of mouse and human IL-22. A hybridoma cell line producing Ab-02 has been deposited on June 5, 2003 with the ATCC and has been assigned ATCC accession number PTA-5254. Additional examples of IL-22 antibodies that reduce, inhibit or antagonize IL-22 activity are found in U.S. Published Patent Application No. 2007-0243589, which describes germlined antibodies identified as GIL50, GIL16, GIL45, GIL60, GIL68, GIL92, 062A09, 087B03, 166B06, 166G05, 354A08, 355B06, 355E04, 356Al 1, and 368D04.

[0159] In certain embodiments, an antibody is provided that binds to the wild-type human IL-22 but fails to bind to a mutant IL-22 comprising one or more point mutations.

In certain embodiments, an antibody is provided that binds to the wild-type human IL-22 but fails to bind to a mutant IL-22 wherein the mutant IL-22 comprises one or more of the following changes relative to wild-type human IL-22(a) the amino acid at position 34 of the mutant IL-22 is alanine; (b) the amino acid at position 52 of the mutant IL-22 is alanine; (c) the amino acid at position 56 of the mutant IL-22 is alanine; (d) the amino acid at position 61 of the mutant IL-22 is alanine; (e) the amino acid at position 66 of the mutant IL-22 is alanine; (f) the amino acid at position 83 of the mutant IL-22 is alanine; (g) the amino acid at position 88 of the mutant IL-22 is alanine; (h) the amino acid at position 113 of the mutant IL-22 is alanine; (i) the amino acid at position 121 of the mutant IL-22 is alanine; (j) the amino acid at position 122 of the mutant IL-22 is alanine; (k) the amino acid at position 125 of the mutant IL-22 is alanine; or (l) the amino acid at position 172 of the mutant IL-22 is alanine.

[0160] In certain embodiments, an antibody is provided that binds to the wild-type human IL-22 but fails to bind to a mutant IL-22 wherein the mutant IL-22 comprises one or more of the following changes relative to wild-type human IL-22: a) the amino
acid at position 57 of the mutant IL-22 is alanine; b) the amino acid at position 59 of the mutant IL-22 is alanine; c) the amino acid at position 67 of the mutant IL-22 is alanine; d) the amino acid at position 72 of the mutant IL-22 is alanine; e) the amino acid at position 159 of the mutant IL-22 is alanine; f) the amino acid at position 161 of the mutant IL-22 is alanine; g) the amino acid at position 162 of the mutant IL-22 is alanine; or h) the amino acid at position 169 of the mutant IL-22 is alanine.

[0161] In certain embodiments, an antibody is provided that binds to the wild-type human IL-22 but fails to bind to a mutant IL-22 wherein the mutant IL-22 comprises one or more of the following changes relative to wild-type human IL-22: a) the amino acid at position 67 of the mutant IL-22 is alanine; b) the amino acid at position 73 of the mutant IL-22 is alanine; c) the amino acid at position 83 of the mutant IL-22 is alanine; or d) the amino acid at position 162 of the mutant IL-22 is alanine.

Methods of Systematic Mutagenesis of Target Proteins

[0162] In certain embodiments, methods of systematic mutagenesis of a target protein are provided.

[0163] The term "systematic mutagenesis" refers to the creation of multiple different mutants of the same target protein. Examples of systematic mutagenesis include, but are not limited to, alanine scanning. In certain embodiments, each amino acid of a target protein is individually mutagenized. For example, and not limitation, if a target protein consists of 400 amino acids, in certain embodiments, 400 different mutants are created. In certain embodiments, less than all of the amino acids in a target protein are mutagenized. For example, and not limitation, in certain embodiments, 90%, 80%, 70%, 60%, 50%, or fewer of the amino acids of a target protein are mutagenized during systematic mutagenesis.

[0164] In certain embodiments, a method of systematic mutagenesis of a target protein comprising generating a series of ten or more different nucleic acid molecules is provided. In certain embodiments, each different nucleic acid molecule comprises a nucleic acid sequence that encodes a polypeptide comprising a different mutant of the target protein. In certain embodiments, each different polypeptide comprising a different mutant of the target protein further comprises a secretory sequence, a first tag, and a second tag. Secretory sequences are known by those of skill in the art and are
commonly used in molecular biology. Tags are also known by those of skill in the art and are commonly used in molecular biology. Examples of tags include, but are not limited to, FLAG tag, His tag, c-myc-tag, Maltose Binding Protein tag, Thioredoxin tag, GFP tag, and Glutathione-S-transferase tag.

[0165] In certain embodiments, ten or more different mutants of the target protein are expressed in separate wells. In certain such embodiments, the ten or more different mutants of the target protein are secreted into the liquid media of the separate wells. In certain embodiments, the ten or more different mutants of the target protein in the liquid media in the separate wells are quantitated using a first tag and a second tag. For example, and not limitation, in certain embodiments, a small sample of the media may be removed and subjected to a sandwich assay using the first tag and the second tag.

[0166] In certain embodiments, the use of two tags added to a polypeptide allows for a more accurate detection of a target protein than using a single tag and an epitope on the target protein. For example, and not limitation, if a target protein has been mutated, one or more of the epitopes that would have been present in the wild-type protein may be disrupted in the mutant protein. If an antibody used to quantitate that mutant protein in an assay happens to overlap with a disrupted epitope, the quantitation of the assay may be inaccurate due to the disrupted epitope. In comparison, if the same mutant were detected with two antibodies, each specific for a separate artificial tag added to the mutant protein, the chances of an inaccurate quantitation of the mutant protein are decreased.

[0167] In certain embodiments, the use of two artificial tags allows for the rapid systematic mutagenesis of a protein. In certain such embodiments, the presence of two artificial tags on each different mutant protein allows for the use of assays to quantitate the different proteins without having to be concerned about the effect of mutations to the target protein on the quantitation assay.

[0168] In certain embodiments, systematic mutagenesis comprises generating a series of 10 or more, 20 or more, 30 or more, 40 or more, 50 or more, 60 or more, 70 or more, 80 or more, 90 or more, or 100 or more different nucleic acid molecules wherein each different nucleic acid molecule comprises a nucleic acid sequence that encodes a polypeptide comprising a different mutant of the target protein.
[0169] In certain embodiments, systematic mutagenesis provides information concerning which residues in a protein are important for a particular function. In certain embodiments, systematic mutagenesis includes testing the mutated target proteins in an assay for a particular function. For example, and not limitation, systematic mutagenesis can be performed on a receptor to determine which amino acids are important for binding of the receptor to the receptor's ligand by testing the mutated target proteins in a ligand binding assay. Examples of systematic mutagenesis include, but are not limited to, analysis of amino acids of a receptor involved in binding to a ligand; analysis of the amino acids of a ligand involved in binding to a receptor; analysis of the amino acids of a first protein involved in binding to a second protein; and analysis of the amino acids of an enzyme that are involved in that enzyme's activity, e.g., the amino acids of a kinase involved in kinase activity.

[0170] In certain embodiments, identification of amino acids involved in a particular function aids in rational design and evaluation of antagonists and/or agonists of the target protein. For example, and not limitation, identification of amino acids of a first protein involved in binding to a second protein provides insight into which portions of the first protein are involved in binding to the second protein. That information can be used in the rational design and evaluation of antagonists and/or agonists of the first protein.

[0171] In certain embodiments, identification of amino acids involved in a particular function aids in identifying amino acids involved in a similar function in related proteins. In certain embodiments, the information from systematic mutagenesis can be combined with other information concerning related proteins to identify amino acids involved in a similar function in those related proteins. Examples of information that can be used to help identify amino acids involved in a similar function in related proteins includes, but is not limited to, sequence alignments, crystal structures of the target protein and/or related proteins, structural modeling based on amino acid sequences, and predicted structure and/or function based on analysis of amino acid sequences.

[0172] In certain embodiments, systematic mutagenesis is used to define which amino acids of the IL-22 protein sequence are important for the binding of IL-22 to IL-22R, IL-10R2, and/or IL-22BP. In certain embodiments, systematic mutagenesis of IL-22 allows for the design of binding fragments, antibodies, and fragments thereof that
specifically block one or more of IL-22 binding to IL-22R, IL-22 binding to IL-10R2, and IL-22 binding to IL-22BP.

[0173] The study of IL-22 as described herein is the first to explore by systematic mutagenesis a structure/ function relationship for an IL-10-like cytokine. The members of this group (i.e., IL-10, IL-19, IL-20, IL-22, IL-24, and IL-26) are proposed to have a conserved six α-helical structural and functional unit that is also shared with the interferons. The receptors for these cytokines and the interferons belong to the cytokine receptor family 2 (CRF 2) and studies have suggested that these receptors share a conserved structure. As described in detail below, single substitutions of amino acids in the DE loop of IL-22 derived from mammalian cells did not reduce binding to either IL-22R or IL-22R/IL-10R2 in the assays performed, indicating that none of the DE loop residues are singularly involved in IL-22 receptor binding. However, dimerization via the DE loop would be quite removed from the receptor binding sites described in this application and therefore dimerization via the DE loop would be compatible with the receptor binding sites described in this application.

Protein Structure and Crystallography

[0174] Structural data describing a crystal can be obtained, for example, by X-ray diffraction. X-ray diffraction data can be collected by a variety of sources, X-ray wavelengths and detectors. In some embodiments, rotating anodes and synchrotron sources (e.g., Advanced Light Source (ALS), Berkeley, California; or Advanced Photon Source (APS), Argonne, Illinois) can be used as the source(s) of X-rays. In certain embodiments, X-rays for generating diffraction data can have a wavelength of from about 0.5 Å to about 1.6 Å (e.g., about 0.7 Å, about 0.9 Å, about 1.0 Å, about 1.1 Å, about 1.3 Å, about 1.4 Å, about 1.5 Å, or about 1.6 Å). In some embodiments, area detectors and/or charge-couple devices (CCDs) can be used as the detector(s).

[0175] X-ray diffraction data of a crystal of a polypeptide can be used to obtain the structural coordinates of the atoms in the complex. The structural coordinates are Cartesian coordinates that describe the location of atoms in three-dimensional space in relation to other atoms in the complex. The structural coordinates can be modified by mathematical manipulation, such as by inversion or integer additions or subtractions. As such, structural coordinates are relative coordinates.
[0176] The structural coordinates of a polypeptide can be used to derive a representation (e.g., a two dimensional representation or three dimensional representation) of the polypeptide or a fragment of the polypeptide. Such representations can be useful for a number of applications, including, for example, the visualization, identification and characterization of an active site of the polypeptide. In certain embodiments, a three-dimensional representation can include the structural coordinates of a polypeptide according to certain coordinates ± a root mean square (rms) deviation from the alpha carbon atoms of amino acids of not more than about 1.5 Å (e.g., not more than about 1.0 Å, not more than about 0.5 Å).

[0177] RMS deviation is the square root of the arithmetic mean of the squares of the deviations from the mean, and is a way of expressing deviation or variation from structural coordinates. Conservative substitutions (see discussion below) of amino acids can result in a molecular representation having structural coordinates within the stated rms deviation. For example, two molecular models of polypeptides that differ from one another by conservative amino acid substitutions can have coordinates of backbone atoms within a stated rms deviation, such as less than about 1.5 Å (e.g., less than about 1.0 Å, less than about 0.5 Å). Backbone atoms of a polypeptide include the alpha carbon (Cα or CA) atoms, carbonyl carbon (C) atoms, and amide nitrogen (N) atoms.

[0178] Various software programs allow for the graphical representation of a set of structural coordinates to obtain a representation of a polypeptide or a fragment of the polypeptide. In general, such a representation should accurately reflect (relatively and/or absolutely) structural coordinates, or information derived from structural coordinates, such as distances or angles between features. In some embodiments, the representation is a two-dimensional figure, such as a stereoscopic two-dimensional figure. In certain embodiments, the representation is an interactive two-dimensional display, such as an interactive stereoscopic two-dimensional display. An interactive two-dimensional display can be, for example, a computer display that can be rotated to show different faces of a polypeptide or a fragment of a polypeptide. In some embodiments, the representation is a three-dimensional representation. As an example, a three-dimensional model can be a physical model of a molecular structure (e.g., a ball-and-stick model). As another example, a three dimensional representation can be a graphical representation of
a molecular structure (e.g., a drawing or a figure presented on a computer display). A two-dimensional graphical representation (e.g., a drawing) can correspond to a three-dimensional representation when the two-dimensional representation reflects three-dimensional information, for example, through the use of perspective, shading, or the obstruction of features more distant from the viewer by features closer to the viewer. In some embodiments, a representation can be modeled at more than one level. As an example, when the three-dimensional representation includes a polypeptide, the polypeptide can be represented at one or more different levels of structure, such as primary (amino acid sequence), secondary (e.g., α-helices and β-sheets), tertiary (overall fold), and quaternary (oligomerization state) structure. A representation can include different levels of detail. For example, the representation can include the relative locations of secondary structural features of a protein without specifying the positions of atoms. A more detailed representation could, for example, include the positions of atoms.

[0179] In some embodiments, a representation can include information in addition to the structural coordinates of the atoms in a polypeptide. For example, a representation can provide information regarding the shape of a solvent accessible surface, the van der Waals radii of the atoms of the model, and the van der Waals radius of a solvent (e.g., water). Other features that can be derived from a representation include, for example, electrostatic potential, the location of voids or pockets within a macromolecular structure, and the location of hydrogen bonds and salt bridges.

[0180] An agent that interacts with (e.g., binds) a polypeptide can be identified or designed by a method that includes using a representation of the polypeptide or a fragment of the polypeptide. Exemplary types of representations include the representations discussed above. In some embodiments, the representation can be of an analog polypeptide or polypeptide fragment. A candidate agent that interacts with the representation can be designed or identified by performing computer fitting analysis of the candidate agent with the representation. In general, an agent is a molecule. Examples of agents include polypeptides, nucleic acids (including DNA or RNA), steroids and non-steroidal organic compounds. An agent that interacts with a polypeptide can interact transiently or stably with the polypeptide. The interaction can be mediated by any of the
forces noted herein, including, for example, hydrogen bonding, electrostatic forces, hydrophobic interactions, and van der Waals interactions.

[0181] As noted above, X-ray crystallography can be used to obtain structural coordinates of a polypeptide. However, such structural coordinates can be obtained using other techniques including NMR techniques. Additional structural information can be obtained from spectral techniques (e.g., optical rotary dispersion (ORD), circular dichroism (CD)), homology modeling, and computational methods (e.g., computational methods that can include data from molecular mechanics, computational methods that include data from dynamics assays).

[0182] In some embodiments, the X-ray diffraction data can be used to construct an electron density map of a polypeptide or a fragment of the polypeptide, and the electron density map can be used to derive a representation (e.g., a two dimensional representation, a three dimensional representation) of the polypeptide. Creation of an electron density map typically involves using information regarding the phase of the X-ray scatter. Phase information can be extracted, for example, either from the diffraction data or from supplementing diffraction experiments to complete the construction of the electron density map. Methods for calculating phase from X-ray diffraction data include, for example, multiwavelength anomalous dispersion (MAD), multiple isomorphous replacement (MIR), multiple isomorphous replacement with anomalous scattering (MIRAS), single isomorphous replacement with anomalous scattering (SIRAS), reciprocal space solvent flattening, molecular replacement, or any combination thereof. These methods generate phase information by making isomorphous structural modifications to the native protein, such as by including a heavy atom or changing the scattering strength of a heavy atom already present, and then measuring the diffraction amplitudes for the native protein and each of the modified cases. If the position of the additional heavy atom or the change in its scattering strength is known, then the phase of each diffracted X-ray can be determined by solving a set of simultaneous phase equations. The location of heavy atom sites can be identified using a computer program, such as SHELXD (Bruker-AXS, Madison, WI) or SHELXS (Sheldrick, Institut Anorg. Chemie, Göttingen, Germany), and XPREP (Bruker-AXS, Madison, WI); and diffraction data can be processed using computer programs such as MOSFLM, SCALA,
SOLOMON, and SHARP ("The CCP4 Suite: Programs for Protein Crystallography," Acta Crystallogr. Sect. D, 54:905-921, 1997; deLa Fortelle and Brigogne, Meth. Enzym. 276:472-494, 1997). The phase of X-ray scatter for a crystalline polypeptide, for example, can be determined by SIRAS using crystals of a platinum derivative of the polypeptide. To create a platinum derivative of a crystalline polypeptide, the crystalline polypeptide can be soaked in a solution containing platinum. Phases obtained by SIRAS from the platinum derivative can then be refined using, for example, non-crystallographic symmetry (NCS) averaging and phase extension in a computer program such as DM (Cowtan and Main, Acta Cryst. D49:148-157, 1993). The resulting model can be further derived by molecular replacement with a second data set. For example, a model derived from a crystalline polypeptide having space group 1222 can be refined using molecule replacement with a data set from a crystalline polypeptide having space group P2_12_12. Phases obtained by SIRAS from crystals of the native crystalline polypeptide and the platinum derivative can then be used to create an electron density map of the polypeptide.

[0183] The electron density map can be used to derive a representation of a polypeptide or a fragment of a polypeptide by aligning a three-dimensional model of a polypeptide with the electron density map. For example, the electron density map corresponding to the native crystalline polypeptide can be aligned with the electron density map corresponding to the platinum derivative of the crystalline polypeptide complex derived by an isomorphous replacement method.

[0184] The alignment process results in a comparative model that shows the degree to which the calculated electron density map varies from the model of the previously known polypeptide or the previously known complex. The comparative model is then refined over one or more cycles (e.g., two cycles, three cycles, four cycles, five cycles, six cycles, seven cycles, eight cycles, nine cycles, ten cycles) to generate a better fit with the electron density map. Software programs such as CNS (Brunger et al., Acta Crystallogr. D54:905-921, 1998) and REFMAC (Collaborative Computational Project, Number 4. The CCP4 suite: Programs for Protein Crystallography, Acta Crystallogr. D50:760-776, 1994) can be used to refine the model. The quality of fit in the comparative model can be measured by, for example, an R_{factor} or R_{free} value. A smaller value of R_{factor} or R_{free} generally indicates a better fit. Misalignments in the
comparative model can be adjusted to provide a modified comparative model and a lower $R_{\text{free}}$ value. The adjustments can be based on information relating to variations of the polypeptide (e.g., sequence variation information, alternative structure information, heavy atom derivative information) as appropriate. As an example, in embodiments in which a model of a heavy atom derivative of a crystalline polypeptide is used, an adjustment can include fitting an approximate model of the native polypeptide over the model of the heavy atom derivative. As another example, in certain embodiments, an adjustment can include replacing an amino acid in the previously known polypeptide with an amino acid having a similar structure (a conservative amino acid change). When adjustments to the modified comparative model satisfy a best fit to the electron density map, the resulting model is that which is determined to describe the polypeptide or complex from which the X-ray data was derived. Methods of such processes are disclosed, for example, in Carter and Sweet, eds., "Macromolecular Crystallography" in Methods in Enzymology, Vol. 277, Part B, New York: Academic Press, 1997, and articles therein, e.g., Jones and Kjeldgaard, "Electron-Density Map Interpretation," p. 173, and Kleywegt and Jones, "Model Building and Refinement Practice," p. 208.

[0185] A machine, such as a computer, can be programmed in memory with the structural coordinates of a polypeptide together with a program capable of generating a graphical representation of the structural coordinates on a display connected to the machine. A software system can also be designed and/or utilized to accept and store the structural coordinates. The software system can be capable of generating a graphical representation of the structural coordinates. The software system can also be capable of accessing external databases to identify one or more candidate agents likely to interact with the polypeptide.

[0186] A machine having a memory containing structure data or a software system containing such data can aid in the rational design or selection of a polypeptide agonist or antagonist of a polypeptide. For example, such a machine or software system can aid in the evaluation of the ability of an agent to associate with the polypeptide, or can aid in the modeling of compounds or proteins related by structural or sequence homology to the polypeptide.
[0187] The machine can produce a representation (e.g., a two dimensional representation, a three dimensional representation) of the polypeptide or a fragment of the polypeptide. A software system, for example, can cause the machine to produce such information. The machine can include a machine-readable data storage medium including a data storage material encoded with machine-readable data. The machine-readable data can include structural coordinates of atoms of the polypeptide. Machine-readable storage media (e.g., data storage material) include, for example, conventional computer hard drives, floppy disks, DAT tape, CD-ROM, DVD, and other magnetic, magneto-optical, optical, and other media which may be adapted for use with a machine (e.g., a computer). The machine can also have a working memory for storing instructions for processing the machine-readable data, as well as a central processing unit (CPU) coupled to the working memory and to the machine-readable data storage medium for the purpose of processing the machine-readable data into the desired three-dimensional representation. A display can be connected to the CPU so that the three-dimensional representation can be visualized by the user. Accordingly, when used with a machine programmed with instructions for using the data (e.g., a computer loaded with one or more programs of the sort described herein) the machine is capable of displaying a graphical representation (e.g., a two dimensional graphical representation, a three-dimensional graphical representation) of any of the polypeptides, polypeptide fragments, complexes, or complex fragments described herein.

[0188] A display (e.g., a computer display) can show a representation of a polypeptide or a fragment of a polypeptide. The user can inspect the representation and, using information gained from the representation, generate a model of the polypeptide or polypeptide fragment bound to a ligand. The model can be generated, for example, by altering a previously existing representation of the polypeptide. Optionally, the user can superimpose a three-dimensional model of an agent on the representation of the polypeptide. The agent can be an agonist or antagonist of the polypeptide. In some embodiments, the agent can be a known compound or a fragment of a known compound. In certain embodiments, the agent can be a previously unknown compound, or a fragment of a previously unknown compound.
It can be desirable for the agent to have a shape that complements the shape of the active site. There can be a preferred distance, or range of distances, between atoms of the agent and atoms of a polypeptide. Distances longer than a preferred distance may be associated with a weak interaction between the agent and active site. Distances shorter than a preferred distance may be associated with repulsive forces that can weaken the interaction between the agent and the polypeptide. A steric clash can occur when distances between atoms are too short. A steric clash occurs when the locations of two atoms are unreasonably close together, for example, when two atoms are separated by a distance less than the sum of their van der Waals radii. If a steric clash exists, the user can adjust the position of the agent relative to the polypeptide (e.g., a rigid body translation or rotation of the agent) until the steric clash is relieved. The user can adjust the conformation of the agent or of the polypeptide in the vicinity of the agent in order to relieve a steric clash. Steric clashes can also be removed by altering the structure of the agent, for example, by changing a "bulky group," such as an aromatic ring, to a smaller group, such as to a methyl or hydroxyl group, or by changing a rigid group to a flexible group that can accommodate a conformation that does not produce a steric clash. Electrostatic forces can also influence an interaction between an agent and a ligand-binding domain. For example, electrostatic properties can be associated with repulsive forces that can weaken the interaction between the agent and the polypeptide. Electrostatic repulsion can be relieved by altering the charge of the agent, e.g., by replacing a positively charged group with a neutral group.

Forces that influence binding strength between a candidate agent and a polypeptide, respectively, can be evaluated in the polypeptide/agent model. These can include, for example, hydrogen bonding, electrostatic forces, hydrophobic interactions, van der Waals interactions, dipole-dipole interactions, π-π interactions. The user can evaluate these forces visually, for example by noting a hydrogen bond donor/acceptor pair arranged with a distance and angle suitable for a hydrogen bond. Based on the evaluation, the user can alter the model to find a more favorable interaction between the polypeptide and the agent. Altering the model can include changing the three-dimensional structure of the polypeptide without altering its chemical structure, for example by altering the conformation of amino acid side chains or...
backbone dihedral angles. Altering the model can include altering the position or
conformation of the agent, as described above. Altering the model can also include
altering the chemical structure of the agent, for example by substituting, adding, or
removing groups. For example, if a hydrogen bond donor on the polypeptide is located
near a hydrogen bond donor on the agent, the user can replace the hydrogen bond donor
on the agent with a hydrogen bond acceptor.

[0191] The relative locations of an agent and a polypeptide, or their
conformations, can be adjusted to find an optimized binding geometry for a particular
agent to the polypeptide. An optimized binding geometry is characterized by, for
example, favorable hydrogen bond distances and angles, maximal electrostatic
attractions, minimal electrostatic repulsions, the sequestration of hydrophobic moieties
away from an aqueous environment, and the absence of steric clashes. The optimized
geometry can have the lowest calculated energy of a family of possible geometries for the
polypeptide/agent complex. An optimized geometry can be determined, for example,
through molecular mechanics or molecular dynamics calculations.

[0192] A series of representations of a polypeptide having different bound
agents can be generated. A score can be calculated for each representation. The score
can describe, for example, an expected strength of interaction between the polypeptide
and the agent. The score can reflect one of the factors described above that influence
binding strength. The score can be an aggregate score that reflects more than one of the
factors. The different agents can be ranked according to their scores.

[0193] Steps in the design of the agent can be carried out in an automated
fashion by a machine. For example, a representation of the polypeptide can be
programmed in the machine, along with representations of candidate agents. The
machine can find an optimized binding geometry for each of the candidate agents to an
active site, and calculate a score to determine which of the agents in the series is likely to
interact most strongly with the polypeptide.

[0194] A software system can be designed and/or implemented to facilitate
these steps. Software systems (e.g., computer programs) used to generate representations
or perform the fitting analyses include, for example: MCSS, Ludi, QUANTA®
(macromolecular X-ray crystallography software), Insight II® (biological compound
modeling and simulation software), Cerius® (modeling and simulation software), CHARMm® (software for simulation of biological macromolecules), and Modeler from Accelrys, Inc. (San Diego, CA); SYBYL® (molecular modeling software), Unity, FleXX, and LEAPFROG from TRIPOS, Inc. (St. Louis, MO); AUTODOCK (Scripps Research Institute, La Jolla, CA); GRID (Oxford University, Oxford, UK); DOCK (University of California, San Francisco, CA); and Flo+ and Flo99 (Thistlesoft, Morris Township, NJ).


The agent can, for example, be selected by screening an appropriate database, can be designed de novo by analyzing the steric configurations and charge potentials of a polypeptide in conjunction with the appropriate software systems, and/or can be designed using characteristics of known ligands. The method can be used to design or select agonists or antagonists of the polypeptide. A software system can be designed and/or implemented to facilitate database searching, and/or agent selection and design.

Once an agent has been designed or identified, it can be obtained or synthesized and further evaluated for its effect on the activity of the polypeptide. For example, the agent can be evaluated by contacting it with the polypeptide and measuring the effect of the agent on an activity of the polypeptide. A method for evaluating the agent can include an activity assay performed in vitro or in vivo.

An activity assay can be a cell-based assay, for example. A cell based assay can include monitoring the effect of a candidate agent on myelin production. Such assays for polypeptide inhibitors may involve contacting a candidate inhibitor with cells
expressing the polypeptide and assaying for an effect on cell morphology. Protein levels can be assayed by standard protein detection techniques, such as immunohistochemistry by Western blot analysis or in situ hybridization in cultured cells or whole tissue sections.

[0198] Depending upon the action of the agent on the polypeptide, the agent can act either as an agonist or antagonist of an activity of the polypeptide. A crystal containing the polypeptide bound to the identified agent can be grown and the structure determined by X-ray crystallography. A second agent can be designed or identified based on the interaction of the first agent with the polypeptide.

[0199] Various molecular analysis and rational drug design techniques are further disclosed in, for example, U.S. Patent Nos. 5,834,228, 5,939,528 and 5,856,116, as well as in PCT Application No. PCT/US98/16879, published as WO 99/09148.

Small Molecules

[0200] Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. These types of non-peptide compounds are termed "peptide mimetics" or "peptidomimetics". Fauchere, J Adv. Drug Res. 15:29 (1986); Veber and Freidinger TINS p.392 (1985); and Evans et al. J Med. Chem. 30:1229 (1987). Such compounds are often developed with the aid of computerized molecular modeling. Peptide mimetics that are structurally similar to therapeutically useful peptides may be used to produce a similar therapeutic or prophylactic effect. In certain embodiments, peptidomimetics are structurally similar to a paradigm polypeptide (i.e., a polypeptide that has a biochemical property or pharmacological activity), such as human antibody, but have one or more peptide linkages optionally replaced by a linkage selected from: -CH₂-NH-, -CH₂-S-, -CH₂-CH₂-CH₂-, -CH=CH-(cis and trans), -COCH₂-CH₂-, -CH(OH)CH₂-, and -CH₂SO₂-, by methods well known in the art. Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (e.g., D-lysine in place of L-lysine) may be used in certain embodiments to generate more stable peptides. In addition, constrained peptides comprising a consensus sequence or a substantially identical consensus sequence variation may be generated by methods known in the art (Rizo and Gierasch Ann. Rev. Biochem. 61:387 (1992)); for example, by adding internal
cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide.

[0201] Methods of designing peptidomimetics can be combined with other methods useful in elucidating the structure of epitopes and/or the identification of small molecules that bind to such epitopes, including, but not limited to, alanine scanning, high throughput screening, Structural Activity Relationship ("SAR") analysis, X-ray crystallography, medicinal chemistry, NMR spectroscopy, computer analysis, sequence alignments, and predictions of three-dimensional protein structure. Examples of methods to screen for small molecules that disrupt protein-protein interactions are known in the art, e.g., Wells et al., *Nature* 450:1001-9 (2007) and Voronkov et al., *Journal of Molecular Graphics and Modeling* 26:1 179-87 (2008).

[0202] In certain embodiments, an epitope defined on a target protein comprises amino acid residues that are not contiguous in the polymer chain. In certain such embodiments, small molecules, such as peptidomimetics can be designed to mimic the structure of the defined epitope. Computer programs are known in the art to aid in designing small molecules, including, but not limited to, peptidomimetics. In certain embodiments, computer programs known in the art can aid in designing peptidomimetics that mimic at least a portion of an epitope based on a crystal structure or predicted structure of a protein. Examples of computer programs that can used to design a peptidomimetic include, but are not limited to, Charmm, InsightII Glide, Maestro and Macromodel. In certain embodiments, a peptidomimetic designed to mimic a defined epitope binds to the same molecule that is naturally bound by the defined epitope.

[0203] In certain embodiments, a peptidomimetic is provided that mimics an epitope comprising two or more of the following amino acids of IL-22: A34, 152, T56, K61, A66, V83, R88, P113, F121, L122, L125, or M172. In certain such embodiments, the peptidomimetic binds to IL-10R2. In certain embodiments, the peptidomimetic that mimics an epitope is a peptidomimetic that mimics an epitope further comprising one or more of the following amino acids of IL-22: Y51, N54, R55, Y114, or E117. In certain embodiments, the peptidomimetic that mimics an epitope is a peptidomimetic that mimics an epitope further comprising one or more of the following amino acids of IL-22: F57, L59, D67, T70, D71, V72, R73, G159, 1161, K162, G165, or L169. In certain
embodiments, the peptidomimetic that mimics an epitope is a peptidomimetic that mimics an epitope further comprising one or more of the following amino acids of IL-22: D67, R73, or K162. In certain embodiments, a peptidomimetic that mimics an epitope comprising two or more amino acids of IL-22 binds to one or more of IL-10R2, IL-22R, or IL-22BP.

[0204] In certain embodiments, a method of making a peptidomimetic that mimics an epitope comprising two or more of the following amino acids of IL-22: A34, 152, T56, K61, A66, V83, R88, P113, F121, L122, L125, or M172, is provided. In certain embodiments, the peptidomimetic that mimics an epitope is a peptidomimetic that mimics an epitope further comprising one or more of the following amino acids of IL-22: Y51, N54, R55, Y114, or E117. In certain embodiments, the peptidomimetic that mimics an epitope is a peptidomimetic that mimics an epitope further comprising one or more of the following amino acids of IL-22: F57, L59, D67, T70, D71, V72, R73, G159, 1161, K162, G165, or L169. In certain embodiments, the peptidomimetic that mimics an epitope is a peptidomimetic that mimics an epitope further comprising one or more of the following amino acids of IL-22: D67, R73, or K162. In certain embodiments, the method of making a peptidomimetic comprises structural modeling of the epitope through the use of a computer.

[0205] In certain embodiments, a peptidomimetic is provided that mimics an epitope comprising two or more of the following amino acids of IL-22: F57, L59, D67, V72, G159, 1161, K162, or L169. In certain such embodiments, the peptidomimetic binds to IL-22R. In certain embodiments, the peptidomimetic that mimics an epitope is a peptidomimetic that mimics an epitope further comprising one or more of the following amino acids of IL-22: T70, D71, R73, or G165. In certain embodiments, the peptidomimetic that mimics an epitope is a peptidomimetic that mimics an epitope further comprising one or more of the following amino acids of IL-22: A34, Y51, 152, N54, R55, T56, K61, A66, V83, R88, P113, Y114, E117, F121, L122, L125, or M172. In certain embodiments, the peptidomimetic that mimics an epitope is a peptidomimetic that mimics an epitope further comprising one or more of the following amino acids of IL-22: R73 or V83. In certain embodiments, a peptidomimetic that mimics an epitope
comprising two or more amino acids of IL-22 binds to one or more of IL-10R2, IL-22R, or IL-22BP.

[0206] In certain embodiments, a method of making a peptidomimetic that mimics an epitope comprising two or more of the following amino acids of IL-22: F57, L59, D67, V72, G159, 1161, K162, or L169. In certain embodiments, the peptidomimetic that mimics an epitope is a peptidomimetic that mimics an epitope further comprising one or more of the following amino acids of IL-22: T70, D71, R73, or G165. In certain embodiments, the peptidomimetic that mimics an epitope is a peptidomimetic that mimics an epitope further comprising one or more of the following amino acids of IL-22: A34, Y51, 152, N54, R55, T56, K61, A66, V83, R88, P113, Y114, E117, F121, L122, L125, or M172. In certain embodiments, the peptidomimetic that mimics an epitope is a peptidomimetic that mimics an epitope further comprising one or more of the following amino acids of IL-22: R73 or V83. In certain embodiments, the method of making a peptidomimetic comprises structural modeling of the epitope through the use of a computer.

[0207] In certain embodiments, a peptidomimetic is provided that mimics an epitope comprising two or more of the following amino acids of IL-22: D67, R73, V83, and K162. In certain such embodiments, the peptidomimetic binds to IL-22BP. In certain embodiments, the peptidomimetic that mimics an epitope is a peptidomimetic that mimics an epitope further comprising one or more of the following amino acids of IL-22: A34, Y51, 152, N54, R55, T56, K61, A66, R88, P113, Y114, E117, F121, L122, L125, or M172. In certain embodiments, the peptidomimetic that mimics an epitope is a peptidomimetic that mimics an epitope further comprising one or more of the following amino acids of IL-22: F57, L59, T70, D71, V72, G159, 1161, G165, or L169. In certain embodiments, a peptidomimetic that mimics an epitope comprising two or more amino acids of IL-22 binds to one or more of IL-10R2, IL-22R, or IL-22BP.

[0208] In certain embodiments, a method of making a peptidomimetic that mimics an epitope comprising two or more of the following amino acids of IL-22: D67, R73, V83, or K162. In certain embodiments, the peptidomimetic that mimics an epitope is a peptidomimetic that mimics an epitope further comprising one or more of the following amino acids of IL-22: A34, Y51, 152, N54, R55, T56, K61, A66, R88, P113,
Y114, E117, F121, L122, L125, or M172. In certain embodiments, the peptidomimetic that mimics an epitope is a peptidomimetic that mimics an epitope further comprising one or more of the following amino acids of IL-22: F57, L59, T70, D71, V72, G159, 1161, G165, or L169. In certain embodiments, the method of making a peptidomimetic comprises structural modeling of the epitope through the use of a computer.

**Pharmaceutical Compositions**

[0209] IL-22 binding agents, e.g., IL-22 antagonists, (e.g., anti-IL-22 antibodies and fragments thereof (e.g., antigen-binding fragments thereof)) can be used as a pharmaceutical composition when combined with a pharmaceutically acceptable carrier. Such a composition may contain, in addition to the IL-22-agonists or antagonists and carrier, various diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration.

[0210] In certain embodiments, a pharmaceutical composition may be in the form of a liposome in which IL-22 antagonists are combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers which in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Pat. No. 4,235,871; U.S. Pat. No. 4,501,728; U.S. Pat. No. 4,837,028; and U.S. Pat. No. 4,737,323, all of which are incorporated herein by reference.

[0211] In certain embodiments, a therapeutically effective amount of an IL-22 antagonist is administered to a subject, e.g., mammal (e.g., a human). An IL-22 antagonist may be administered either alone or in combination with other therapies, e.g., anti-inflammatory agents described in more detail below. When co-administered with one or more agents, an IL-22 antagonist may be administered either simultaneously with the second agent, or sequentially. If administered sequentially, the attending physician
will decide on the appropriate sequence of administering an IL-22 antagonist in
combination with other agents.

[0212] Administration of an IL-22 antagonist used in a pharmaceutical
composition can be carried out in a variety of conventional ways, such as oral ingestion,
inhalation, or cutaneous, subcutaneous, or intravenous injection. In certain embodiments, intravenous administration to the patient is preferred.

[0213] When a therapeutically effective amount of an IL-22 antagonist is administered orally, the binding agent will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, a pharmaceutical composition may additionally contain a solid carrier such as a gelatin. The tablet, capsule, and powder contain from about 5 to 95% binding agent, and preferably from about 25 to 90% binding agent. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of the binding agent, and preferably from about 1 to 50% the binding agent.

[0214] When a therapeutically effective amount of an IL-22 antagonist is administered by intravenous, cutaneous or subcutaneous injection, binding agent will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. In certain embodiments, a pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to binding agent an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. In certain embodiments, a pharmaceutical composition may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art.
In certain embodiments, the amount of an IL-22 binding agent in a pharmaceutical composition will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments that the patient has undergone. In certain embodiments, the attending physician will decide the amount of binding agent with which to treat each individual patient. In certain embodiments, the attending physician will initially administer low doses of binding agent and observe the patient's response. In certain embodiments, larger doses of binding agent may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not generally increased further. In certain embodiments, the various pharmaceutical compositions should contain about 0.1 µg to about 100 mg IL-22 binding agent per kg body weight.

In certain embodiments, the duration of intravenous therapy will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. In certain embodiments, the duration of each application of the IL-22 binding agent will be in the range of 12 to 24 hours of continuous intravenous administration. In certain embodiments, the attending physician will decide on the appropriate duration of intravenous therapy using a pharmaceutical composition.

Uses of IL-22 Agonists and IL-22 Antagonists

IL-22 is a cytokine involved in pro-inflammatory actions, e.g., inducing an acute phase response. As described in U.S. Published Patent Application No. 2005-0042220, IL-22 induces changes associated with those caused by inflammatory cytokines (such as IL-1 and TNFα), and inhibitors of IL-22 ameliorate symptoms of rheumatoid arthritis. Therefore, IL-22, and/or agents that increase levels of IL-22 or mimic the actions of IL-22 are useful as agonists in certain clinical indications, and antagonists of this molecule are useful in other clinical situations, particularly in those in which modulation of an inflammatory state is desired. Whether the agonist or antagonist is preferred depends on the particular aspects of the disease pathology, such as the cell types involved, the nature of the stimulus and the cellular microenvironment.

Human IL-22 agonists include without limitation human IL-22 proteins and fragments, deletion mutants and addition mutants thereof; and peptide and small
molecule compounds that interact with the receptor or other target to which human IL-22 is directed. Human IL-22 antagonists include without limitation antibodies directed to human IL-22 proteins; soluble forms of the receptor or other target to which human IL-22 is directed; antibodies directed to the receptor or other target to which human IL-22 is directed; and peptide and small molecule compounds that inhibit or interfere with the interaction of human IL-22 with its receptor or other target.

[0219] In certain embodiments, a method is provided of inhibiting at least one IL-22-associated activity, by contacting a cell, e.g., an epithelial cell, with an IL-22 antagonist (e.g., an anti-IL-22 antibody or an antigen-binding fragment thereof), in an amount sufficient to inhibit the activity. Antagonists of IL-22 (e.g., a neutralizing antibody, as described herein) can also be administered to subjects for which inhibition of an immune IL-22-associated activity is desired. For example, inhibition of IL-22-associated activities would be desired in order to prevent, treat, ameliorate or reduce at least one symptom of an IL-22-associated disorder, such as, for example, autoimmune disorders, e.g., arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis, lupus-associated arthritis or ankylosing spondylitis), scleroderma, systemic lupus erythematosus, HIV, Sjogren's syndrome, vasculitis, multiple sclerosis, autoimmune thyroiditis, dermatitis (including atopic dermatitis and eczematous dermatitis), myasthenia gravis, inflammatory bowel disease (IBD), Crohn's disease, colitis, diabetes mellitus (type I); inflammatory conditions of, e.g., the skin (e.g., psoriasis), cardiovascular system (e.g., atherosclerosis), nervous system (e.g., Alzheimer's disease), liver (e.g., hepatitis), kidney (e.g., nephritis) and pancreas (e.g., pancreatitis); cardiovascular disorders, e.g., cholesterol metabolic disorders, oxygen free radical injury, ischemia; disorders associated with wound healing; respiratory disorders, e.g., asthma and COPD (e.g., cystic fibrosis); acute inflammatory conditions (e.g., endotoxemia, sepsis and septicaemia, toxic shock syndrome and infectious disease); transplant rejection and allergy. In one embodiment, the IL-22-associated disorder is, an arthritic disorder, e.g., a disorder chosen from one or more of rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis, or ankylosing spondylitis; a respiratory disorder (e.g., asthma, chronic obstructive pulmonary disease (COPD); or an inflammatory condition of, e.g., the skin (e.g., psoriasis), cardiovascular system (e.g.,
atherosclerosis), nervous system (e.g., Alzheimer's disease), liver (e.g., hepatitis), kidney (e.g., nephritis), pancreas (e.g., pancreatitis), and gastrointestinal organs, e.g., colitis, Crohn's disease and IBD.

[0220] Reduction of IL-22 activity by using a neutralizing anti-IL-22 antibody has been shown to ameliorate inflammatory symptoms in mouse collagen-induced arthritis (CIA) animal models. In addition, it has been demonstrated that expression of IL-22 mRNA is upregulated in the paws of CIA mice (See, e.g., U.S. Published Patent Application No. 2005-0042220, Examples 9 and 10). Accordingly, IL-22 antagonists can be used to induce immune suppression in vivo, e.g., for treating or preventing IL-22-associated disorders, in a subject.

[0221] As used herein, the term "subject" is intended to include human and non-human animals. Human animals include a human patient having a disorder characterized by abnormal IL-22 activity. The term "non-human animals" includes all vertebrates, e.g., mammals and non-mammals, such as non-human primates, rodents, sheep, llamas, camels, dogs, cows, sharks, fish, chickens, amphibians, reptiles, etc.

[0222] Non-limiting examples of IL-22-associated disorders that can be treated or prevented include, but are not limited to, transplant rejection, autoimmune diseases (including, for example, diabetes mellitus, arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis), multiple sclerosis, encephalomyelitis, myasthenia gravis, systemic lupus erythematosus, autoimmune thyroiditis, dermatitis (including atopic dermatitis and eczematous dermatitis), Sjogren's Syndrome, Crohn's disease, aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, spondyloarthopathy, ankylosing spondylitis, intrinsic asthma, allergic asthma, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug eruptions, leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, allergic encephalomyelitis, acute necrotizing hemorrhagic encephalopathy, idiopathic bilateral progressive sensorineural hearing loss, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Stevens-Johnson syndrome, idiopathic sprue, lichen planus, Graves' disease, sarcoidosis, primary biliary cirrhosis, uveitis posterior, and interstitial lung fibrosis); respiratory disorders, e.g., asthma or COPD; inflammatory conditions of the skin (e.g., psoriasis),...
liver (e.g., hepatitis), kidney (e.g., nephritis) and pancreas (e.g., pancreatitis); graft-versus-host disease, and allergy such as, atopic allergy; cancers (e.g., solid or soft tissue tumors), arthritic disorders (e.g., rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis, and ankylosing spondylitis), multiple sclerosis, type I diabetes, lupus (SLE), IBD, ulcerative colitis, Crohn's disease, COPD, asthma, vasculitis, allergy, scleroderma and inflammatory conditions of the skin (e.g., psoriasis), liver (e.g., hepatitis), kidney (e.g., nephritis) and pancreas (e.g., pancreatitis).

[0223] In certain embodiments, IL-22 antagonists, alone or in combination with, other therapeutic agents as described herein (e.g., TNF antagonists) can be used to treat multiple myeloma and related B lymphocytic malignancies (Brenne, A. et al. (2002) BloodNol. 99(10):3756-3762).

[0224] In certain embodiments, the IL-22 antagonists, e.g., pharmaceutical compositions thereof, are administered in combination therapy, i.e., combined with other agents, e.g., therapeutic agents that are useful for treating pathological conditions or disorders, such as immune and inflammatory disorders.

[0225] For example, the combination therapy can include one or more IL-22 antagonists, e.g., an antibody or an antigen-binding fragment thereof as described herein (e.g., a chimeric, humanized, human, or in vitro generated antibody or antigen-binding fragment thereof against IL-22) co-formulated with, and/or co-administered with, one or more additional therapeutic agents, e.g., one or more cytokine and growth factor inhibitors, immunosuppressants, anti-inflammatory agents, metabolic inhibitors, enzyme inhibitors, and/or cytotoxic or cytostatic agents, as described in more detail below. Furthermore, one or more IL-22 antagonists described herein may be used in combination with two or more of the therapeutic agents described herein.

[0226] Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies.

[0227] Accordingly, inhibition of IL-22 activity using, e.g., an anti-IL22 antibody or fragment thereof described herein, may provide a more effective tissue-specific, anti-inflammatory activity than systemic anti-inflammatory modalities as described herein. Furthermore, inhibition of local IL-22 activity using, e.g., an anti-IL22
antibody or fragment thereof described herein, may provide a useful candidate for combination with systemic anti-inflammatory modalities described herein.

[0228] In certain embodiments, one or more IL-22 antagonist described herein may be co-formulated with, and/or co-administered with, one or more additional agents such as other cytokine or growth factor antagonists (e.g., soluble receptors, peptide inhibitors, small molecules, ligand fusions); or antibodies or antigen-binding fragments thereof that bind to other targets (e.g., antibodies that bind to other cytokines or growth factors, their receptors, or other cell surface molecules); and anti-inflammatory cytokines or agonists thereof. Non-limiting examples of the agents that can be used in combination with the IL-22 antagonists described herein, include, but are not limited to, antagonists of one or more interleukins (ILs) or their receptors, e.g., antagonists of IL-1, IL-2, IL-6, IL-7, IL8, IL-12, IL-13, IL-15, IL-16, IL-18, and IL-21/IL-21R; antagonists of cytokines or growth factors or their receptors, such as tumor necrosis factor (TNF), LT, EMAP-II, GM-CSF, FGF and PDGF. IL-22 antagonists can also be combined with inhibitors of, e.g., antibodies to, cell surface molecules such as CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD80 (B7.1), CD86 (B7.2), CD90, or their ligands, including CD154 (gp39 or CD40L), or LFA-1/ICAM-1 and VLA-4/VCAM-1 (Yusuf-Makagiansar H. et al. (2002) Med Res Rev 22(2): 146-67). In certain embodiments, antagonists that can be used in combination with IL-22 antagonists described herein include antagonists of IL-1, IL-12, TNFα, IL-15, IL-17, IL-18, and IL-21/IL-21R.

[0229] Examples of those agents include, but are not limited to, IL-12 antagonists, such as chimeric, humanized, human or in vitro generated antibodies (or antigen-binding fragments thereof) that bind to IL-12 (preferably human IL-12), e.g., the antibody disclosed in WO 00/56772, Genetics Institute/BASF); IL-12 receptor inhibitors, e.g., antibodies to human IL-12 receptor; and soluble fragments of the IL-12 receptor, e.g., human IL-12 receptor. Examples of IL-15 antagonists include antibodies (or antigen-binding fragments thereof) against IL-15 or its receptor, e.g., chimeric, humanized, human or in vitro generated antibodies to human IL-15 or its receptor, soluble fragments of the IL-15 receptor, and IL-15-binding proteins. Examples of IL-18 antagonists include antibodies, e.g., chimeric, humanized, human or in vitro generated antibodies (or antigen-binding fragments thereof), to human IL-18, soluble fragments of
the IL-18 receptor, and IL-18 binding proteins (IL-18BP, Mallet et al. (2001) Circ. Res. 28). Examples of IL-1 antagonists include Interleukin-1-converting enzyme (ICE) inhibitors, such as Vx740, IL-1 antagonists, e.g., IL-IRA (ANIKINRA, AMGEN), sIL1RII (Immunex), and anti-IL-1 receptor antibodies (or antigen-binding fragments thereof).

[0230] Examples of TNF antagonists include, but are not limited to, chimeric, humanized, human or in vitro generated antibodies (or antigen-binding fragments thereof) to TNF (e.g., human TNFα antibody, US. 6,258,562; BASF), CDP-571/CDP-870/BAY-10-3356 (humanized anti-TNFα antibody; Celltech/Pharmacia), cA2 (chimeric anti-TNFa antibody; RemicadeTM, Centocor); anti-TNF antibody fragments (e.g., CPD870); soluble fragments of the TNF receptors, e.g., p55 or p75 human TNF receptors or derivatives thereof, e.g., 75 kdTNFR-IgG (75 kD TNF receptor-IgG fusion protein, EnbrelTM; Immunex; see e.g., Arthritis & Rheumatism (1994) Vol. 37, S295; J. Invest. Med. (1996) Vol. 44, 235A), p55 kdTNFR-IgG (55 kD TNF receptor-IgG fusion protein (Lenercept)); enzyme antagonists, e.g., TNFα converting enzyme (TACE) inhibitors (e.g., an alpha-sulfonyl hydroxamic acid derivative, WO 01/551 12, and N-hydroxyformamide TACE inhibitor GW 3333, -005, or -022); and TNF-bp/s-TNFR (soluble TNF binding protein; see e.g., Arthritis & Rheumatism (1996) Vol. 39, No. 9 (supplement), S284; Amer. J. Physiol. - Heart and Circulatory Physiology (1995) Vol. 268, pp. 37-42). In certain embodiments, TNF antagonists are soluble fragments of the TNF receptors, e.g., p55 or p75 human TNF receptors or derivatives thereof, e.g., 75 kdTNFR-IgG, and TNFα converting enzyme (TACE) inhibitors.

[0231] In certain embodiments, the IL-22 antagonists described herein can be administered in combination with one or more of the following: IL-13 antagonists, e.g., soluble IL-13 receptors (sIL-13) and/or antibodies against IL-13; IL-2 antagonists, e.g., DAB 486-IL-2 and/or DAB 389-IL-2 (IL-2 fusion proteins; Seragen; see e.g., Arthritis & Rheumatism (1993) Vol. 36, 1223), and/or antibodies to IL-2R, e.g., anti-Tac (humanized anti-IL-2R; Protein Design Labs, Cancer Res. 1990 Mar l;50(5):1495-502). Yet another combination includes IL-21 antagonists in combination with non-depleting anti-CD4 inhibitors (IDEC-CE9.1/SB 210396 (non-depleting primatized anti-CD4 antibody;
IDEC/SmithKline). In certain embodiments, combinations include antagonists of the co-stimulatory pathway CD80 (B7.1) or CD86 (B7.2) including antibodies, soluble receptors or antagonistic ligands; as well as p-selectin glycoprotein ligand (PSGL), anti-inflammatory cytokines, e.g., IL-4 (DNAX/Schering); IL-10 (SCH 52000; recombinant IL-10 DNAX/Schering); IL-13 and TGFβ, and agonists thereof (e.g., agonist antibodies).

[0232] In certain embodiments, one or more IL-22 antagonists can be co-formulated with, and/or co-administered with, one or more anti-inflammatory drugs, immunosuppressants, or metabolic or enzymatic inhibitors. Non-limiting examples of the drugs or inhibitors that can be used in combination with the IL-22 antagonists described herein, include, but are not limited to, one or more of: non-steroidal anti-inflammatory drug(s) (NSAIDs), e.g., ibuprofen, Tenidap (see e.g., Arthritis & Rheumatism (1996) Vol. 39, No. 9 (supplement), S280)), Naproxen (see e.g., Neuro Report (1996) Vol. 7, pp. 1209-1213), Meloxicam, Piroxicam, Diclofenac, and Indomethacin; Sulfasalazine (see e.g., Arthritis & Rheumatism (1996) Vol. 39, No. 9 (supplement), S281); corticosteroids such as prednisolone; cytokine suppressive anti-inflammatory drug(s) (CSAIDs); inhibitors of nucleotide biosynthesis, e.g., inhibitors of purine biosynthesis, folate antagonists (e.g., methotrexate (N-[4-[[2,4-diamino-6-pteridinyl)methyl]methylamino]benzoyl]-L-glutamic acid); and inhibitors of pyrimidine biosynthesis, e.g., dihydroorotate dehydrogenase (DHODH) inhibitors (e.g., leflunomide (see e.g., Arthritis & Rheumatism (1996) Vol. 39, No. 9 (supplement), S131; Inflammation Research (1996) Vol. 45, pp. 103-107). In certain embodiments, therapeutic agents for use in combination with IL-22 antagonists include NSAIDs, CSAIDs, (DHODH) inhibitors (e.g., leflunomide), and folate antagonists (e.g., methotrexate).

[0233] Examples of additional inhibitors include, but are not limited to, one or more of: corticosteroids (oral, inhaled and local injection); immunosuppressants, e.g., cyclosporin, tacrolimus (FK-506); and mTOR inhibitors, e.g., sirolimus (rapamycin) or rapamycin derivatives, e.g., soluble rapamycin derivatives (e.g., ester rapamycin derivatives, e.g., CCI-779 (Elit. L. (2002) Current Opinion Investig. Drugs 3(8): 1249-53; Huang, S. et al. (2002) Current Opinion invest. Drugs 3(2):295-304); agents which interfere with signaling by proinflammatory cytokines such as TNFα or IL-1 (e.g. IRAK,
NIX, IKK, p38 or MAP kinase inhibitors); C0X2 inhibitors, e.g., celecoxib and variants thereof, MK-966, see e.g., Arthritis & Rheumatism (1996) Vol. 39, No. 9 (supplement), S81); phosphodiesterase inhibitors, e.g., R973401 (phosphodiesterase Type IV inhibitor; see e.g., Arthritis & Rheumatism (1996) Vol. 39, No. 9 (supplement), S282)); phospholipase inhibitors, e.g., inhibitors of cytosolic phospholipase 2 (cPLA2) (e.g., trifluoromethyl ketone analogs (U.S. 6,350,892)); inhibitors of vascular endothelial cell growth factor or growth factor receptor, e.g., VEGF inhibitor and/or VEGF-R inhibitor; and inhibitors of angiogenesis. In certain embodiments, therapeutic agents for use in combination with IL-22 antagonists immunosuppressants, e.g., cyclosporin, tacrolimus (FK-506); and mTOR inhibitors, e.g., sirolimus (rapamycin) or rapamycin derivatives, e.g., soluble rapamycin derivatives (e.g., ester rapamycin derivatives, e.g., CCI-779; COX2 inhibitors, e.g., celecoxib and variants thereof; and phospholipase inhibitors, e.g., inhibitors of cytosolic phospholipase 2 (cPLA2) (e.g., trifluoromethyl ketone analogs).

[0234] Additional examples of therapeutic agents that can be combined with an IL-22 antagonist include, but are not limited to, one or more of: 6-mercaptopurines (6-MP); azathioprine sulphasalazine; mesalazine; olsalazine chloroquine/hydroxychloroquine; pencillamine; aurothiomalate (intramuscular and oral); azathioprine; cochicine; beta-2 adrenoreceptor agonists (salbutamol, terbutaline, salmeteral); xanthines (theophylline, aminophylline); cromoglycate; nedocromil; ketotifen; ipratropium and oxtropium; mycophenolate mofetil; adenosine agonists; antithrombotic agents; complement inhibitors; and adrenergic agents.

[0235] The use of the IL-22 antagonists disclosed herein in combination with other therapeutic agents to treat or prevent specific immune disorders is discussed in further detail below.

[0236] Non-limiting examples of agents for treating or preventing arthritic disorders (e.g., rheumatoid arthritis, inflammatory arthritis, rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis and psoriatic arthritis), with which an IL-22 antagonists can be combined include one or more of the following: IL-12 antagonists as described herein, NSAIDs; CSAIDs; TNF's, e.g., TNFα, antagonists as described herein; non-depleting anti-CD4 antibodies as described herein; IL-2 antagonists as described herein; anti-inflammatory cytokines, e.g., IL-4, IL-10, IL-13 and TGFα, or agonists
thereof; IL-1 or IL-1 receptor antagonists as described herein); phosphodiesterase inhibitors as described herein; COX-2 inhibitors as described herein; Iloprost (see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S82); methotrexate; thalidomide (see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S282) and thalidomide-related drugs (e.g., Celgen); leflunomide; inhibitor of plasminogen activation, e.g., tranexamic acid; see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S284); cytokine inhibitor, e.g., T-614; see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S282); prostaglandin El (see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S282); corticosteroid anti-inflammatory drugs (e.g., SB203580); TNF-convertase inhibitors; interleukin-i i (see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S296); interleukin-13 (see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S308); interleukin-17 inhibitors (see e.g., *Arthritis & Rheumatism* (1996) Vol. 39, No. 9 (supplement), S120); gold; penicillamine; chloroquine; hydroxychloroquine; chlorambucil; cyclophosphamide; cyclosporine; total lymphoid irradiation; anti-thymocyte globulin; CD5-toxins; orally-administered peptides and collagen; lobenzarit disodium; Cytokine Regulating Agents (CRAs) HP228 and HP466 (Houghten Pharmaceuticals, Inc.); ICAM-I antisense phosphorothioate oligodeoxynucleotides (ISIS 2302; Isis Pharmaceuticals, Inc.); soluble complement receptor 1 (TPIO; T Cell Sciences, Inc.); prednisone; orgotein; glycosaminoglycan polysulphate; minocycline; anti-IL2R antibodies; marine and botanical lipids (fish and plant seed fatty acids; see e.g., DeLuca et al. (1995) Rheum. Dis. Clin. North Am. 21:759-777); auranofin; phenylbutazone; meclofenamic acid; flufenamic acid; intravenous immune globulin; zileuton; mycophenolic acid (RS61443); tacrolimus (FK-506); sirolimus (rapamycin); amiprilose (therafectin); cladribine (2-chlorodeoxyadenosine); and azaribine. In certain embodiments, combinations include
one or more IL-21 antagonists in combination with methotrexate or leflunomide, and in moderate or severe rheumatoid arthritis cases, cyclosporine.

[0237] In certain embodiments, examples of inhibitors to use in combination with IL-22 antagonists to treat arthritic disorders include TNF antagonists (e.g., chimeric, humanized, human or in vitro generated antibodies, or antigen-binding fragments thereof, that bind to TNF; soluble fragments of a TNF receptor, e.g., p55 or p75 human TNF receptor or derivatives thereof, e.g., 75 kdTNFR-IgG (75 kD TNF receptor-IgG fusion protein, Enbrel™), p55 kD TNF receptor-IgG fusion protein; TNF enzyme antagonists, e.g., TNFa converting enzyme (TACE) inhibitors); antagonists of IL-12, IL-15, IL-17, IL-18, IL-21/IL-21R; T cell and B cell depleting agents (e.g., anti-CD4 or anti-CD22 antibodies); small molecule inhibitors, e.g., methotrexate and leflunomide; sirolimus (rapamycin) and analogs thereof, e.g., CCI-779; Cox-2 and cPLA2 inhibitors; NSAIDs; p38 inhibitors, TPL-2, Mk-2 and NFkb inhibitors; RAGE or soluble RAGE; P-selectin or PSGL-1 inhibitors (e.g., small molecule inhibitors, antibodies thereto, e.g., antibodies to P-selectin); estrogen receptor beta (ERB) agonists or ERB-NFkβ antagonists. Most additional therapeutic agents that can be co-administered and/or co-formulated with one or more IL-22 antagonists include one or more of: a soluble fragment of a TNF receptor, e.g., p55 or p75 human TNF receptor or derivatives thereof, e.g., 75 kd TNFRIgG (75 kD TNF receptor-IgG fusion protein, Enbrel™) methotrexate, leflunomide, or a sirolimus (rapamycin) or an analog thereof, e.g., CCI-779.

[0238] Non-limiting examples of agents for treating or preventing multiple sclerosis with which an IL-22 antagonists can be combined include the following: interferons, e.g., interferon-alphal α (e.g., Avonex™; Biogen) and interferon-1 β (Betason® Chiron/Berlex); Copolymer 1 (Cop-1; Copaxone™ Teva Pharmaceutical Industries, Inc.); hyperbaric oxygen; intravenous immunoglobulin; clabirinet; TNF antagonists as described herein; corticosteroids; prednisolone; methylprednisolone; azathioprine; cyclophosphamide; cyclosporine; methotrexate; 4-aminopyridine; and tizanidine. Additional antagonists that can be used in combination with IL-22 antagonists include antibodies to or antagonists of other human cytokines or growth factors, for example, TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-12 IL-15, IL-16, IL-18, EMAP-I 1, GMCSF, FGF, and PDGF. IL-21 antagonists as described herein can be combined with
antibodies to cell surface molecules such as CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD80, CD86, CD90 or their ligands. The IL-22 antagonists may also be combined with agents, such as methotrexate, cyclosporine, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, for example, ibuprofen, corticosteroids such as prednisolone, phosphodiesterase inhibitors, adenosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, agents which interfere with signaling by proinflammatory cytokines as described herein, IL-1β converting enzyme inhibitors (e.g., VX-740), anti-P7s, PSGL, TACE inhibitors, T-cell signaling inhibitors such as kinase inhibitors, metal loproteinase inhibitors, sulphasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors and derivatives thereof, as described herein, and anti-inflammatory cytokines (e.g. IL-4, IL-10, IL-13 and TGF).

[0239] Examples of therapeutic agents for multiple sclerosis with which the IL-22 antagonists can be combined include, but are not limited to, interferon-β, for example, IFNβ-1α and IFNβ-1β; Copaxone, corticosteroids, IL-1 inhibitors, TNF inhibitors, antibodies to CD40 ligand and CD80, IL-12 antagonists.

[0240] Non-limiting examples of agents for treating or preventing inflammatory bowel disease or Crohn's disease with which an IL-22 antagonist can be combined include the following: budesonide; epidermal growth factor; corticosteroids; cyclosporin, sulphasalazine; aminosalicylates; 6-mercaptopurine; azathioprine; metronidazole; lipoxygenase inhibitors; mesalamine; olsalazine; balsalazide; antioxidants; thromboxane inhibitors; IL-1 receptor antagonists; anti-IL-1 monoclonal antibodies; anti-IL-6 monoclonal antibodies; growth factors; elastase inhibitors; pyridinyl-imidazole compounds; TNF antagonists as described herein; IL-4, IL-10, IL-13 and/or TGFβ cytokines or agonists thereof (e.g., agonist antibodies); interleukin-11; glucuronide- or dextran-conjugated prodrugs of prednisolone, dexamethasone or budesonide; ICAM-I antisense phosphorothioate oligodeoxynucleotides (ISIS 2302; Isis Pharmaceuticals, Inc.); soluble complement receptor 1 (TPIO; T Cell Sciences, Inc.); slow-release mesalazine; methotrexate; antagonists of Platelet Activating Factor (PAF); ciprofloxacin; and lignocaine.
[0241] In certain embodiments, an IL-22 antagonists can be used in combination with one or more antibodies directed at other targets involved in regulating immune responses, e.g., transplant rejection or graft-v-host disease. Non-limiting examples of agents for treating or preventing immune responses with which an IL-22 antagonist can be combined include the following: antibodies against cell surface molecules, including but not limited to CD25 (interleukin-2 receptor-α), CD1 Ia (LFA-1), CD54 (ICAM-1), CD4, CD45, CD28/CTLA4, CD80 (B7-2) and/or CD86 (B7-2). In yet another embodiment, an IL-22 antagonist is used in combination with one or more general immunosuppressive agents, such as cyclosporin A or FK506.

[0242] In certain embodiments, kits for carrying out the combined administration of the IL-22 antagonists with other therapeutic compounds are provided. In certain embodiments, the kit comprises one or more binding agents formulated in a pharmaceutical carrier, and at least one agent, e.g., therapeutic agent, formulated as appropriate, in one or more separate pharmaceutical preparations.

**Diagnostic Assays**

[0243] Antibodies may also be used to detect the presence of IL-22 in biological samples. By correlating the presence or level of these proteins with a medical condition, one of skill in the art can diagnose the associated medical condition. For example, IL-22 induces changes associated with those caused by inflammatory cytokines (such as IL-1 and TNFα), and inhibitors of IL-22 ameliorate symptoms of rheumatoid arthritis (WO 2005/000897 A2). Exemplary medical conditions that may be diagnosed by the antibodies include, but are not limited to, multiple sclerosis, rheumatoid arthritis, psoriasis, inflammatory bowel disease, pancreatitis, and transplant rejection.

[0244] Antibody-based detection methods are well known in the art, and include ELISA, radioimmunoassays, immunoblots, Western blots, flow cytometry, immunofluorescence, immunoprecipitation, and other related techniques. The antibodies may be provided in a diagnostic kit that incorporates at least one of these procedures to detect IL-22. The kit may contain other components, packaging, instructions, or other material to aid the detection of the protein and use of the kit.

[0245] Antibodies may be modified with detectable markers, including ligand groups (e.g., biotin), fluorophores and chromophores, radioisotopes, electron-dense
reagents, or enzymes. Enzymes are detected by their activity. For example, horseradish peroxidase is detected by its ability to convert tetramethylbenzidine (TMB) to a blue pigment, quantifiable with a spectrophotometer. Other suitable binding partners include biotin and avidin, IgG and protein A, and other receptor-ligand pairs known in the art.

[0246] Antibodies can also be functionally linked (e.g., by chemical coupling, genetic fusion, non-covalent association or otherwise) to at least one other molecular entity, such as another antibody (e.g., a bispecific or a multispecific antibody), toxins, radioisotopes, cytotoxic or cytostatic agents, among others. Other permutations and possibilities are apparent to those of ordinary skill in the art.

**Assaying Effects of IL-22 Agonists or Antagonists**

[0247] The activity of an IL-22 agonist or antagonist can be measure by the following methods:


[0250] Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described in: Measurement of Human and Murine Interleukin 2 and Interleukin 4, Bottomly, K., Davis, L.S. and Lipsky, P.E. In Current Protocols in Immunology. J.E.e.a. Coligan eds. VoI 1 pp. 6.3.1-

Antibodies and Specific Binding Fragments against IL-19, IL-20, IL-24 and IL-26

In certain embodiments, a specific binding fragment or antibody against IL-19 is provided. IL-19 is a cytokine that belongs to the IL-10 cytokine subfamily. IL-19 is preferentially expressed in monocytes. It can bind the IL20 receptor complex and lead to the activation of the signal transducer and activator of transcription 3 (STAT3). A similar cytokine in mouse is reported to up-regulate the expression of IL-6 and TNF-alpha and induce apoptosis, which suggests a role of this cytokine in inflammatory responses. Alternatively spliced transcript variants encoding the distinct isoforms have been described.
Non-limiting examples of IL-19-associated disorders that can be treated or prevented include, but are not limited to, transplant rejection, autoimmune diseases (including, for example, diabetes mellitus, arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis), multiple sclerosis, encephalomyelitis, myasthenia gravis, systemic lupus erythematosi, autoimmune thyroiditis, dermatitis (including atopic dermatitis and eczematous dermatitis), Sjogren’s Syndrome, Crohn’s disease, aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, spondyloarthropathy, ankylosing spondylitis, intrinsic asthma, allergic asthma, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug eruptions, leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, allergic encephalomyelitis, acute necrotizing hemorrhagic encephalopathy, idiopathic bilateral progressive sensorineural hearing loss, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener’s granulomatosis, chronic active hepatitis, Stevens-Johnson syndrome, idiopathic sprue, lichen planus, Graves’ disease, sarcoidosis, primary biliary cirrhosis, uveitis posterior, and interstitial lung fibrosis); respiratory disorders, e.g., asthma or COPD; inflammatory conditions of the skin (e.g., psoriasis), liver (e.g., hepatitis), kidney (e.g., nephritis) and pancreas (e.g., pancreatitis); graft-versus-host disease, and allergy such as, atopic allergy; cancers (e.g., solid or soft tissue tumors), arthritic disorders (e.g., rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis, and ankylosing spondylitis), multiple sclerosis, type I diabetes, lupus (SLE), IBD, ulcerative colitis, Crohn's disease, COPD, asthma, vasculitis, allergy, scleroderma and inflammatory conditions of the skin (e.g., psoriasis), liver (e.g., hepatitis), kidney (e.g., nephritis) and pancreas (e.g., pancreatitis).

The amino acid sequence of the human IL-19 polypeptide is provided in Figure 17(a) and is identified as SEQ ID NO. 5. Where particular amino acids of IL-19 are identified by position, SEQ ID NO. 5 should be used as the reference IL-19 amino acid sequence.

In certain embodiments, an IL-19 specific binding agent that binds to the wild-type human IL-19 but fails to bind to a mutant IL-19 wherein the mutant IL-19 comprises one or more of the following changes relative to wild-type human IL-19: H36,

[0256] In certain embodiments, a peptidomimetic is provided that mimics an epitope comprising two or more of the following amino acids of IL-19: H36, 137, E39, S40, F41, Q42, 144, R46, K51, D52, P55, N56, V57, T58, 168, V74, R102, K103, S106, S110, Fi11, M114, A152, 1541, 155K, G158, V162, or A165.

[0257] In certain embodiments, a method of making a peptidomimetic that mimics an epitope comprising two or more of the following amino acids of IL-19: H36, 137, E39, S40, F41, Q42, 144, R46, K51, D52, P55, N56, V57, T58, 168, V74, R102, K103, S106, S110, Fi11, M114, A152, 1541, 155K, G158, V162, or A165, is provided. In certain embodiments, the method of making a peptidomimetic comprises structural modeling of the epitope through the use of a computer.

[0258] IL-19 antibodies and specific binding fragments can be generated and used according to the techniques described above for IL-22. Pharmaceutical compositions comprising IL-19 antibodies and specific binding fragments can be generated and used according to the techniques described above for IL-22.

[0259] In certain embodiments, a method of selecting a specific binding agent to an IL-19 polypeptide is provided. In certain such embodiments, the specific binding agent binds to at least a portion of an epitope on an IL-19 polypeptide. In certain embodiments, an IL-19 polypeptide is contacted with an agent. In certain embodiments, the affinity of the agent for the IL-19 polypeptide is determined. In certain embodiments, a mutant IL-19 polypeptide is contacted with the agent, wherein the mutant IL-19 polypeptide comprises at least one point mutation at at least one amino acid position selected from H36, 137, E39, S40, F41, Q42, 144, R46, K51, D52, P55, N56, V57, T58, 168, V74, R102, K103, S106, S110, Fi11, M114, A152, 1541, 155K, G158, V162, or A165. In certain embodiments, the affinity of the agent for the mutant polypeptide is determined. In certain embodiments, the agent is selected if the affinity for the IL-19 polypeptide is greater than the affinity for the mutant IL-19 polypeptide.

[0260] In certain embodiments, a specific binding fragment or antibody against IL-20 is provided. IL-20 is a cytokine structurally related to IL-10. IL-20 has been shown to transduce its signal through signal transducer and activator of transcription 3
(STAT3) in keratinocytes. A specific receptor for this cytokine is found to be expressed in skin and upregulated dramatically in psoriatic skin, suggesting a role for this protein in epidermal function and psoriasis.

[0261] Non-limiting examples of IL-20-associated disorders that can be treated or prevented include, but are not limited to, transplant rejection, autoimmune diseases (including, for example, diabetes mellitus, arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis), multiple sclerosis, encephalomyelitis, myasthenia gravis, systemic lupus erythematosus, autoimmune thyroiditis, dermatitis (including atopic dermatitis and eczematous dermatitis), Sjogren's Syndrome, Crohn's disease, aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, spondyloarthropathy, ankylosing spondylitis, intrinsic asthma, allergic asthma, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug eruptions, leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, allergic encephalomyelitis, acute necrotizing hemorrhagic encephalopathy, idiopathic bilateral progressive sensorineural hearing loss, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Stevens-Johnson syndrome, idiopathic sprue, lichen planus, Graves' disease, sarcoidosis, primary biliary cirrhosis, uveitis posterior, and interstitial lung fibrosis); respiratory disorders, e.g., asthma or COPD; inflammatory conditions of the skin (e.g., psoriasis), liver (e.g., hepatitis), kidney (e.g., nephritis) and pancreas (e.g., pancreatitis); graft-versus-host disease, and allergy such as, atopic allergy; cancers (e.g., solid or soft tissue tumors), arthritic disorders (e.g., rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis, and ankylosing spondylitis), multiple sclerosis, type I diabetes, lupus (SLE), IBD, ulcerative colitis, Crohn's disease, COPD, asthma, vasculitis, allergy, scleroderma and inflammatory conditions of the skin (e.g., psoriasis), liver (e.g., hepatitis), kidney (e.g., nephritis) and pancreas (e.g., pancreatitis).

[0262] The amino acid sequence of the human IL-20 polypeptide is provided in Figure 17(b) and is identified as SEQ ID NO. 6. Where particular amino acids of IL-20 are identified by position, SEQ ID NO. 6 should be used as the reference IL-20 amino acid sequence.
In certain embodiments, an IL-20 specific binding agent that binds to the wild-type human IL-20 but fails to bind to a mutant IL-20 wherein the mutant IL-20 comprises one or more of the following changes relative to wild-type human IL-20: E41, 142, N44, G45, F46, S47, 149, G51, D57, 160, D61, 162, R63, 164, L65, D73, R79, R107, K108, S111, S115, F116, 1119, 157A, V159, K160, G163, 1170, or Q173.

In certain embodiments, a peptidomimetic is provided that mimics an epitope comprising two or more of the following amino acids of IL-20: E41, 142, N44, G45, F46, S47, 149, G51, D57, 160, D61, 162, R63, 164, L65, D73, R79, R107, K108, S111, S115, F116, 1119, 157A, V159, K160, G163, 1170, or Q173.

In certain embodiments, a method of making a peptidomimetic that mimics an epitope comprising two or more of the following amino acids of IL-20: E41, 142, N44, G45, F46, S47, 149, G51, D57, 160, D61, 162, R63, 164, L65, D73, R79, R107, K108, S111, S115, F116, 1119, 157A, V159, K160, G163, 1170, or Q173 is provided. In certain embodiments, the method of making a peptidomimetic comprises structural modeling of the epitope through the use of a computer.

IL-20 antibodies and specific binding fragments can be generated and used according to the techniques described above for IL-22. Pharmaceutical compositions comprising IL-20 antibodies and specific binding fragments can be generated and used according to the techniques described above for IL-22.

In certain embodiments, a method of selecting a specific binding agent to an IL-20 polypeptide is provided. In certain such embodiments, the specific binding agent binds to at least a portion of an epitope on an IL-20 polypeptide. In certain embodiments, an IL-20 polypeptide is contacted with an agent. In certain embodiments, the affinity of the agent for the IL-20 polypeptide is determined. In certain embodiments, a mutant IL-20 polypeptide is contacted with the agent, wherein the mutant IL-20 polypeptide comprises at least one point mutation at at least one amino acid position selected from E41, 142, N44, G45, F46, S47, 149, G51, D57, 160, D61, 162, R63, 164, L65, D73, R79, R107, K108, S111, S115, F116, 1119, 157A, V159, K160, G163, 1170, or Q173. In certain embodiments, the affinity of the agent for the mutant polypeptide is determined. In certain embodiments, the agent is selected if the affinity for the IL-20 polypeptide is greater than the affinity for the mutant IL-20 polypeptide.
In certain embodiments, a specific binding fragment or antibody against IL-24 is provided. IL-24 was identified as a gene induced during terminal differentiation in melanoma cells. The protein encoded by this gene can induce apoptosis selectively in various cancer cells. Overexpression of this gene leads to elevated expression of several GADD family genes, which correlates with the induction of apoptosis. The phosphorylation of mitogen-activated protein kinase 14 (MAPK7/P38), and heat shock 27kDa protein 1 (HSPB2/HSP27) are found to be induced by this gene in melanoma cells, but not in normal immortal melanocytes. Alternatively spliced transcript variants encoding distinct isoforms have been reported. IL-24 signals through two distinct receptor complexes - an IL-22R1 and IL-20R2 receptor complex and an IL-20R1 and IL-22R2 receptor complex (U.S. Published Application Nos. 20030078381, 20030023033, and 20060013815).

Non-limiting examples of IL-24-associated disorders that can be treated or prevented include, but are not limited to, transplant rejection, autoimmune diseases (including, for example, diabetes mellitus, arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis), multiple sclerosis, encephalomyelitis, myasthenia gravis, systemic lupus erythematosus, autoimmune thyroiditis, dermatitis (including atopic dermatitis and eczematous dermatitis), Sjogren's Syndrome, Crohn's disease, aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, spondyloarthropathy, ankylosing spondylitis, intrinsic asthma, allergic asthma, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug eruptions, leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, allergic encephalomyelitis, acute necrotizing hemorrhagic encephalopathy, idiopathic bilateral progressive sensorineural hearing loss, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Stevens-Johnson syndrome, idiopathic sprue, lichen planus, Graves' disease, sarcoidosis, primary biliary cirrhosis, uveitis posterior, and interstitial lung fibrosis); respiratory disorders, e.g., asthma or COPD; inflammatory conditions of the skin (e.g., psoriasis), liver (e.g., hepatitis), kidney (e.g., nephritis) and pancreas (e.g., pancreateitis); graft-versus-host disease, and allergy such as, atopic allergy; cancers (e.g., solid or soft tissue tumors), arthritic disorders (e.g., rheumatoid arthritis, juvenile rheumatoid arthritis,
osteoarthritis, psoriatic arthritis, and ankylosing spondylitis), multiple sclerosis, type I diabetes, lupus (SLE), IBD, ulcerative colitis, Crohn’s disease, COPD, asthma, vasculitis, allergy, scleroderma and inflammatory conditions of the skin (e.g., psoriasis), liver (e.g., hepatitis), kidney (e.g., nephritis) and pancreas (e.g., pancreatitis).

[0270] The amino acid sequence of the human IL-24 polypeptide is provided in Figure 18(a) and is identified as SEQ ID NO. 7. Where particular amino acids of IL-24 are identified by position, SEQ ID NO. 7 should be used as the reference IL-24 amino acid sequence.

[0271] In certain embodiments, an IL-24 specific binding agent that binds to the wild-type human IL-24 but fails to bind to a mutant IL-24 wherein the mutant IL-24 comprises one or more of the following changes relative to wild-type human IL-24: K68, L69, E71, A72, F73, W74, V76, D78, Q83, D84, T87, S88, A89, R90, V100, S105, K135, S136, T139, N143, F144, 1147, A185, T187, K188, G191, 1195, or T198.

[0272] In certain embodiments, a peptidomimetic is provided that mimics an epitope comprising two or more of the following amino acids of IL-24: K68, L69, E71, A72, F73, W74, V76, D78, Q83, D84, T87, S88, A89, R90, V100, S105, K135, S136, T139, N143, F144, 1147, A185, T187, K188, G191, 1195, or T198.

[0273] In certain embodiments, a method of making a peptidomimetic that mimics an epitope comprising two or more of the following amino acids of IL-24: K68, L69, E71, A72, F73, W74, V76, D78, Q83, D84, T87, S88, A89, R90, V100, S105, K135, S136, T139, N143, F144, 1147, A185, T187, K188, G191, 1195, or T198, is provided. In certain embodiments, the method of making a peptidomimetic comprises structural modeling of the epitope through the use of a computer.

[0274] IL-22 antibodies and specific binding fragments can be generated and used according to the techniques described above for IL-22. Pharmaceutical compositions comprising IL-24 antibodies and specific binding fragments can be generated and used according to the techniques described above for IL-22.

[0275] In certain embodiments, a method of selecting a specific binding agent to an IL-24 polypeptide is provided. In certain such embodiments, the specific binding agent binds to at least a portion of an epitope on an IL-24 polypeptide. In certain embodiments, an IL-24 polypeptide is contacted with an agent.
the affinity of the agent for the IL-24 polypeptide is determined. In certain embodiments, a mutant IL-24 polypeptide is contacted with the agent, wherein the mutant IL-24 polypeptide comprises at least one point mutation at at least one amino acid position selected from K68, L69, E71, A72, F73, W74, V76, D78, Q83, D84, T87, S88, A89, R90, V100, S105, K135, S136, T139, N143, F144, 1147, A185, T187, K188, G191, 1195, or T198. In certain embodiments, the affinity of the agent for the mutant polypeptide is determined. In certain embodiments, the agent is selected if the affinity for the IL-24 polypeptide is greater than the affinity for the mutant IL-24 polypeptide.

[0276] In certain embodiments, a specific binding fragment or antibody against IL-26 is provided. IL-26 was identified by its overexpression specifically in herpesvirus samimiri-transformed T cells (Knappe et al. Journal of Virology, 74:3881-3887 (2000)). The encoded protein is a member of the IL-10 family of cytokines. IL-26 is a secreted protein and may function as a homodimer. IL-26 may contribute to the transformed phenotype of T cells after infection by herpesvirus samimiri. IL-26 signals through a receptor complex of IL-20R1 and IL-10R2 (Sheikh et al. Journal of Immunology, 172:2006-2010 (2004) and Hør et al. J Biol. Chem. 279 (32): 33343-51 (2004)).

[0277] Non-limiting examples of IL-26-associated disorders that can be treated or prevented include, but are not limited to, transplant rejection, autoimmune diseases (including, for example, diabetes mellitus, arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis), multiple sclerosis, encephalomyelitis, myasthenia gravis, systemic lupus erythematosus, autoimmune thyroiditis, dermatitis (including atopic dermatitis and eczematous dermatitis), Sjogren's Syndrome, Crohn's disease, aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, spondyloarthopathy, ankylosing spondylitis, intrinsic asthama, allergic asthma, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug eruptions, leprosy reversal reactions, erythema nodosum leprous, autoimmune uveitis, allergic encephalomyelitis, acute necrotizing hemorrhagic encephalopathy, idiopathic bilateral progressive sensorineural hearing loss, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Stevens-Johnson syndrome, idiopathic sprue, lichen planus, Graves' disease, sarcoidosis,
primary biliary cirrhosis, uveitis posterior, and interstitial lung fibrosis); respiratory disorders, e.g., asthma or COPD; inflammatory conditions of the skin (e.g., psoriasis), liver (e.g., hepatitis), kidney (e.g., nephritis) and pancreas (e.g., pancreatitis); graft-versus-host disease, and allergy such as, atopic allergy; cancers (e.g., solid or soft tissue tumors), arthritic disorders (e.g., rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis, and ankylosing spondylitis), multiple sclerosis, type I diabetes, lupus (SLE), IBD, ulcerative colitis, Crohn's disease, COPD, asthma, vasculitis, allergy, scleroderma and inflammatory conditions of the skin (e.g., psoriasis), liver (e.g., hepatitis), kidney (e.g., nephritis) and pancreas (e.g., pancreatitis).

[0278] The amino acid sequence of the human IL-26 polypeptide is provided in Figure 18(b) and is identified as SEQ ID NO. 8. Where particular amino acids of IL-26 are identified by position, SEQ ID NO. 8 should be used as the reference IL-26 amino acid sequence.

[0279] In certain embodiments, an IL-26 specific binding agent that binds to the wild-type human IL-26 but fails to bind to a mutant IL-26 wherein the mutant IL-26 comprises one or more of the following changes relative to wild-type human IL-26: Q40, A41, D43, A44, L45, Y46, K48, A50, T55, D59, 161, K62, N63, 164, F75, N78, R106, F107, D110, L114, R115, L118, G148, Y150, K151, S154, 1158, or S161.

[0280] In certain embodiments, a peptidomimetic is provided that mimics an epitope comprising two or more of the following amino acids of IL-26: Q40, A41, D43, A44, L45, Y46, K48, A50, T55, D59, 161, K62, N63, 164, F75, N78, R106, F107, D110, L114, R115, L118, G148, Y150, K151, S154, 1158, or S161.

[0281] In certain embodiments, a method of making a peptidomimetic that mimics an epitope comprising two or more of the following amino acids of IL-26: Q40, A41, D43, A44, L45, Y46, K48, A50, T55, D59, 161, K62, N63, 164, F75, N78, R106, F107, D110, L114, R115, L118, G148, Y150, K151, S154, 1158, or S161, is provided. In certain embodiments, the method of making a peptidomimetic comprises structural modeling of the epitope through the use of a computer.

[0282] IL-26 antibodies and specific binding fragments can be generated and used according to the techniques described above for IL-22. Pharmaceutical
compositions comprising IL-26 antibodies and specific binding fragments can be generated and used according to the techniques described above for IL-22.

[0283] In certain embodiments, a method of selecting a specific binding agent to an IL-26 polypeptide is provided. In certain such embodiments, the specific binding agent binds to at least a portion of an epitope on an IL-26 polypeptide. In certain embodiments, an IL-26 polypeptide is contacted with an agent. In certain embodiments, the affinity of the agent for the IL-26 polypeptide is determined. In certain embodiments, a mutant IL-26 polypeptide is contacted with the agent, wherein the mutant IL-26 polypeptide comprises at least one point mutation at least one amino acid position selected from Q40, A41, D43, A44, L45, Y46, K48, A50, T55, D59, 161, K62, N63, 164, F75, N78, R106, F107, D110, L114, R115, L118, G148, Y150, K151, S154, 1158, or S161. In certain embodiments, the affinity of the agent for the mutant polypeptide is determined. In certain embodiments, the agent is selected if the affinity for the IL-26 polypeptide is greater than the affinity for the mutant IL-26 polypeptide.

EXAMPLES

Evaluation of 146 distinct point substitutions in IL-22

[0284] A panel of IL-22 mutants was generated by a method employing three polymerase chain reactions (PCR) for each point mutation (Ho et al. Gene 11, 51-9 (1989)). A mammalian expression plasmid that encoded IL-22 with an N-terminal hexahistidine/FLAG® octapeptide (H/F) tag was used as template for the first two of three PCR steps (Li et al. International Immunopharmacology 4, 693-708 (2004)). The third PCR generated a linear DNA expression cassette that encoded a CMV promoter, H/F-IL-22 protein with a single point substitution, and an SV40 polyadenylation sequence. This 1816 bp linear DNA encoded a mammalian promoter, translation start and secretory leader, the latter fused in-frame to an N-terminal H/F-tagged IL-22, and then followed by a polyadenylation sequence. Using this PCR-based method, the seven alanines of the mature IL-22 ORF were individually mutated, either to glycine or a different alanine codon. The remaining amino acids of IL-22 were individually changed to alanines.
The panel of mutated IL-22 linear DNA expression cassettes was used directly, without purification, for transfection of mammalian cells (Liang et al. *Journal of Biological Chemistry* 277, 3593-8 (2002)). Transfection of suspension HEK293FT cells (Invitrogen, Carlsbad, CA) was performed in 48 deep-well plates using 293fectin™ (Invitrogen, Carlsbad, CA) and conditioned media was collected after five days. For purification of select IL-22 mutants, point-substituted cDNA was subcloned into a mammalian expression plasmid and the resulting subcloning products were used for transient transfection of adherent HEK293. H/F-tagged and mutated IL-22 protein was purified using Ni-NTA resin (Qiagen, Valencia, CA). All together, 146 distinct point mutants encompassing the mature IL-22 ORF, as well as seven silent alanine substitution controls, were expressed in mammalian cells.

IL-22 in the conditioned media was quantitated by sandwich ELISA, exploiting both entities of the H/F tag. Using standard methods with very gentle wash steps, H/F-IL-22 was captured with anti-FLAG HS M2 antibody covalently coated to plates (P2983; Sigma, St. Louis, MO) and detected with HRP-conjugated HisProbe (Pierce, Rockford, IL).

To determine which IL-22 amino acids are important for binding to IL-22 cell surface and soluble receptors, we evaluated our panel of control IL-22 and point mutants in five distinct ELISAs. The five ELISAs used were 1) an IL-22BP-Fc homodimer binding assay; 2) an IL-22R-Fc homodimer binding assay; 3) an IL-22R-Fc/IL-10R2-Fc heterodimer binding assay; 4) an IL-22 antibody (IL22-02) binding assay; and a second IL-22 antibody (IL22-04) binding assay (Li et al. *International Immunopharmacology* 4, 693-708 (2004)). The two antibody assays, which detect distinct IL-22 epitopes (Li et al. *International Immunopharmacology* 4, 693-708 (2004)), were used in concert with the three IL-22 receptor assays to identify those IL-22 mutations that affect the overall stability of IL-22 secondary and/or tertiary structure and only indirectly affect receptor or antibody binding. As described previously by Li et al., ELISAs for HF-IL-22 binding to receptors used plates coated indirectly with either 25 ng/ml IL-22BP-Fc or 50 ng/ml IL-22R-Fc homodimers or 50 ng/ml IL-22R-Fc/IL-10R2-Fc heterodimers, the latter two containing only the extracellular domains of the corresponding cell surface receptor subunits (Li et al. *International Immunopharmacology* 4, 693-708 (2004)).
Immimopharmacology 4, 693-708 (2004). Mutant and control IL-22 cytokines in conditioned media were tested in duplicate for binding at either 200 ng/ml (IL-22R) or 20 ng/ml (IL-22R-Fc/IL-10R2-Fc and IL-22BP-Fc). Rat IL22-02 and IL22-04 antibodies were coated directly at 3 μg/ml and 1 μg/ml, respectively, and IL-22 mutants were tested in duplicate for binding at 200 ng/ml and 20 ng/ml, respectively. Bound H/F-IL-22 was detected by conventional methods using HRP-conjugated HisProbe. Serial dilutions of purified IL-22 mutants were evaluated in at least two independent experiments. Approximately 800 independent data points were collected in duplicate that described the binding characteristics of single amino acid substitutions spanning the mature primary sequence of IL-22.

[0288] The binding of silent alanine substitutions, derived from thirteen separate transfections, served as the control IL-22 cytokine for the high-throughput method (open squares in Figure 9(a)). The signal threshold for the control IL-22 binding was set at 1.8 standard deviations below the average signal in a given assay and is a one-sided 95% confidence interval for individual observations. IL-22 mutants that gave a binding signal below the threshold were defined as being weaker than normal for binding to a given receptor or antibody.

[0289] Sixty-five of the 146 (45%) substitutions bound comparably to control IL-22 in the five IL-22 receptor and antibody assays (see Figure 12). The IL-22 amino acid side chains defined by this group of substitutions range from entirely surface exposed to buried, and their individual integrity was not essential for normal binding to IL-22R, IL-10R2, or IL-22BP.

[0290] Thirty of the 146 (20%) substitutions had statistically significant weaker than normal binding compared to control IL-22, in four or five assays (Figure 12). Twenty alanine substitutions had weaker than normal binding in all five binding assays (Figure 12). Alanine substitutions at 175, L100, C132, E166, and C178 showed the strongest inhibition of binding in the five assays. The ten additional substitutions classified as statistically significant in only four assays (Figure 12) may also have had a subtle impact in the IL-22R binding assay, since those mutants had a binding value that was just above the statistical threshold for the IL-22R assay. Considering that relatively few of the 30 corresponding IL-22 side chains are exposed to the surface (see solvent
accessibility values in Supplementary Table II and surface visualization of these in Supplementary Fig. Ib), these IL-22 side chains may not contribute specifically to the receptor binding sites. Rather, these 30 amino acids may be important for maintaining IL-22 secondary and/or tertiary structures.

29 side chains in IL-22 are involved in binding to IL-22BP, IL-22R, and/or IL-10R2.

Twelve of the 146 (8%) amino acid substitutions in the IL-22 protein were determined to be involved in the binding of IL-22 to IL-22R due to the fact that these mutants did not bind as well to the IL-22R as the control IL-22 protein did in the IL-22R binding assay (F57A, L59A, D67A, T70A, D71A, V72A, R73A, G159A, 1161A, K162A, G165A, and L169A substitutions in Figure 11 and corresponding residues in Figure 1(a); IL-22R in Figure 9(a)). Alanine substitution of 1161, V72, G165, D71, and L169 had the most deleterious effect in the IL-22R-Fc binding assay. Three of the twelve individual substitutions (D67A, R73A, and K162A) were also shown to not bind as well as the control IL-22 to IL-22BP (Figure 11 and Figure 1(a); IL-22BP in Figure 9(a)), indicating that the corresponding IL-22 side chains are involved in binding to IL-22BP. Collectively, these data suggest that the IL-22R and IL-22BP binding sites on IL-22 are overlapping.

IL-10R2 binds to a pre-formed IL-22/IL-22R complex (Logsdon et al. *Journal of Interferon & Cytokine Research* 22, 1099-112 (2002) and Li et al. *International Immunopharmacology* 4, 693-708 (2004)). Seventeen of the 146 (12%) substitutions in IL-22 were determined to be involved in binding to IL-10R2. Twenty-nine amino acid substitutions did not bind as well as control IL-22 in the IL-22R-Fc/IL-10R2-Fc binding assay. As twelve of these 29 mutants were also less effective in the IL-22R-Fc binding assay, we inferred that the remaining seventeen mutants correspond to IL-22 side chains that are involved in binding to IL-10R2 (A34G, Y51A, I52A, N54A, R55A, T56A, K61A, A66G, V83A, R88A, P113A, Y114A, E117A, F121A, L122A, L125A, and M172A substitutions in Figure 11 and corresponding residues in Figure 1(a); IL-10R2 in Figure 9(a)). Alanine substitution of T56, Y51, R55, N54, F121, and E117 had the most deleterious and specific effect in the IL-22R-Fc/IL-10R2 binding assay. The alanine substitution of V83 bound less well in both the IL-22R-Fc/IL-10R2-Fc and
IL-22BP-Fc assays (Figure 11 and Figure 1; Figure 9(a)), indicating that the corresponding IL-22 side chain may be involved in both IL-10R2 and IL-22BP binding.

[0293] None of the 146 point substitutions in IL-22 had a uniquely deleterious effect in the IL-22BP binding assay. Rather, the four IL-22 side chains that were involved in binding to IL-22BP were also involved in binding to IL-22R or IL-10R2 (D67, R73, V83, and K162 residues/solid symbols, respectively, in Figure 11, Figure 1(a), and Figure 9(a)).

[0294] To study the activity of certain IL-22 substitutions in more detail, thirteen IL-22 point mutants were purified and evaluated over a three-log range of concentrations in the five receptor and antibody binding assays. The binding data collected with purified IL-22 point mutants (Figure 2) confirmed the weaker than normal effects on binding observed in the high throughput evaluation (summarized in Figure 11 and Figure 9(a)). IL-22 mutants that contained alanine substitution of D67, V72, R73, 1161, K162, or L169 were approximately 50-fold less effective than control cytokine for binding to IL-22R-Fc (Figure 2(a), D67A, V72A, R73A, I161A, K162A, and L169A substitutions). Those mutants that blocked binding to IL-22R were also less effective, to varying degrees, in the IL-22R-Fc/IL-10R2-Fc binding assay (Figure 2(b), D67A, V72A, R73A, 1161A, K162A, and L169A substitutions). As expected, three of these purified IL-22 substitutions (D67A, R73A, and K162A) were also deleterious for binding to IL-22BP-Fc (Figure 2(c)). Mutants that were specifically defective for binding to IL-10R2 in the high-throughput screen were also weaker than normal for binding to IL-22R-Fc/IL-10R2-Fc when purified and evaluated at different concentrations (Figure 2(b), Y51A, R55A, and E117A substitutions) and bound normally to IL-22R-Fc or IL-22BP-Fc (Figures 2(a) and (c)). Accordingly, the data collected with the high-throughput methods were predictive of observations that were subsequently obtained with purified mutants. IL-22 side chains involved in binding to IL-22R or IL-10R2 are also involved in signaling into a cell.

[0295] The purified IL-22 substitutions were evaluated for the ability to induce proliferation of an IL-22-dependent BaF3 cell line that over-expressed both human IL-22R and IL-10R2 (Figure 3). A BaF3 cell line that expressed IL-10R2/YFP and IL-
22R/GFP was generated by sequential retroviral transductions and proliferated in response to IL-22. Serial dilutions of IL-22, control or mutated, were added to 5 x 10^3 GFP^+YFP^+ cells in RPMI with 10 % FCS and standard concentrations of penicillin, streptomycin, and glutamine. Cells were incubated at 37°C and 5% CO_2 for 72 hours, and then evaluated for proliferation by conventional methods using 3H-thymidine incorporation.

[0296] The eleven mutants that did not bind as well as control IL-22 to IL-22R or IL-10R2 in the ELISA binding assays (Figure 2A and B) were, in general, similarly weaker than normal for inducing IL-22-dependent cell proliferation (Figure 3). One exception was the R73A substitution that bound poorly to IL-22R-Fc/IL-10R2-Fc (R73A, Figure 2b). The R73A substitution mutant effected a signal into a cell when a 40-fold higher concentration was added relative to control IL-22 (R73A, Figure 3). The two other exceptions were the alanine substitutions of 1161 and L169. In the IL-22R-Fc/IL-10R2-Fc binding assay, the 1161A mutant IL-22 was a stronger binder than the V72A and L169A mutants (Figure 2b). In contrast, in the IL-22-dependent cell-based assay, the V72A, 1161A, and L169A substitutions were similarly less effective at lower concentrations with the L169A substitution inducing more proliferation at higher concentrations (Figure 3 and data not depicted). Overall, however, the above studies in a cell-based assay indicate that how effectively an IL-22 mutant interacts with IL-22R and IL-10R2 by ELISA is predictive of its effectiveness for signaling into a cell.

Side chains mostly in IL-22 helices A, D, and F, and loop AB are involved in binding IL-22BP, IL-22R, and/or IL-10R2 and define the cell surface receptor binding sites.

[0297] To explore the structural implications of mutagenesis results, the IL-22 residues that have been previously reported as being involved in cell signaling and/or receptor binding were considered in the context of existing IL-22 crystal structure (Nagem et al. Structure 10, 1051-62 (2002) and Xu et al. Acta Crystallographica Section D-Biological Crystallography 61, 942-50 (2005)). Based on the new data described in this application, the proposed IL-22R binding site is defined by IL-22 side chains within helices A and F, and loop AB (Figure 1b and CPK (Figure 4) renderings of structure). The IL-10R2 binding site is adjacent and defined by IL-22 side chains in helices A and D.
with contributions from loops BC and CD, and helices C and F. The binding site for IL-22BP involves four IL-22 side chains, three of which are in loop AB and helix F, and transect the proposed IL-22R binding site. Sequential 90° rotations of IL-22, counterclockwise around the vertical axis, are shown in the CPK structures of Figure 4 (a-d) and (e-h), and emphasize the restriction of the IL-22 cell surface and soluble receptor binding sites to certain surface regions of the IL-22 structure. IL-22R binding is dependent on the integrity of IL-22 side chains in helix A, and F and loop AB.

[0298] IL-22 binds first to IL-22R, its high affinity receptor subunit (Logsdon et al., Journal of Interferon & Cytokine Research 22, 1099-112 (2002) and Li, et al. International Immunopharmacology 4, 693-708 (2004)). The twelve IL-22 amino acids identified as being involved in its binding to IL-22R are located in helix A (F57, L59), loop AB (D67, T70, D71, V72, R73), and helix F (G159, 1161, K162, G165, L169) (Figure 1). Eight of these amino acids (F57, D67, T70, D71, V72, R73, G165, and L169) have 12-86% solvent accessibility (see Figure 14) suggesting that each may contribute atoms to the IL-22R receptor interface. The remaining four amino acids (i.e., L59, G159, 1161, K162) are almost or completely buried, suggesting that these contribute indirectly by facilitating a local surface structure. The proposed IL-22R binding site on IL-22 is illustrated in CPK (F57, D67, T70, D71, V72, R73, 1161, K162, and L169 residues, Figure 4(a-d)) and solvent accessibility (F57, L59, D67, T70, D71, V72, R73, G159, K161, K162, G165, and L169 residues, Figure 6(a)) renderings.

[0299] Nagem et al. superimposed their IL-22 crystal structure to the cytokine within IL-10/IL-10RIECD and IFN-γ/IFN-γRIECD co-crystal structures, and proposed that T70 and D71, as well as four other side chains of IL-22, are important for the recognition of IL-22’s corresponding high affinity receptor subunit (Nagem et al., Structure 10, 1051-62 (2002)). Logsdon et al., using a model of IL-22/IL-22R based on their solved IL-10/IL-10RIECD co-crystal structure, subsequently proposed that D71, R73, and G165, as well as seven other side chains of IL-22, may contribute to IL-22R binding (Logsdon et al., Journal of Interferon & Cytokine Research 22, 1099-112 (2002) and Josephson et al., Immunity 15, 35-46 (2001)). The studies in this application prove experimentally that
four of those previously proposed side chains (i.e., T70, D71, R73, and G165), as well as eight additional side-chains identified in the present application, are part of the generation of the IL-22R binding interface. Five amino acids proposed by Logsdon et al. to contribute to IL-22R binding are surface neighbors (K61, S64, N68, E166, and D168 residues in Figure 6(a)) to the residues identified in the present application as contributory (F57, L59, D67, T70, D71, V72, R73, G159, K161, K162, G165, and L169 residues; Figure 6(a)) (Logsdon et al. Journal of Interferon & Cytokine Research 22, 1099-112 (2002)). Further, it is possible that K61, S64, N68, E166, and D168 may also contribute to the IL-22R binding interface.

[0300] The high affinity receptor binding interface for IL-10 was elucidated from IL-10/IL-10R1 ECD co-crystal structure (Josephson et al. Immunity 15, 35-46 (2001) and Yoon et al. Journal of Biological Chemistry 281, 35088-96 (2006)). The IL-10R1 binding site encompasses much of IL-10's helix A, loop AB and helix F (see IL-10R1-a and -b dashed bars in alignment of Figure 5, and the renderings in Figure 6b). Nine of the IL-22R amino acids identified as contributing to the IL-22R binding interface (i.e., L59, D67, T70, D71, V72, R73, K162, G165, and L169) align with the IL-I OR1-a region. The three remaining residues (i.e., F57, G159, and 1161) are immediately to the N terminal side of the IL-10R1-a sites in helix A and F (Figure 5). Accordingly, the IL-22R binding site may be slightly expanded in helix A and translated upstream in helix F of IL-22 (see IL-22R dashed bars in Figure 5) relative to the IL-I OR1-a binding site in these same helices of IL-10.

[0301] None of the IL-22 amino acids that align with the IL-I OR1-b regions of IL-10 (Figure 5 and Figure 6b) appear to be involved in IL-22's interaction with IL-22R. This absence may be due to the use of different methods, as described above, to explore the structure and function of IL-10 and IL-22. Or, the identified amino acids may reflect a more compact IL-22R binding site than the corresponding IL-10R1 binding site on IL-10, as shown by comparing the IL-22 and IL-10 side chains in the superimposed ribbon structures of Figure 6b. In consideration of the distinct methods used to study the interaction between IL-10 or IL-22 and their respective high affinity receptors, a gross conservation of function as dictated by structure appears to exist between these two cytokines (see alignments and structures shown in Figure 5 and 6b, respectively).
IL-22 surface structure that is involved in IL-22R binding is also involved in binding IL-22BP.

[0302] IL-22BP specifically blocks an interaction between IL-22 and IL-22R, suggesting that IL-22BP has at least a partially overlapping binding site with IL-22R (Li et al. International Immunopharmacology 4, 693-708 (2004)). The mutagenesis analysis described above demonstrated that four IL-22 side chains in loops AB (D67 and R73), and BC (V83) and helix F (K162), are involved in binding to IL-22BP as well as IL-22R. The side chains D67, R73, and K162 may contribute directly to those binding sites (Figure 6a and c). V83 may contribute to those binding sites indirectly since it has only 3% solvent accessibility. Amino acids corresponding to D67, R73, V83, and K162 are conserved between IL-22 and IL-24. IL-22BP, however, is specific for IL-22. Thus, those four amino acids of IL-22 may not be sufficient for binding to IL-22BP. In consideration of IL-22BP’s high affinity for IL-22, other IL-22 side chains in the vicinity of D67, R73, and K162 (see Figure 6c) may also contribute to the IL-22BP binding site but may contribute to a lesser extent than the four identified amino acids, and thus were not detected in the mutagenesis screens described above. The existence of IL-22BP, its specificity and high affinity for IL-22, and the fact that it interferes with binding to IL-22R, by a mechanism proposed above, indicates that it has a physiologic role, yet to be explored, in vivo.

IL-10R2 binding to IL-22 requires the integrity of side chains mostly in helix A and D.

[0303] IL-10R2 binds to a surface created by the interaction of IL-22 and IL-22R (Logsdon et al. Journal of Interferon & Cytokine Research 22, 1099-12 (2002) and Li et al. International Immunopharmacology 4, 693-708 (2004)). This surface is proposed to include a conformational change in IL-22 that is induced by binding to IL-22R (Li et al. International Immunopharmacology 4, 693-708 (2004)). The mutagenesis analysis described above determined that IL-22 binding to IL-10R2 involves at least seventeen side chains, located in pre-helix A (A34); helix A (Y51, 152, N54, R55, T56, K61); loop AB (A66); loop BC (V83); helix C (R88); loop CD (P13); helix D (Y14, E117, F121, L122, L125); and helix F (M172) (Figure 1b). Solvent accessibility values (see Figure 14) indicate that most of these IL-22 side chains could contribute atoms to the
interface with IL-10R2, as is shown in the solvent accessible rendering of IL-22 in Figure 7a. A conformational change in IL-10 has already been determined to occur with binding to its high affinity receptor, IL-10R1 (Yoon et al. Journal of Biological Chemistry 281, 35088-96 (2006)). With this conformational change, the solvent accessibility of the five IL-10 side chains of helix A that contribute to the IL-10R2 interface - N39, M40, R42, S49, R50 - are altered by 9 to 21%, in one direction or the other. Determining how significantly the putative conformational change in IL-22 will modify the surface displayed in Figure 7a may involve the determination of an IL-22/IL-22R structure.

[0304] If a conformational change in IL-22 does occur with binding to IL-22R, then certain side chain atoms on the surface of IL-22 may contribute to both the IL-22R and IL-10R2 binding sites. Yoon et al. demonstrated, using surface plasmon resonance methods, that R42 of IL-10 is important for both IL-10R1 and IL-10R2 binding (Yoon et al. Journal of Biological Chemistry 281, 35088-96 (2006)). To interpret the mutagenesis data, an amino acid substitution of IL-22 that was weaker than normal for binding to IL-22R was assumed to bind similarly less effectively to a complex of IL-22R/IL-10R2. Substitutions that had a deleterious impact on binding to IL-22R/IL-10R2, and not IL-22R, were assumed to affect only IL-10R2 binding. This interpretation does not, however, consider those IL-22 amino acids that might be involved in the binding of both the high and low affinity receptor subunits.

[0305] D67, R73, and K162 of IL-22, which are centrally located within the IL-22R binding site and are involved in IL-22BP recognition, may also contribute to the IL-10R2 binding site. To validate the high-throughput screen, the binding of nine purified IL-22 substitutions was evaluated over a three-log range of concentrations. Six of these substitutions bind very poorly to IL-22R at relatively low concentrations of cytokine (i.e., 10-100 ng/ml; D67A, V72A, R73A, I161A, K162A, and L169A substitutions in Figure 2A). However, these six substitutions do not behave comparably in the presence of both IL-22R and IL-10R2, in either a receptor binding (i.e., 10-100 ng/ml, Figure 2b) or cell signaling (0.1-1 ng/ml, Figure 3) assay. Three of the substitutions (V72A, I161A, L169A) bind or signal relatively well, suggesting that the presence of IL-10R2 shifts an equilibrium, compensating for an initial IL-22 defect on IL-22R binding. The other three substitutions (D67A, R73A, and K162A) still bind poorly in the presence of IL-10R2,
indicating that the presence of IL-10R2 cannot compensate for the poor binding of these substitutions. These observations suggest that D67, R73, and K162 also contribute, directly or indirectly, to the interaction between IL-22/IL-22R and IL-10R2.

[0306] To validate their structure-function model for IL-22, Logsdon et al. evaluated the impact of fifteen alanine or cysteine point substitutions, demonstrating that Q48, Y51, N54, R55 in helix A (i.e., bold residues in QQPYITNR) and Y114, E117 in helix D (i.e., YMQE) are important for the interaction between IL-22/IL-22R and IL-10R2 (Logsdon et al. Journal of Molecular Biology 342, 503-14 (2004)). Subsequently, Wolk et al. determined that soluble IL-10R2 binds to surface coupled IL-22 peptides that include either QQPYITNR of helix A or LARLS of helix D (Wolk et al. Genes & Immunity 6, 8-18 (2005)). While the mutagenesis analysis described above did not detect an impact of the Q48A substitution, the mutagenesis analysis supports these prior observations and demonstrates that twelve additional side chains of IL-22 contribute to the recognition of IL-22/IL-22R by IL-10R2.

Comparison of surface regions of IL-10 and IL-22 that are recognized by IL-10R2

[0307] All cells express the IL-10R2 receptor subunit (Kotenko et al. Cytokine & Growth Factor Reviews 13, 223-40 (2002)). The expression of IL-10R2 suggests that IL-10R2 is a receptor subunit for several type II cytokines (i.e., IL-10, IL-22, IL-26 and IFN-λ) (Kotenko et al. International Immunopharmacology 4, 593-608 (2004)). Yoon et al. determined that that M40, R42, and R50 in helix A are critical for IL-10R2 binding to IL-10/IL10RI, with additional side chains (i.e., N39 and S49 in helix A and H108 and S111 in helix D; see dashed bars under IL-10 sequence in Figure 5) having subtler impacts in some assays (Yoon et al. Journal of Biological Chemistry 281, 35088-96 (2006)). In Figure 7(b), helix A and D structures from IL-22 and IL-10 are superimposed. Figure 7(b) shows the IL-22 (Y51, 152, N54, R55, T56, L59, K61, A66, R88, P113, Y114, E117, F121, L122, L125, G159, and M172) and IL-10 (N39, M40, R42, S49, R50, H108, and S111) side chains that may contribute to the binding site for IL-10R2. While the surface regions that are recognized by IL-10R2 are grossly overlapping, the systematic evaluation of IL-22 mutants suggests that IL-22' s binding site for IL-10R2 covers a broader surface than that elucidated to date for IL-10. The study of
a more comprehensive panel of IL-IO substitutions, similar to the systematic evaluation of IL-22 mutants described above, may elucidate a similar aspect of IL-IO surface contributing to the IL-10R2 binding site.

IL-22 structure-function model suggests the high and low affinity binding sites for other IL-10-like cytokines

[0308] A monomeric structure of IL-10 (PDB:1LK3) (Josephson et al. Structure 10, 981-7 (2002)) was used for superimposition with the monomeric crystal structures of IL-22 (PDB:1M4R) (Nagem et al. Structure 10, 1051-62 (2002)) and IL-19 (PDB:1N1F) (Chang et al. Journal of Biological Chemistry 278, 3308-13 (2003)), using the least squares method based on Ca positions as implemented by the Malign3D routine of Modeler (Martí-Renom et al. Annual Review of Biophysics & Biomolecular Structure 29, 291-325 (2000)). Structural models for IL-20 (NP_061194.2) (Blumberg et al. Cell 104, 9-19 (2001)) and IL-24 (NP_006841.1) (Jiang et al. Oncogene 11, 2477-86 (1995)) were generated using helices B through G of IL-19 (PDB:1N1F) as the template. Helical secondary structure for these three cytokines, as shown in Figures 1(a), 5 and 10, were derived from Discovery Studio Visualizer 2.0 evaluation of the above structure files. Structural models for IL-26 (NP_060872.1) (Knappe et al. Journal of Virology IA, 3881-7 (2000)) were generated using the monomeric IL-10 structure (PDB:1LK3) as the template. A virtual construct of IL-26 containing a six-residue flexible linker (GGGSGG) inserted in the predicted DE loop between residues E130 and M131 was used in model building to mimic the engineered monomeric IL-10 (Josephson et al. Structure 10, 981-7 (2002)). From the 100 initial models for IL-20, IL-24 and IL-26, the model with the lowest restraint violations, as defined by the molecular probability density function, was chosen for further optimization. For model optimization, an energy minimization cascade consisting of Steepest Descent, Conjugate Gradient and Adopted Basis Newton Raphson methods was performed until an RMS gradient of 0.01 was satisfied using CHARMM force field (Accelrys Software Inc.) and Generalized Born implicit solvation as implemented in Discovery Studio 1.7 (Accelrys Software Inc.). During energy minimization, backbone atom movements were restrained using a harmonic constraint of 10 mass force.
The systematic mutagenesis analysis of IL-22 described above defines at the molecular level those localized regions of IL-22 that are involved in binding to IL-22R and IL-10R2. Side chains, mostly from helices A, D, and F, and loop AB, are individually involved in recognition by IL-22's high affinity and low affinity receptor subunits. In consideration of the actual (i.e., IL-10, IL-19, IL-22) and modeled (IL-20, IL-24, IL-26) structural homology between the IL-10-like cytokines (summarized in Figure 10) and the present demonstration that there is functional conservation between the IL-22 and IL-10 structures (Figure 6b, 7b), positional transposition of the experimentally-defined IL-22 receptor binding sites is a first step towards the elucidation of other IL-10-like cytokines' receptor binding sites. The IL-19, IL-20, IL-24 and IL-26 residues that align (see Figure 10) with the IL-22R and IL-10R2 binding sites are shown as ball and stick models on the ribbon structures in Figure 8. Accordingly, based on information known in the art and that provided by the present invention, protein and small molecule therapeutics can also be designed for targeting and/or interrupting the IL-19, IL-20, IL-24 and IL-26 cytokine signaling pathways, therefore providing a method for treating and/or preventing disorders associated with these cytokines.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments described herein. Such equivalents are encompassed by the following claims.
What is claimed is:

1. An IL-22 specific binding agent that binds to the wild-type human IL-22 but fails to bind to a mutant IL-22 wherein the mutant IL-22 comprises one or more of the following changes relative to wild-type human IL-22:
   a) the amino acid at position 34 of the mutant IL-22 is alanine;
   b) the amino acid at position 52 of the mutant IL-22 is alanine;
   c) the amino acid at position 56 of the mutant IL-22 is alanine;
   d) the amino acid at position 61 of the mutant IL-22 is alanine;
   e) the amino acid at position 66 of the mutant IL-22 is alanine;
   f) the amino acid at position 83 of the mutant IL-22 is alanine;
   g) the amino acid at position 88 of the mutant IL-22 is alanine;
   h) the amino acid at position 113 of the mutant IL-22 is alanine;
   i) the amino acid at position 121 of the mutant IL-22 is alanine;
   j) the amino acid at position 122 of the mutant IL-22 is alanine;
   k) the amino acid at position 125 of the mutant IL-22 is alanine; or
   l) the amino acid at position 172 of the mutant IL-22 is alanine.

2. The IL-22 specific binding agent of claim 1, wherein the mutant IL-22 further comprises one or more of the following changes relative to wild-type human IL-22:
   a) the amino acid at position 51 of the mutant IL-22 is alanine;
   b) the amino acid at position 54 of the mutant IL-22 is alanine;
   c) the amino acid at position 55 of the mutant IL-22 is alanine;
   d) the amino acid at position 114 of the mutant IL-22 is alanine; or
   e) the amino acid at position 117 of the mutant IL-22 is alanine.

3. The IL-22 specific binding agent of claim 1, wherein the mutant IL-22 further comprises one or more of the following changes relative to wild-type human IL-22:
   a) the amino acid at position 57 of the mutant IL-22 is alanine;
   b) the amino acid at position 59 of the mutant IL-22 is alanine;
   c) the amino acid at position 67 of the mutant IL-22 is alanine;
   d) the amino acid at position 72 of the mutant IL-22 is alanine;
e) the amino acid at position 159 of the mutant IL-22 is alanine;
f) the amino acid at position 161 of the mutant IL-22 is alanine;
g) the amino acid at position 162 of the mutant IL-22 is alanine; or
h) the amino acid at position 169 of the mutant IL-22 is alanine.

4. The IL-22 specific binding agent of claim 1, wherein the mutant IL-22 further comprises one or more of the following changes relative to wild-type human IL-22:
   a) the amino acid at position 70 of the mutant IL-22 is alanine;
   b) the amino acid at position 71 of the mutant IL-22 is alanine;
   c) the amino acid at position 73 of the mutant IL-22 is alanine; or
   d) the amino acid at position 165 of the mutant IL-22 is alanine.

5. The IL-22 specific binding agent of claim 1, wherein the IL-22 specific binding agent is an antibody.

6. An IL-22 specific binding agent that binds to the wild-type human IL-22 but fails to bind to a mutant IL-22 wherein the mutant IL-22 comprises one or more of the following changes relative to wild-type human IL-22:
   a) the amino acid at position 57 of the mutant IL-22 is alanine;
   b) the amino acid at position 59 of the mutant IL-22 is alanine;
   c) the amino acid at position 67 of the mutant IL-22 is alanine;
   d) the amino acid at position 72 of the mutant IL-22 is alanine;
   e) the amino acid at position 159 of the mutant IL-22 is alanine;
   f) the amino acid at position 161 of the mutant IL-22 is alanine;
   g) the amino acid at position 162 of the mutant IL-22 is alanine; or
   h) the amino acid at position 169 of the mutant IL-22 is alanine.

7. The IL-22 specific binding agent of claim 6, wherein the mutant IL-22 further comprises one or more of the following changes relative to wild-type human IL-22:
   a) the amino acid at position 70 of the mutant IL-22 is alanine;
   b) the amino acid at position 71 of the mutant IL-22 is alanine;
c) the amino acid at position 73 of the mutant IL-22 is alanine; or
d) the amino acid at position 165 of the mutant IL-22 is alanine.

8. The IL-22 specific binding agent of claim 6, wherein the IL-22 specific binding agent is an antibody.

9. An IL-22 specific binding agent that binds to the wild-type human IL-22 but fails to bind to a mutant IL-22 wherein the mutant IL-22 comprises one or more of the following changes relative to wild-type human IL-22:
   a) the amino acid at position 67 of the mutant IL-22 is alanine;
   b) the amino acid at position 73 of the mutant IL-22 is alanine;
   c) the amino acid at position 83 of the mutant IL-22 is alanine; or
   d) the amino acid at position 162 of the mutant IL-22 is alanine.

10. The IL-22 specific binding agent of claim 9, wherein the IL-22 specific binding agent is an antibody.

11. A method of selecting a specific binding agent to an IL-22 polypeptide, wherein the specific binding agent binds to at least a portion of an epitope on an IL-22 polypeptide, comprising:
   a) contacting an IL-22 polypeptide with an agent;
   b) determining the affinity of the agent for the IL-22 polypeptide;
   c) contacting a mutant IL-22 polypeptide with the agent, wherein the mutant IL-22 polypeptide comprises at least one point mutation at at least one amino acid position selected from A34, 152, T56, K61, A66, V83, R88, P113, F121, L122, L125, or M172;
   d) determining the affinity of the agent for the mutant IL-22 polypeptide; and
   e) selecting the agent if the affinity for the IL-22 polypeptide is greater than the affinity for the mutant IL-22 polypeptide.
12. The method of claim 11, wherein the mutant IL-22 polypeptide is the same as the IL-22 polypeptide except for the at least one mutation.

13. The method of claim 11, wherein the agent is selected from a protein, peptide, nucleic acid, carbohydrate, lipid, and small molecule compound.

14. The method of claim 13, wherein the agent is an antibody.

15. The method of claim 13, wherein the agent is a small molecule compound.

16. The method of claim 11, wherein the amount of binding to the IL-22 polypeptide is at least 2-fold greater than the amount of binding to the mutant IL-22 polypeptide.

17. The method of claim 11, wherein the affinity for the IL-22 polypeptide is at least 5-fold greater than the affinity for the mutant IL-22 polypeptide.

18. The method of claim 11, wherein the affinity for the IL-22 polypeptide is at least 10-fold greater than the affinity for the mutant IL-22 polypeptide.

19. The method of claim 11, wherein the mutant IL-22 polypeptide comprises at least one point mutation selected from Y51, N54, R55, Y114, or E117.

20. The method of claim 11, wherein the mutant IL-22 polypeptide comprises at least one point mutation selected from F57, L59, D67, T70, D71, V72, R73, G159, 1161, K162, G165, or L169.

21. The method of claim 11, wherein the mutant IL-22 polypeptide comprises at least one point mutation selected from D67, R73, and K162.
22. A method of selecting a specific binding agent to an IL-22 polypeptide, wherein the specific binding agent binds to at least a portion of an epitope on an IL-22 polypeptide, comprising:
   a) contacting an IL-22 polypeptide with an agent;
   b) determining the affinity of the agent for the IL-22 polypeptide;
   c) contacting a mutant IL-22 polypeptide with the agent, wherein the mutant IL-22 polypeptide comprises at least one point mutation at least one amino acid position selected from F57, L59, D67, V72, G159, 1161, K162, or L169;
   d) determining the affinity of the agent for the mutant IL-22 polypeptide; and
   e) selecting the agent if the affinity for the IL-22 polypeptide is greater than the affinity for the mutant IL-22 polypeptide.

23. The method of claim 22, wherein the mutant IL-22 polypeptide is the same as the IL-22 polypeptide except for the at least one mutation.

24. The method of claim 22, wherein the agent is selected from a protein, peptide, nucleic acid, carbohydrate, lipid, and small molecule compound.

25. The method of claim 24, wherein the agent is an antibody.

26. The method of claim 24, wherein the agent is a small molecule compound.

27. The method of claim 22, wherein the amount of binding to the IL-22 polypeptide is at least 2-fold greater than the amount of binding to the mutant IL-22 polypeptide.

28. The method of claim 22, wherein the affinity for the IL-22 polypeptide is at least 5-fold greater than the affinity for the mutant IL-22 polypeptide.

29. The method of claim 22, wherein the affinity for the IL-22 polypeptide is at least 10-fold greater than the affinity for the mutant IL-22 polypeptide.
30. The method of claim 22, wherein the mutant IL-22 polypeptide comprises at least one point mutation selected from T70, D71, R73, or G165.

31. The method of claim 22, wherein the mutant IL-22 polypeptide comprises at least one point mutation selected from A34, Y51, 152, N54, R55, T56, K61, A66, V83, R88, P113, Y114, E117, F121, L122, L125, or M172.

32. The method of claim 22, wherein the mutant IL-22 polypeptide comprises at least one point mutation selected from R73 or V83.

33. A method of selecting a specific binding agent to an IL-22 polypeptide, wherein the specific binding agent binds to at least a portion of an epitope on an IL-22 polypeptide, comprising:
   a) contacting an IL-22 polypeptide with an agent;
   b) determining the affinity of the agent for the IL-22 polypeptide;
   c) contacting a mutant IL-22 polypeptide with the agent, wherein the mutant IL-22 polypeptide comprises at least one point mutation at at least one amino acid position selected from D67, R73, V83, or K162;
   d) determining the affinity of the agent for the mutant IL-22 polypeptide; and
   e) selecting the agent if the affinity for the IL-22 polypeptide is greater than the affinity for the mutant IL-22 polypeptide.

34. The method of claim 33, wherein the mutant IL-22 polypeptide is the same as the IL-22 polypeptide except for the at least one mutation.

35. The method of claim 33, wherein the agent is selected from a protein, peptide, nucleic acid, carbohydrate, lipid, and small molecule compound.

36. The method of claim 35, wherein the agent is an antibody.
37. The method of claim 35, wherein the agent is a small molecule compound.

38. The method of claim 33, wherein the amount of binding to the IL-22 polypeptide is at least 2-fold greater than the amount of binding to the mutant IL-22 polypeptide.

39. The method of claim 33, wherein the affinity for the IL-22 polypeptide is at least 5-fold greater than the affinity for the mutant IL-22 polypeptide.

40. The method of claim 33, wherein the affinity for the IL-22 polypeptide is at least 10-fold greater than the affinity for the mutant IL-22 polypeptide.

41. The method of claim 33, wherein the mutant IL-22 polypeptide comprises at least one point mutation selected from A34, Y51, 152, N54, R55, T56, K61, A66, R88, P113, Y114, E117, F121, L122, L125, or M172.

42. The method of claim 33, wherein the mutant IL-22 polypeptide comprises at least one point mutation selected from F57, L59, T70, D71, V72, G159, 1161, G165, or L169.

43. A pharmaceutical composition comprising the IL-22 specific binding agent of any of claims 1, 5, 6, 8, 9, or 10.

44. The pharmaceutical composition of claim 43, further comprising a cytokine inhibitor, a growth factor inhibitor, an immunosuppressant, an anti-inflammatory agent, a metabolic inhibitor, an enzyme inhibitor, a cytotoxic agent, or a cytostatic agent.

45. The pharmaceutical composition of claim 44, wherein the therapeutic agent is a TNF antagonist, an IL-12 antagonist, an IL-15 antagonist, an IL-17 antagonist, an IL-18 antagonist, an IL-21R antagonist, a T cell depleting agent, a B cell depleting...
agent, methotrexate, leflunomide, sirolimus (rapamycin) or an analog thereof, a Cox-2 inhibitor, a cPLA2 inhibitor, an NSAID, or a p38 inhibitor.

46. A method of treating or preventing an IL22-associated disorder, in a subject, comprising, administering to the subject the antibody or fragment thereof of any of claims 1, 5, 6, 8, 9, or 10, in an amount sufficient to treat or prevent the IL22-associated disorder.

47. The method of claim 46, wherein the IL22-associated disorder is an autoimmune disorder, a respiratory disorder, or an inflammatory condition.

48. The method of claim 46, wherein the IL22-associated disorder is rheumatoid arthritis, osteoarthritis, multiple sclerosis, myasthenia gravis, Crohn's disease, inflammatory bowel disease, lupus, diabetes, psoriasis, asthma, chronic obstructive pulmonary disease (COPD), cardiovascular inflammation, pancreatitis, hepatitis or nephritis.

49. The method of claim 46, further comprising administering to the subject a cytokine inhibitor, a growth factor inhibitor, an immunosuppressant, an anti-inflammatory agent, a metabolic inhibitor, an enzyme inhibitor, a cytotoxic agent, or a cytostatic agent.

50. The method of claim 49, wherein the therapeutic agent is a TNF antagonist, an IL-12 antagonist, an IL-15 antagonist, an IL-17 antagonist, an IL-18 antagonist, an IL-21R antagonist, a T cell depleting agent, a B cell depleting agent, methotrexate, leflunomide, sirolimus (rapamycin) or an analog thereof, a Cox-2 inhibitor, a cPLA2 inhibitor, an NSAID, or a p38 inhibitor.

51. An IL-19 specific binding agent that binds to the wild-type human IL-19 but fails to bind to a mutant IL-19 wherein the mutant IL-19 comprises one or more of the following changes relative to wild-type human IL-19:
a) the amino acid at position 36 of the mutant IL-19 is alanine;
b) the amino acid at position 37 of the mutant IL-19 is alanine;
c) the amino acid at position 39 of the mutant IL-19 is alanine;
d) the amino acid at position 40 of the mutant IL-19 is alanine;
e) the amino acid at position 41 of the mutant IL-19 is alanine;
f) the amino acid at position 42 of the mutant IL-19 is alanine;
g) the amino acid at position 44 of the mutant IL-19 is alanine;
h) the amino acid at position 46 of the mutant IL-19 is alanine;
i) the amino acid at position 51 of the mutant IL-19 is alanine;
j) the amino acid at position 52 of the mutant IL-19 is alanine;
k) the amino acid at position 55 of the mutant IL-19 is alanine;
l) the amino acid at position 56 of the mutant IL-19 is alanine;
m) the amino acid at position 57 of the mutant IL-19 is alanine;
n) the amino acid at position 58 of the mutant IL-19 is alanine;
o) the amino acid at position 68 of the mutant IL-19 is alanine;
p) the amino acid at position 74 of the mutant IL-19 is alanine;
q) the amino acid at position 102 of the mutant IL-19 is alanine;
r) the amino acid at position 103 of the mutant IL-19 is alanine;
s) the amino acid at position 106 of the mutant IL-19 is alanine;
t) the amino acid at position 110 of the mutant IL-19 is alanine;
u) the amino acid at position 111 of the mutant IL-19 is alanine;
v) the amino acid at position 114 of the mutant IL-19 is alanine;
w) the amino acid at position 152 of the mutant IL-19 is alanine;
x) the amino acid at position 154 of the mutant IL-19 is alanine;
y) the amino acid at position 155 of the mutant IL-19 is alanine;
z) the amino acid at position 158 of the mutant IL-19 is alanine;
aa) the amino acid at position 162 of the mutant IL-19 is alanine; or
bb) the amino acid at position 165 of the mutant IL-19 is alanine.

52. The IL-19 specific binding agent of claim 51, wherein the IL-19 specific binding agent is an antibody.
53. A method of selecting a specific binding agent to an IL-19 polypeptide, wherein the specific binding agent binds to at least a portion of an epitope on an IL-19 polypeptide, comprising:
   a) contacting an IL-19 polypeptide with an agent;
   b) determining the affinity of the agent for the IL-19 polypeptide;
   c) contacting a mutant IL-19 polypeptide with the agent, wherein the mutant IL-19 polypeptide comprises at least one point mutation at least one amino acid position selected from H36, 137, E39, S40, F41, Q42, 144, R46, K51, D52, P55, N56, V57, T58, 168, V74, R102, K103, S106, S110, F111, M114, A152, 1541, 155K, G158, V162, or A165
   d) determining the affinity of the agent for the mutant IL-19 polypeptide; and
   e) selecting the agent if the affinity for the IL-19 polypeptide is greater than the affinity for the mutant IL-19 polypeptide.

54. The method of claim 53, wherein the mutant IL-19 polypeptide is the same as the IL-19 polypeptide except for at least one mutation.

55. The method of claim 53, wherein the agent is selected from a protein, peptide, nucleic acid, carbohydrate, lipid, and small molecule compound.

56. The method of claim 55, wherein the agent is an antibody.

57. The method of claim 55, wherein the agent is a small molecule compound.

58. The method of claim 53, wherein the amount of binding to the IL-19 polypeptide is at least 2-fold greater than the amount of binding to the mutant IL-19 polypeptide.

59. The method of claim 53, wherein the affinity for the IL-19 polypeptide is at least 5-fold greater than the affinity for the mutant IL-19 polypeptide.
60. The method of claim 53, wherein the affinity for the IL-19 polypeptide is at least 10-fold greater than the affinity for the mutant IL-19 polypeptide.

61. A pharmaceutical composition comprising the IL-19 specific binding agent of any of claims 51 or 52.

62. A method of treating or preventing an IL-19-associated disorder, in a subject, comprising, administering to the subject the antibody or fragment thereof of any of claims 51 or 52, in an amount sufficient to treat or prevent the IL-19-associated disorder.

63. An IL-20 specific binding agent that binds to the wild-type human IL-20 but fails to bind to a mutant IL-20 wherein the mutant IL-20 comprises one or more of the following changes relative to wild-type human IL-20:
   a) the amino acid at position 41 of the mutant IL-20 is alanine;
   b) the amino acid at position 42 of the mutant IL-20 is alanine;
   c) the amino acid at position 44 of the mutant IL-20 is alanine;
   d) the amino acid at position 45 of the mutant IL-20 is alanine;
   e) the amino acid at position 46 of the mutant IL-20 is alanine;
   f) the amino acid at position 47 of the mutant IL-20 is alanine;
   g) the amino acid at position 49 of the mutant IL-20 is alanine;
   h) the amino acid at position 51 of the mutant IL-20 is alanine;
   i) the amino acid at position 56 of the mutant IL-20 is alanine;
   j) the amino acid at position 57 of the mutant IL-20 is alanine;
   k) the amino acid at position 60 of the mutant IL-20 is alanine;
   l) the amino acid at position 61 of the mutant IL-20 is alanine;
   m) the amino acid at position 62 of the mutant IL-20 is alanine;
   n) the amino acid at position 63 of the mutant IL-20 is alanine;
   o) the amino acid at position 73 of the mutant IL-20 is alanine;
   p) the amino acid at position 79 of the mutant IL-20 is alanine;
q) the amino acid at position 107 of the mutant IL-20 is alanine; 
r) the amino acid at position 108 of the mutant IL-20 is alanine; 
s) the amino acid at position 111 of the mutant IL-20 is alanine; 
t) the amino acid at position 115 of the mutant IL-20 is alanine; 
u) the amino acid at position 116 of the mutant IL-20 is alanine; 
v) the amino acid at position 119 of the mutant IL-20 is alanine; 
w) the amino acid at position 157 of the mutant IL-20 is alanine; 
x) the amino acid at position 159 of the mutant IL-20 is alanine; 
y) the amino acid at position 160 of the mutant IL-20 is alanine; 
z) the amino acid at position 163 of the mutant IL-20 is alanine 
aa) the amino acid at position 170 of the mutant IL-20 is alanine; or 
bb) the amino acid at position 173 of the mutant IL-20 is alanine.

64. The IL-20 specific binding agent of claim 63, wherein the IL-20 specific binding agent is an antibody.

65. A method of selecting a specific binding agent to an IL-20 polypeptide, 
wherein the specific binding agent binds to at least a portion of an epitope on an IL-20 polypeptide, comprising:
   a) contacting an IL-20 polypeptide with an agent;
   b) determining the affinity of the agent for the IL-20 polypeptide;
   c) contacting a mutant IL-20 polypeptide with the agent, wherein the mutant IL-20 polypeptide comprises at least one point mutation at least one amino acid position selected from E41, 142, N44, G45, F46, S47, 149, G51, D57, 160, D61, 162, R63, 164, L65, D73, R79, R107, K108, S111, S115, F116, 1119, A157, V159, K160, G163, 1170, or Q173
   d) determining the affinity of the agent for the mutant IL-20 polypeptide; and
   e) selecting the agent if the affinity for the IL-20 polypeptide is greater than
the affinity for the mutant IL-20 polypeptide.
66. The method of claim 65, wherein the mutant IL-20 polypeptide is the same as the IL-20 polypeptide except for the at least one mutation.

67. The method of claim 65, wherein the agent is selected from a protein, peptide, nucleic acid, carbohydrate, lipid, and small molecule compound.

68. The method of claim 67, wherein the agent is an antibody.

69. The method of claim 67, wherein the agent is a small molecule compound.

70. The method of claim 65, wherein the amount of binding to the IL-20 polypeptide is at least 2-fold greater than the amount of binding to the mutant IL-20 polypeptide.

71. An IL-24 specific binding agent that binds to the wild-type human IL-24 but fails to bind to a mutant IL-24 wherein the mutant IL-24 comprises one or more of the following changes relative to wild-type human IL-24:
   a) the amino acid at position 68 of the mutant IL-24 is alanine;
   b) the amino acid at position 69 of the mutant IL-24 is alanine;
   c) the amino acid at position 71 of the mutant IL-24 is alanine;
   d) the amino acid at position 72 of the mutant IL-24 is alanine;
   e) the amino acid at position 73 of the mutant IL-24 is alanine;
   f) the amino acid at position 74 of the mutant IL-24 is alanine;
   g) the amino acid at position 76 of the mutant IL-24 is alanine;
   h) the amino acid at position 78 of the mutant IL-24 is alanine;
   i) the amino acid at position 83 of the mutant IL-24 is alanine;
   j) the amino acid at position 84 of the mutant IL-24 is alanine;
   k) the amino acid at position 87 of the mutant IL-24 is alanine;
   l) the amino acid at position 88 of the mutant IL-24 is alanine;
   m) the amino acid at position 89 of the mutant IL-24 is alanine;
   n) the amino acid at position 90 of the mutant IL-24 is alanine;
o) the amino acid at position 100 of the mutant IL-24 is alanine;  
p) the amino acid at position 105 of the mutant IL-24 is alanine;  
q) the amino acid at position 135 of the mutant IL-24 is alanine;  
r) the amino acid at position 136 of the mutant IL-24 is alanine;  
s) the amino acid at position 139 of the mutant IL-24 is alanine;  
t) the amino acid at position 143 of the mutant IL-24 is alanine;  
u) the amino acid at position 144 of the mutant IL-24 is alanine;  
v) the amino acid at position 147 of the mutant IL-24 is alanine;  
w) the amino acid at position 185 of the mutant IL-24 is alanine;  
x) the amino acid at position 187 of the mutant IL-24 is alanine;  
y) the amino acid at position 188 of the mutant IL-24 is alanine;  
z) the amino acid at position 191 of the mutant IL-24 is alanine  
aa) the amino acid at position 195 of the mutant IL-24 is alanine; or  
bb) the amino acid at position 198 of the mutant IL-24 is alanine.

72. The IL-24 specific binding agent of claim 71, wherein the IL-24 specific binding agent is an antibody.

73. A method of selecting a specific binding agent to an IL-24 polypeptide, wherein the specific binding agent binds to at least a portion of an epitope on an IL-24 polypeptide, comprising:
   a) contacting an IL-24 polypeptide with an agent;
   b) determining the affinity of the agent for the IL-24 polypeptide;
   c) contacting a mutant IL-24 polypeptide with the agent, wherein the mutant IL-24 polypeptide comprises at least one point mutation at least one amino acid position selected from K68, L69, E71, A72, F73, W74, V76, D78, Q83, D84, T87, S88, A89, R90, VIOO, S105, K135, S136, T139, N143, F144, 1147, A185, T187, K188, G191, 1195, or T198;
   d) determining the affinity of the agent for the mutant IL-24 polypeptide; and
   e) selecting the agent if the affinity for the IL-24 polypeptide is greater than the affinity for the mutant IL-24 polypeptide.
74. The method of claim 73, wherein the mutant IL-24 polypeptide is the same as the IL-24 polypeptide except for the at least one mutation.

75. The method of claim 73, wherein the agent is selected from a protein, peptide, nucleic acid, carbohydrate, lipid, and small molecule compound.

76. The method of claim 75, wherein the agent is an antibody.

77. The method of claim 75, wherein the agent is a small molecule compound.

78. The method of claim 73, wherein the amount of binding to the IL-24 polypeptide is at least 2-fold greater than the amount of binding to the mutant IL-24 polypeptide.

79. The method of claim 73, wherein the affinity for the IL-24 polypeptide is at least 5-fold greater than the affinity for the mutant IL-24 polypeptide.

80. The method of claim 73, wherein the affinity for the IL-24 polypeptide is at least 10-fold greater than the affinity for the mutant IL-24 polypeptide.

81. A pharmaceutical composition comprising the IL-24 specific binding agent of any of claims 71 or 72.

82. A method of treating or preventing an IL-24-associated disorder, in a subject, comprising, administering to the subject the antibody or fragment thereof of any of claims 71 or 72, in an amount sufficient to treat or prevent the IL-24-associated disorder.
83. An IL-26 specific binding agent that binds to the wild-type human IL-26 but fails to bind to a mutant IL-26 wherein the mutant IL-26 comprises one or more of the following changes relative to wild-type human IL-26:

a) the amino acid at position 40 of the mutant IL-26 is alanine;
b) the amino acid at position 41 of the mutant IL-26 is alanine;
c) the amino acid at position 43 of the mutant IL-26 is alanine;
d) the amino acid at position 44 of the mutant IL-26 is alanine;
e) the amino acid at position 45 of the mutant IL-26 is alanine;
f) the amino acid at position 46 of the mutant IL-26 is alanine;
g) the amino acid at position 48 of the mutant IL-26 is alanine;
h) the amino acid at position 50 of the mutant IL-26 is alanine;
i) the amino acid at position 55 of the mutant IL-26 is alanine;
j) the amino acid at position 59 of the mutant IL-26 is alanine;
k) the amino acid at position 61 of the mutant IL-26 is alanine;
l) the amino acid at position 62 of the mutant IL-26 is alanine;
m) the amino acid at position 63 of the mutant IL-26 is alanine;
n) the amino acid at position 64 of the mutant IL-26 is alanine;
o) the amino acid at position 75 of the mutant IL-26 is alanine;
p) the amino acid at position 78 of the mutant IL-26 is alanine;
q) the amino acid at position 106 of the mutant IL-26 is alanine;
r) the amino acid at position 107 of the mutant IL-26 is alanine;
s) the amino acid at position 110 of the mutant IL-26 is alanine;
t) the amino acid at position 114 of the mutant IL-26 is alanine;
u) the amino acid at position 115 of the mutant IL-26 is alanine;
v) the amino acid at position 118 of the mutant IL-26 is alanine;
w) the amino acid at position 148 of the mutant IL-26 is alanine;
x) the amino acid at position 150 of the mutant IL-26 is alanine;
y) the amino acid at position 151 of the mutant IL-26 is alanine;
z) the amino acid at position 154 of the mutant IL-26 is alanine
aa) the amino acid at position 158 of the mutant IL-26 is alanine; or
bb) the amino acid at position 161 of the mutant IL-26 is alanine.
84. The IL-26 specific binding agent of claim 83, wherein the IL-26 specific binding agent is an antibody.

85. A method of selecting a specific binding agent to an IL-26 polypeptide, wherein the specific binding agent binds to at least a portion of an epitope on an IL-26 polypeptide, comprising:
   a) contacting an IL-26 polypeptide with an agent;
   b) determining the affinity of the agent for the IL-26 polypeptide;
   c) contacting a mutant IL-26 polypeptide with the agent, wherein the mutant IL-26 polypeptide comprises at least one point mutation at least one amino acid position selected from Q40, A41, D43, A44, L45, Y46, K48, A50, T55, D59, 161, K62, N63, 164, F75, N78, R106, F107, D110, L114, R115, L118, G148, Y150, K151, S154, 1158, or S161;
   d) determining the affinity of the agent for the mutant IL-26 polypeptide; and
   e) selecting the agent if the affinity for the IL-26 polypeptide is greater than the affinity for the mutant IL-26 polypeptide.

86. The method of claim 85, wherein the mutant IL-26 polypeptide is the same as the IL-26 polypeptide except for the at least one mutation.

87. The method of claim 85, wherein the agent is selected from a protein, peptide, nucleic acid, carbohydrate, lipid, and small molecule compound.

88. The method of claim 87, wherein the agent is an antibody.

89. The method of claim 87, wherein the agent is a small molecule compound.

90. The method of claim 85, wherein the amount of binding to the IL-26 polypeptide is at least 2-fold greater than the amount of binding to the mutant IL-26 polypeptide.
91. The method of claim 85, wherein the affinity for the IL-26 polypeptide is at least 5-fold greater than the affinity for the mutant IL-26 polypeptide.

92. The method of claim 85, wherein the affinity for the IL-26 polypeptide is at least 10-fold greater than the affinity for the mutant IL-26 polypeptide.

93. A pharmaceutical composition comprising the IL-26 specific binding agent of any of claims 83 or 84.

94. A method of treating or preventing an IL-26-associated disorder, in a subject, comprising, administering to the subject the antibody or fragment thereof of any of claims 83 or 84, in an amount sufficient to treat or prevent the IL-26-associated disorder.

95. A method of systematic mutagenesis of a target protein comprising:
   a) generating a series of or more different nucleic acid molecules, wherein each different nucleic acid molecule comprises a nucleic acid sequence that encodes a polypeptide comprising a different mutant of the target protein; wherein each polypeptide comprising a different mutant further comprises a secretory sequence; a first tag, and a second tag;
   b) introducing each of the ten or more different nucleic acid molecules into a different group of cells, wherein each different group of cells is in a separate well comprising liquid media;
   c) expressing ten or more different mutants of the target protein in the separate wells; wherein the ten or more different mutants of the target protein are secreted into the liquid media of the separate wells;
   d) quantitating the ten or more different mutants of the target protein in the liquid media in the separate wells using the first tag and the second tag;
   e) subjecting the ten or more different mutants of the target protein to at least one assay.
96. The method of claim 95, wherein the cells are eukaryotic cells.

97. The method of claim 96, wherein the introducing each of the ten or more different nucleic acid molecules into a different group of cells comprises transfecting each of the ten or more different nucleic acid molecules into a different group of cells.

98. The method of claim 95, wherein the cells are bacterial cells.

99. The method of claim 95, wherein each amino acid of the target protein is mutated in a different mutant target protein.

100. The method of claim 95, wherein each amino acid of the target protein that is not alanine is mutated to alanine in a different mutant target protein.

101. The method of claim 95, wherein the first tag is a six histidine tag and the second tag is a FLAG tag.

102. A peptidomimetic that mimics an epitope comprising two or more of the following amino acids of IL-22: A34, 152, T56, K61, A66, V83, R88, P113, F121, L122, L125, or M172.

103. The peptidomimetic of claim 102 that mimics an epitope further comprising one or more of the following amino acids of IL-22: Y51, N54, R55, Y114, or E117.

104. The peptidomimetic of claim 102 that mimics an epitope further comprising one or more of the following amino acids of IL-22: F57, L59, D67, T70, D71, V72, R73, G159, 1161, K162, G165, or L169.
105. The peptidomimetic of claim 102 that mimics an epitope further comprising one or more of the following amino acids of IL-22: D67, R73, or K162.

106. A peptidomimetic that mimics an epitope comprising two or more of the following amino acids of IL-22: F57, L59, D67, V72, G159, 1161, K162, or L169.

107. The peptidomimetic of claim 106 that mimics an epitope further comprising one or more of the following amino acids of IL-22: T70, D71, R73, or G165.

108. The peptidomimetic of claim 106 that mimics an epitope further comprising one or more of the following amino acids of IL-22: A34, Y51, 152, N54, R55, T56, K61, A66, V83, R88, P113, Y114, E117, F121, L122, L125, or M172.

109. The peptidomimetic of claim 106 that mimics an epitope further comprising one or more of the following amino acids of IL-22: R73 or V83.

110. A peptidomimetic that mimics an epitope comprising two or more of the following amino acids of IL-22: D67, R73, V83, or K162.

111. The peptidomimetic of claim 110 that mimics an epitope further comprising one or more of the following amino acids of IL-22: A34, Y51, 152, N54, R55, T56, K61, A66, R88, P113, Y114, E117, F121, L122, L125, or M172.

112. The peptidomimetic of claim 110 that mimics an epitope further comprising one or more of the following amino acids of IL-22: F57, L59, T70, D71, V72, G159, 1161, G165, or L169.

113. A peptidomimetic that mimics an epitope comprising two or more of the following amino acids of IL-19: H36, 137, E39, S40, F41, Q42, 144, R46, K51, D52, P55, N56, V57, T58, 168, V74, R102, K103, S106, S110, F11, M114, A152, 1541, 155K, G158, V162, or A165.

115. A peptidomimetic that mimics an epitope comprising two or more of the following amino acids of IL-24: K68, L69, E71, A72, F73, W74, V76, D78, Q83, D84, T87, S88, A89, R90, V100, S105, K135, S136, T139, N143, F144, 1147, A185, T187, K188, G191, I195, or T198.

116. A peptidomimetic that mimics an epitope comprising two or more of the following amino acids of IL-26: Q40, A41, D43, A44, L45, Y46, K48, A50, T55, D59, 161, K62, N63, 164, F75, N78, R106, F107, D110, L114, R115, L118, G148, Y150, K151, S154, 1158, or S161.

117. A method for increasing antagonistic activity of an antagonist of IL-22 comprising the step of altering a structure of the antagonist such that the antagonist binds to one or more of the following amino acids of IL-22: F57, L59, D67, T70, D71, V72, R73, G159, 1161, K162, G165, or L169.

118. The method of claim 117 wherein the step of altering the structure is performed by computer modeling.

119. The method of claim 117 where in the structure is an amino acid sequence.

120. The method of claim 119 wherein the antagonist is an antibody.

121. The method of claim 117 wherein the structure is a crystal structure.

122. The method of claim 121 wherein the antagonist is a small molecule.
123. A method for increasing antagonistic activity of an antagonist of IL-22 comprising the step of altering a structure of the antagonist such that the antagonist binds to one or more of the following amino acids of IL-22: A34, 152, T56, K61, A66, V83, R88, P113, F121, L122, L125, or M172.

124. The method of claim 123 wherein the step of altering the structure is performed by computer modeling.

125. The method of claim 123 where in the structure is an amino acid sequence.

126. The method of claim 125 wherein the antagonist is an antibody.

127. The method of claim 123 wherein the structure is a crystal structure.

128. The method of claim 127 wherein the antagonist is a small molecule.

129. A method for increasing antagonistic activity of an antagonist of IL-22 comprising the step of altering a structure of the antagonist such that the antagonist binds to one or more of the following amino acids of IL-22: D67, R73, V83, or K162.

130. The method of claim 129 wherein the step of altering the structure is performed by computer modeling.

131. The method of claim 129 where in the structure is an amino acid sequence.

132. The method of claim 131 wherein the antagonist is an antibody.

133. The method of claim 129 wherein the structure is a crystal structure.

134. The method of claim 133 wherein the antagonist is a small molecule.
135. A method for designing an antagonist of IL-19 comprising the step of altering a structure of the antagonist such that the antagonist binds to one or more of the following amino acids of IL-19: H36, 137, E39, S40, F41, Q42, 144, R46, K51, D52, P55, N56, V57, T58, 168, V74, R102, K103, S106, S110, F111, M114, A152, 1541, 155K, G158, V162, or A165.

136. The method of claim 135 wherein the step of altering the structure is performed by computer modeling.

137. The method of claim 135 where in the structure is an amino acid sequence.

138. The method of claim 137 wherein the antagonist is an antibody.

139. The method of claim 135 wherein the structure is a crystal structure.

140. The method of claim 139 wherein the antagonist is a small molecule.

141. A method for designing an antagonist of IL-20 comprising the step of altering a structure of the antagonist such that the antagonist binds to one or more of the following amino acids of IL-20: E41, 142, N44, G45, F46, S47, 149, G51, D57, 160, D61, 162, R63, 164, L65, D73, R79, R107, K108, S111, S115, F116, 1119, 157A, V159, K160, G163, 1170, or Q173.

142. The method of claim 141 wherein the step of altering the structure is performed by computer modeling.

143. The method of claim 141 where in the structure is an amino acid sequence.

144. The method of claim 143 wherein the antagonist is an antibody.
145. The method of claim 141 wherein the structure is a crystal structure.

146. The method of claim 145 wherein the antagonist is a small molecule.

147. A method for designing an antagonist of IL-24 comprising the step of altering a structure of the antagonist such that the antagonist binds to one or more of the following amino acids of IL-24: K68, L69, E71, A72, F73, W74, V76, D78, Q83, D84, T87, S88, A89, R90, V100, S105, K135, S136, T139, N143, F144, 1147, A185, T187, K188, G191, 1195, or T198.

148. The method of claim 147 wherein the step of altering the structure is performed by computer modeling.

149. The method of claim 147 where in the structure is an amino acid sequence.

150. The method of claim 149 wherein the antagonist is an antibody.

151. The method of claim 147 wherein the structure is a crystal structure.

152. The method of claim 151 wherein the antagonist is a small molecule.

153. A method for designing an antagonist of IL-26 comprising the step of altering a structure of the antagonist such that the antagonist binds to one or more of the following amino acids of IL-26: Q40, A41, D43, A44, L45, Y46, K48, A50, T55, D59, 161, K62, N63, 164, F75, N78, R106, F107, D110, L114, R115, L118, G148, Y150, K151, S154, 1158, or S161.

154. The method of claim 153 wherein the step of altering the structure is performed by computer modeling.

155. The method of claim 153 where in the structure is an amino acid sequence.
156. The method of claim 155 wherein the antagonist is an antibody.

157. The method of claim 153 wherein the structure is a crystal structure.

158. The method of claim 157 wherein the antagonist is a small molecule.
FIG. 1
FIG. 3
FIG. 5
FIG. 9A

FIG. 9B
IL-22 point substitutions impact binding
to IL-22BP, IL-22R and/or IL-10R2

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-22BP-Fc</td>
<td>D67A, R73A, V83A, K162A</td>
</tr>
</tbody>
</table>

a. IL-22 amino acid substitutions F57A, L59A, T70A, D71A, V72A, G159A, I161A, G165A, and L169A were suboptimal for binding, relative to control IL-22, in both the IL-22R-Fc and IL-22R-Fc/IL-10R2-Fc binding assays. Substitutions D67A, R73A, and K162A were also suboptimal in the IL-22BP-Fc assay.
b. An amino acid substitution is represented by its numerical position in the mature open reading frame of IL-22, as shown in Figure 1a, flanked by the un-mutated (before) and substituted (after) amino acid in single letter nomenclature.
c. Substitutions that are in a larger, bold font indicate point mutants that were purified and evaluated in more detail.
d. IL-22 amino acid substitutions A34G, Y51A, I52A, N54A, R55A, T56A, K61A, A66G, R88A, P113A, Y114A, E117A, F121A, L122A, L125A, and M172A were suboptimal for binding, compared to control IL-22, in the IL-22R-Fc/IL-10R2-Fc assay. One substitution, V83A, was also suboptimal in the IL-22BP-Fc binding assay.
e. IL-22 amino acid substitutions that were suboptimal in the IL-22BP-Fc binding assay were also suboptimal for binding to IL-22R-Fc (D67A, R73A, and K162A) or IL-10R2-Fc (V83A), the latter in the context of IL-22R-Fc.

**FIG. 11**
IL-22 point substitutions impact binding to receptor subunits and/or antibodies or do not hinder binding in any assay

<table>
<thead>
<tr>
<th>Proposed effect on overall IL-22 structure</th>
<th>Suboptimal binding in all five assays</th>
<th>Suboptimal binding in four assays and probable subtle impact in the IL-22R binding assay</th>
<th>Suboptimal binding for IL-22 antibodies only</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>35, 36, 37, 38, 39, 43, 44, 45, 46, 48, 53, 58, 62, 64, 65, 68, 69, 76, 77, 78, 81, 84, 85, <strong>S86A</strong>&lt;sup&gt;c&lt;/sup&gt;, 87, 90, 93, <strong>Q94A</strong>&lt;sup&gt;a&lt;/sup&gt;, 97, 99, 105, 106, 107, 108, 109, 110, 112, 115, 116, 119, 124, 131, 133, 134, 135, 136, 137, 138, 140, 142, 143, 144, 145, 146, 147, 149, 150, 151, 153, 154, 155, 157, 158, 160, 176</td>
<td>47, 60, 74, 75, 80, 92, 96, <strong>L100A</strong>&lt;sup&gt;b&lt;/sup&gt;, 111, 129, <strong>C132A</strong>, 141, 148, 163, 164, 166, 168, 174, 177, 178</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40, 42, 79, 89, 95, 98, 104, 156, 170, 171</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> In addition to the IL-22BP-Fc, IL-22R-Fc and IL-22R-Fc/IL-10R2-Fc binding assays, IL-22 point mutants were also evaluated for binding to two IL-22 antibodies, IL22-02 and IL22-04. Thus, each IL-22 substitution was evaluated in five distinct binding assays.

<sup>b</sup> Mutants with point substitutions at these positions bound as well as control IL-22 in all five binding assays.

<sup>c</sup> Substitutions that are in a larger, bold font indicate point mutants that were purified and evaluated in more detail.

<sup>d</sup> Substitutions that had suboptimal binding in four and five assays were deemed to have a general deleterious effect on IL-22 structure.

<sup>e</sup> Substitutions at these positions bound suboptimally, compared to control IL-22, in all five binding assays.

<sup>f</sup> These substitutions were statistically suboptimal for binding in four of the assays. In the remaining assay – IL-22R binding – the values obtained with these substitutions were just above the cut-off for statistically ‘suboptimal binding’. The IL-22R-Fc binding assay is the lowest affinity cytokine-receptor binding assay and had the widest spread of binding values for control IL-22 (see Supplementary Figure 1a). In consideration of data collected with these mutants in the other four assays, we concluded that there is probably a subtle impact of these mutants in the IL-22R assay.

<sup>g</sup> Mutants with these point substitutions bound suboptimally to one or more of the IL-22 antibodies, IL22-02 or IL22-04. These mutants had optimal binding in all three of the receptor binding assays.

**FIG. 12**
The estimated solvent accessibility of IL-22 amino acids, substitutions of which were suboptimal for binding, relative to control IL-22, in four or five assays

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>% SA&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>11</td>
</tr>
<tr>
<td>42</td>
<td>0</td>
</tr>
<tr>
<td>47</td>
<td>2</td>
</tr>
<tr>
<td>60</td>
<td>1</td>
</tr>
<tr>
<td>74</td>
<td>7</td>
</tr>
<tr>
<td>75</td>
<td>10</td>
</tr>
<tr>
<td>79</td>
<td>2</td>
</tr>
<tr>
<td>80</td>
<td>19</td>
</tr>
<tr>
<td>89</td>
<td>2</td>
</tr>
<tr>
<td>92</td>
<td>0</td>
</tr>
<tr>
<td>95</td>
<td>2</td>
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<tr>
<td>96</td>
<td>0</td>
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<td>98</td>
<td>5</td>
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<td>104</td>
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<td>111</td>
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<tr>
<td>129</td>
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<td>132</td>
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<td>141</td>
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<td>156</td>
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</tr>
<tr>
<td>177</td>
<td>17</td>
</tr>
<tr>
<td>178</td>
<td>0</td>
</tr>
</tbody>
</table>

a. % estimated solvent accessibility with 1.4 angstrom probe radius

**FIG. 13**
Estimated solvent accessibility of amino acids defined as critical for optimal IL-22 binding to IL-22BP, IL-22R and/or IL-10R2

<table>
<thead>
<tr>
<th>Location</th>
<th>IL-22R</th>
<th>% SA(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>F57</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>L59</td>
<td>0</td>
</tr>
<tr>
<td>AB</td>
<td>D67</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>T70</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>D71</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>V72</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>R73</td>
<td>59</td>
</tr>
<tr>
<td>F</td>
<td>G159</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>I161</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>K162</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>G165</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>L169</td>
<td>26</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Location</th>
<th>IL-10R2</th>
<th>% SA(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre-A</td>
<td>A34</td>
<td>nd(^c)</td>
</tr>
<tr>
<td>A</td>
<td>Y51</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>T52</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>N54</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>R55</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>T56</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>K61</td>
<td>56</td>
</tr>
<tr>
<td>AB</td>
<td>A66</td>
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<tr>
<td>BC</td>
<td>V83</td>
<td>5</td>
</tr>
<tr>
<td>C</td>
<td>R88</td>
<td>21</td>
</tr>
<tr>
<td>CD</td>
<td>P113</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>Y114</td>
<td>23</td>
</tr>
<tr>
<td>D</td>
<td>E117</td>
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<tr>
<td></td>
<td>F121</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>L122</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>L125</td>
<td>2</td>
</tr>
<tr>
<td>F</td>
<td>M172</td>
<td>39</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Location</th>
<th>IL-22BP</th>
<th>% SA</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB</td>
<td>D67</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>R73</td>
<td>59</td>
</tr>
<tr>
<td>BC</td>
<td>V83</td>
<td>5</td>
</tr>
<tr>
<td>F</td>
<td>K162</td>
<td>3</td>
</tr>
</tbody>
</table>

\(^a\) Location in indicated IL-22 helix or loop

\(^b\) % estimated solvent accessibility with 1.4 angstrom probe radius

\(^c\) A34 has not been resolved in IL-22 crystal structure

**FIG. 14**
Human IL-22 Nucleotide Sequence

1 GAATTCCGCC AAAGAGCCCT ACAAGTCTCCT CTTCCCCAGT CACCAGTTGC
51 TGAGATTTAGA ATGAGTCGCA ATGCCGGCAGC GGAAGAAATC TGGAGCTTCT
101 GTCCTATAGG GCCAGGCTGC CACAGCTTCA GCTCTCTCAG TGCCCTGCTT
151 GGTCAGCCGG CACAGAGGCTG CGCCCATCGC ACTCCACTGC AGGCTTGGAC
201 AGTCCAACTC CCAGACCGCA TATATCCAGC ACGCATCTTC TACCTGCTGCT
251 AAGGAGCACTA GTTGGCTACA TAAACACACA GACGCCGCTG TCATTGCGGA
301 GAAAGCTGTTG CAGGGATGCA TGTGAGTGGC GCCGCTGACT CTGATGGAAC
351 AGTTGCGTAAA GTCACCTCTT GAAAAAGTGC TCTGCTCTCA ATCTCGATAG
401 TCCCGACCTT ATATGGAAGG GCCGGCTGCC TCTCCGGCCA GCTCAGCCCC
451 CAGGCAATAGC ACCACATGTA TGGAGGTTAG TGAACCTGCGA ATCCAGAGCA
501 ATGTGCAAAA GCTGAGAGAC ACGTGAAGAA AGCTTGAGGA GAGTGGAGAG
551 ATCAAAGCAA TGGAGAACTT GAATGTGCCG TATTGTCTCT TGGAAAATGC
601 CTGCAATTTGA CCAGGCACAA GCTGAAAATA GAATAACTAA CCCCTTTCC
651 CTGACTAGAA TAAACAATTAG ATGCCCCAAA CGATATTTTT TGAACCAAAA
701 GGAAGAAGGGG AAGCCAAACT CATCATGATG TGGAGATCCC CAAATGAAAC
751 CCTGCGTTAG TACAAAGGGA AACCAATGCC ACTTTTGTTC ATAAAGAACC
801 AAAGCTGACCT TCTAAGAAGAT AGATTTTTAT AGTAACATTT ATGTCTGGAAC
851 TGCTGTCTGT AAGACAGATA ACAATTAGTT TTTAAATAAT TGCTTCTGTTT
901 CCATAAAAAA GATTACTTTC CATCCTTTTA GGGAAAAA ACCCTAAAATA
951 GCCCTAGTGC TCGCTATTCA GTAATTAATA TTTATAATAT TTTATTTTAT
1001 TATATATAGA CTGCCATTTT TTTATTATAT TTTATTATAT TTAGCTTTAT
1051 ATAGAAACA ATGCTCTGTA TGCTACTTGT ACGTGAAGGC TATATGTTAT
1101 ATATATATAG AATATATATAG ATGTATACAC GGTGTTATG TACCTGATAA
1151 ACACCTGGAT ATCCGAAAAA AAAAAAAA AAAGCGGCGC

(SEQ ID NO: 1)

Human IL-22 Amino Acid Sequence

1 MAALQKSVSS FMLGTLATSC LLLLALLVQG GAAAPISSHG RLDKSNFPQQP
51 YITNRTFMLA KEASLADNNT DVRLIGEKLFL HGVMSERCY LMKQVLNFTL
101 EEVLPFQSDR FQPYMQEVVVP FLARLSNRSL TCHIEGDILH IQRNVQLKLD
151 TVKKGVESGE IKAIGELDLL FMSLNRACI (SEQ ID NO: 2)

FIG. 15
Mouse IL-22 Nucleotide Sequence

1  GAATTCGCCC AAACAGGCTT ACCTAAACAG GCTCTCCTCT CAGTTATCAA
51  CTTTGGACAC TTGTTGAGATC TCTGAGTGGCT GTTCTCGAGA AATCTATGGAG
101  TTTTTCCTCT ATGAGGGACCT TGCGGCGGCA CTGCTGTGCTT CTCACTGGCC
151  TGCGGGCAGA GGAGCAGAAT GCGCTGGCCG TCAACGCCCG GTGCAAGGCTT
201  GAGCTTGTCA ACTCAGCAGCA GCCATACAGT GTCACCCGCA CTTTTAGCTCT
251  GCCAACGGAG GCCAGCCTTG CAGATAAGAC CACAGATAGC CGGCTCATCG
301  GGAGAAGACT GTTCCGAGGA GTGAGTGCTA AGGATCACTG TACCTGATAG
351  AAGCAGGCTGC TCAACTCTAC CTCGGGAGAC GTTCTGCTCC CCCAGTCA
401  CAGGTTCAG CCCTACATGC AGAGGCTGCT GCCCTCTCCT ACAAACCTCA
451  GCATTCCTAAG AGCTCTGTTA CACATCCAG GTAGCCAGCC ACCCATCCCG
501  AGAGATGCTCA CAGGCTGAAA GGAGCCAGTG AAAAAAGCTT GAGAGAGTGG
551  AGAGATCAAGA GGCTGGGGGA AACTGGACCT GCTGTATTAG TCTCTGAGAA
601  ATGATGGCACT GGTAGCCGAGA AGAAGCTAGA AAACGAAAGA CGCTCTCTTC
651  CTGCTTCTCA AAAAAAGAAA TAAGATCCCT GAAATGGACTT TTITTTACTAAA
701  GCAGAATGGA AAGCTAAGCT CCATCTATAT TAAAGATTTT CACATCAACC
751  CTGCTCGGATG TGAAGAAAGA AATAGTAGCA AGTCTGACCT GAGACAGAG
801  GTAGACTTAGA TACACCAAAATA GATGTAAAACA AAATGGTTGA AAAGAGGTGT
901  ACCCTATTTT CTTGTAAGAA AAAAAATCAT TGAACCTGTG TTCCATCTCC
951  AATATTTTAT ATATATGATAA TTATTTTATTA TAATGATACAA TTTATTTTTAT
1001  GTCTCTTATTT TAAATAGGTA ATATTATGAA TAAAATATTCA GTATTATTTA
1051  ATTATATATTT TTATATATTT AAGAAATATGA TAATATATTTA GAAACAGATG
1101  ATCTTTAGGCT TTAAGAACA CATGGATAAT ATAAAGAATAA AAAAAAAA
1151  CAGAGGCGC (SEQ ID NO:3)

Mouse IL-22 Amino Acid Sequence

1  MAVLQLMSMSF LGTTLAAASC LLILALWAQEE ANALPVNTREC KLEVSNFQQP
51  YIVNRTFNLK KEASLADNTT DVRLIIEKLF RGVSAKDQCY LMKQQLNFTL
101  EDVLLPQSDR FQPYMQBVTP FLTQLSNQLS SCHISGDDQNY IQKNVRLKE
151  TVKCLKGESGE IKAIGELDDL FMSLRNACAV* (SEQ ID NO:4)

FIG. 16
Human IL-19 Amino Acid Sequence

1  mklqcvslwl lgtililcsv dnhglrcclq stdmhhiees fqeikraiga
51  kdtfpnvtil stletlqilk pldvccvtkn llafyvdrvf kdhgcpnpki
101  lrkissians flymqktrrq cqeqrqchcr qeatnatrvi hdnydqlevh
151  aaiaksltgel dvflawinkn hevmssa (SEQ ID NO. 5)

FIG. 17A

Human IL-20 Amino Acid Sequence

1  mkasslafsl lsafyllwlt psqglktlnl gscviatnlq eirngfseir
51  gsvgakdgni dirilrrtes lqdtkpanrc cllrlrlrlry idrvfksnyqt
101  pdhytlrkis slansfltik kdrlchahm tchcgseekm kysgilshfe
151  klepqaavvk algeldillg wmeete (SEQ ID NO. 6)

FIG. 17B
Human IL-24 Amino Acid Sequence

1  mnfqgrlqsl wtlarpcfpp llatasqmgm vvlpclgftl llwsqvgsgaq
51  ggefhegpec vkgvvpqklw eafwavkdtn qaqdnitsar llqgevqlqv
101 sdaescylvh tlefeylktv fknynrtve vrtlksfetl annfvlvsq
151 lgpqenemf sirdsahrf llfrarfkql dveaaltkal gevdlitwml
201 qkfykl (SEQ ID NO. 7)

FIG. 18A

Human IL-26 Amino Acid Sequence

1  mlvnlfrlrcg lllvtlslai akhkqssftk scyprgtlsq avdalyikaa
51  wkatipedr iknirllkkk tkkqfkmncq fqeqlslffm edvfgqlqlq
101 gckkicrved fhs1rqlksh ciscassare mksitmkkri fyrlgnkqly
151 kaiseldill swikkllless q (SEQ ID NO. 8)

FIG. 18B