INTERLEUKIN-21 RECEPTOR BINDING PROTEINS

Related Applications

[0001] This application claims the benefit of priority from U.S. Provisional Patent Application No. 61/055,500, filed May 23, 2008, the content of which is hereby incorporated by reference herein in its entirety.

BACKGROUND OF THE INVENTION

Field of the Invention

[0002] The present invention relates to binding proteins and antigen-binding fragments thereof that bind interleukin-21 receptor (IL-21R), in particular, human IL-21R, and their use in regulating IL-21R-associated activities. The binding proteins disclosed herein are useful in treating and/or diagnosing IL-21R-associated disorders, e.g., inflammatory disorders, autoimmune diseases, allergies, transplant rejection, hyperproliferative disorders of the blood, and other immune system disorders.

Related Background Art

[0003] Antigens initiate immune responses and activate the two largest populations of lymphocytes: T cells and B cells. After encountering antigen, T cells proliferate and differentiate into effector cells, while B cells proliferate and differentiate into antibody-secreting plasma cells. These effector cells secrete and/or respond to

cytokines, which are small proteins (less than about 30 kDa) secreted by lymphocytes and other cell types.

[0004] Human IL-21 is a cytokine that shows sequence homology to IL-2, IL-4 and IL-15 (Parrish-Novak et al. (2000) *Nature* 408:57-63). Despite low sequence homology among interleukin cytokines, cytokines share a common fold into a "four-helix-bundle" structure that is representative of the family. Most cytokines bind either class I or class II cytokine receptors. Class II cytokine receptors include the receptors for IL-10 and the interferons, whereas class I cytokine receptors include the receptors for IL-2 through IL-7, IL-9, IL-11, IL-12, IL-13, and IL-15, as well as hematopoietic growth factors, leptin, and growth hormone (Cosman (1993) *Cytokine* 5:95-106).

[0005] Human IL-21R is a class I cytokine receptor. The nucleotide and amino acid sequences encoding human IL-21 and its receptor (IL-21R) are described in International Application Publication Nos. WO 00/053761 and WO 01/085792; Parrish-Novak et al. (2000) *supra*; and Ozaki et al. (2000) *Proc. Natl. Acad. Sci. USA* 97:11439-44. IL-21R has the highest sequence homology to the IL-2 receptor β chain and the IL-4 receptor α chain (Ozaki et al. (2000) *supra*). Upon ligand binding, IL-21R associates with the common gamma cytokine receptor chain (γc) that is shared by receptor complexes for IL-2, IL-3, IL-4, IL-7, IL-9, IL-13 and IL-15 (Ozaki et al. (2000) *supra*; Asao et al. (2001) *J. Immunol.* 167:1-5).

[0006] IL-21R is expressed in lymphoid tissues, particularly on T cells, B cells, natural killer (NK) cells, dendritic cells (DC) and macrophages (Parrish-Novak et al. (2000) *supra*), which allows these cells to respond to IL-21 (Leonard and Spolski (2005) *Nat. Rev. Immunol.* 5:688-98). The widespread lymphoid distribution of IL-21R indicates that IL-21 plays an important role in immune regulation. *In vitro* studies have shown that IL-21 significantly modulates the function of B cells, $CD4^+$ and $CD8^+$ T cells, and NK cells (Parrish-Novak et al. (2000) *supra*; Kasaian et al. (2002) *Immunity* 16:559-69). Recent evidence suggests that IL-21–mediated signaling can have antitumor activity (Sivakumar et al. (2004) *Immunology*

112:177-82), and that IL-21 can prevent antigen-induced asthma in mice (Shang et al. (2006) *Cell. Immunol.* 241:66-74).

[0007] In autoimmunity, disruption of the IL-21 gene and injection of recombinant IL-21 have been shown to modulate the progression of experimental autoimmune myasthenia gravis (EAMG) and experimental autoimmune encephalomyelitis (EAE), respectively (King et al. (2004) *Cell* 117:265-77; Ozaki et al. (2004) *J. Immunol.* 173:5361-71; Vollmer et al. (2005) *J. Immunol.* 174:2696-2701; Liu et al. (2006) *J. Immunol.* 176:5247-54). In these experimental systems, it has been suggested that the manipulation of IL-21–mediated signaling directly altered the function of $CD8^+$ cells, B cells, T helper cells, and NK cells.

SUMMARY OF THE INVENTION

[0008] The present invention describes the isolation and characterization of binding proteins, for example, human antibodies and fragments thereof, that specifically bind to the human and murine IL-21R. The binding proteins described herein are derived from antibody 18A5, which is disclosed in U.S. Patent No. 7,495,085, the entirety of which is hereby incorporated by reference herein. The binding proteins of the present invention have a much greater degree of affinity to human and/or murine IL-21R than does the parental 18A5 antibody.

[0009] The invention provides, at least in part, IL-21R binding agents (such as binding proteins and antigen-binding fragments thereof) that bind to IL-21R, in particular, human IL-21R, with high affinity and specificity. The binding proteins, and antigen-binding fragments thereof, of the present invention are also referred to herein as "anti-IL-21R binding proteins" and "fragments thereof," respectively. In one embodiment, the binding protein or fragment thereof reduces, inhibits, or antagonizes IL-21R activity. Such binding proteins can be used to regulate immune responses or IL-21R-associated disorders by antagonizing IL-21R activity. In other embodiments, the anti-IL-21R binding protein can be used diagnostically, or as a targeting binding protein to deliver a therapeutic or cytotoxic agent to an IL-21R-

expressing cell. Thus, the anti-IL-21R binding proteins of the invention are useful in diagnosing and treating IL-21R-associated disorders, e.g., inflammatory disorders, autoimmune diseases, allergies, transplant rejection, hyperproliferative disorders, and other immune system disorders.

[0010] Accordingly, in one aspect, the binding proteins of the invention feature an isolated binding protein (e.g., an isolated antibody) or antigen-binding fragment thereof that binds to IL-21R, in particular, human IL-21R. In certain embodiments, the anti-IL-21R binding protein (e.g., antibody) can have one or more of the following characteristics: (1) it is a monoclonal or single specificity binding protein; (2) it is a human binding protein; (3) it is an *in vitro*-generated binding protein; (4) it is an *in vivo*-generated (for example, a transgenic mouse system) binding protein; (5) it inhibits the binding of IL-21 to IL-21R; (6) it is an IgG1; (7) it binds to human IL-21R with an association constant of at least about 10^5 $M^{-1}s^{-1}$; (8) it binds to murine IL-21R with an association constant of at least about $5x10^4$ $M^{-1}s^{-1}$; (9) it binds to human IL-21R with a dissociation constant of about $10^{-3}s^{-1}$ or less; (10) it binds to murine IL-21R with a dissociation constant of about $10^{-2}s^{-1}$ or less; (11) it inhibits human IL-21R-mediated proliferation of human IL-21R-expressing BaF3 cells with an IC_{50} of about 1.75 nM or less; (12) it inhibits murine IL-21R-mediated proliferation of murine IL-21R-expressing BaF3 cells with an IC_{50} of about 0.5 nM or less; (13) it inhibits human IL-21R-mediated proliferation of human IL-21R-expressing TF1 cells with an IC_{50} of about 14.0 nM or less; (14) it inhibits IL-21-mediated proliferation of human primary B cells with an IC_{50} of about 1.9 nM or less; (15) it inhibits IL-21-mediated proliferation of human primary $CD4^+$ T cells with an IC_{50} of about 1.5 nM or less; and (16) it inhibits IL-21-mediated proliferation of murine primary $CD4^+$ T cells with an IC_{50} of about 5.0 nM or less.

[0011] Nonlimiting illustrative embodiments of the binding proteins of the invention (the term "binding proteins" also includes and refers to antigen-binding fragments thereof, as appropriate) are referred to herein as AbA-AbZ, and correlation of these terms with terms used in U.S. Provisional Patent Application No. 61/055,500 is

presented in **Table 2A**. Other illustrative embodiments of the binding proteins of the present invention, i.e., scFv, are referred to herein as H3-H6, L1-L6, L8-L21, and L23-L25, as detailed in **Table 2B**.

[0012] One embodiment of the present invention is an isolated binding protein or antigen-binding fragment thereof that binds to IL-21R, wherein the binding protein or antigen-binding fragment thereof comprises at least one amino acid sequence that is at least about 95% identical to an amino acid sequence(s) selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 165-168, 171-193, 213-229, 240, 242, 244, 246, and 248. The isolated binding protein or antigen-binding fragment can be, for example, an antibody, an scFv, a V_H, a V_L, or a CDR.

[0013] Another embodiment of the present invention is an isolated binding protein or antigen-binding fragment thereof that binds to IL-21R, wherein the binding protein or antigen-binding fragment thereof comprises at least one amino acid sequence encoded by a nucleotide sequence that is at least about 95% identical to a nucleotide sequence(s) selected from the group consisting of SEQ ID NOs:13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 239, 241, 243, 245, and 247.

[0014] A further embodiment of the present invention is an isolated binding protein or antigen-binding fragment comprising at least one amino acid sequence selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116,

118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 165-168, 171-193, 213-229, 240, 242, 244, 246, and 248.

[0015] Yet another embodiment of the present invention is an isolated binding protein or antigen-binding fragment thereof that binds to IL-21R, wherein the binding protein or antigen-binding fragment thereof comprises at least one amino acid sequence that is at least about 95% identical to an amino acid sequence(s) selected from the group consisting of SEQ ID NOs:6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162-195, 213-229, 240, 242, 244, 246, and 248, and wherein, if the binding protein or antigen-binding fragment comprises at least one amino acid sequence that is at least about 95% identical to the sequence(s) selected from the group consisting of SEQ ID NOs:6, 8, 10, 12, 163, 164, 169, 170, 194, and 195, then the binding protein or antigen-binding fragment must also comprise at least one amino acid sequence that is at least about 95% identical to the amino acid sequence(s) selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 165-168, 171-193, 213-229, 240, 242, 244, 246, and 248.

[0016] Yet another embodiment of the present invention is an isolated binding protein or antigen-binding fragment thereof that binds to IL-21R, wherein the binding protein or antigen-binding fragment thereof comprises at least one amino acid sequence encoded by a nucleotide sequence that is at least about 95% identical to a nucleotide sequence(s) selected from the group consisting of SEQ ID NOs:5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53,

55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 239, 241, 243, 245, and 247, and wherein, if the binding protein or antigen-binding fragment comprises at least one amino acid sequence encoded by a nucleotide sequence that is at least about 95% identical to the sequence(s) selected from the group consisting of SEQ ID NOs:5, 7, 9, and 11, then the binding protein or antigen-binding fragment must also comprise at least one amino acid sequence encoded by a nucleotide sequence that is at least about 95% identical to the nucleotide sequence(s) selected from the group consisting of SEQ ID NOs:13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 239, 241, 243, 245, and 247.

[0017] Yet a further embodiment of the present invention is an isolated binding protein or antigen-binding fragment comprising at least one amino acid sequence selected from the group consisting of SEQ ID NOs:6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162-195, 213-229, 240, 242, 244, 246, and 248, wherein, if the binding protein or antigen-binding fragment comprises at least one amino acid sequence that is at least about 95% identical to the sequence(s) selected from the group consisting of SEQ ID NOs:6, 8, 10, 12, 163, 164, 169, 170, 194, and 195, then the binding protein or antigen-binding fragment must also comprise at least one amino acid sequence that is at least about 95% identical to the amino acid sequence(s) selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102,

104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 165-168, 171-193, 213-229, 240, 242, 244, 246, and 248.

[0018] Another embodiment of the present invention is an isolated binding protein or antigen-binding fragment thereof that binds to IL-21R, wherein the binding protein or antigen-binding fragment thereof comprises a light chain and a heavy chain, and wherein the heavy chain comprises at least one amino acid sequence selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 68, 70, 72, 88, 90, 92, 94, 213, 218, 219, 240, and 242, or a sequence substantially identical thereto (e.g., a sequence substantially identical thereto includes a sequence at least about 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more identical thereto), or a sequence substantially homologous thereto (e.g., a sequence substantially homologous thereto includes a sequence at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more identical thereto). In a further embodiment, the binding protein or antigen-binding fragment comprises a V_L domain and a V_H domain, and the V_H domain comprises at least one amino acid sequence selected from the group consisting of SEQ ID NOs:14, 16, 18, and 20, or a sequence substantially identical or homologous thereto. Another embodiment of the present invention is an isolated binding protein or antigen-binding fragment thereof that binds to IL-21R, wherein the binding protein or antigen-binding fragment thereof comprises a light chain and a heavy chain, and wherein the light chain comprises at least one amino acid sequence selected from the group consisting of SEQ ID NOs:22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 74, 76, 78, 80, 82, 84, 86, 96, 98, 100, 102, 104, 106, 108, 214-217, 220-229, 244, 246, and 248, or a sequence substantially identical or homologous thereto. In a further embodiment, the binding protein or antigen-binding fragment comprises a V_L domain and a V_H domain, and the V_L domain comprises at least one amino acid sequence selected from the group consisting of SEQ ID NOs:22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 215, 217, 221, 223,

225, 227, and 229, or a sequence substantially identical or homologous thereto. In yet another embodiment, the heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:88, 90, 92, 94, 213, 218, 219, 240, and 242, and the light chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:96, 98, 100, 102, 104, 106, 108, 214, 216, 220, 222, 224, 226, 228, 244, 246, and 248, or a sequence substantially identical or homologous thereto.

[0019] The binding proteins of the present invention, e.g., antibodies, can be germlined or nongermlined. They may specifically bind to the same IL-21R epitope or a similar epitope (e.g., an overlapping epitope) to which AbA-AbZ, H3-H6, L1-L6, L8-L21, or L23-L25 bind. In other embodiments, the binding proteins specifically bind to a fragment of IL-21R, e.g., a fragment of at least 10, 20, 50, 75, 100, 150, or 200 amino acids contiguous to the amino acid sequence set forth in SEQ ID NOs:2 or 4, or a sequence that is at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or more identical thereto.

[0020] Another embodiment of the present invention is an isolated binding protein or antigen-binding fragment thereof that binds to an IL-21R epitope that is recognized by a binding protein selected from the group consisting of AbA-AbZ, H3-H6, L1-L6, L8-L21, and L23-L25, wherein the binding protein or antigen-binding fragment competitively inhibits the binding of a binding protein selected from the group consisting of AbA-AbZ, H3-H6, L1-L6, L8-L21, and L23-L25 to human IL-21R. In another embodiment, the binding protein or antigen-binding fragment comprises a heavy chain, a light chain, or an F_v fragment comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 165-168, 171-193, 213-229, 240, 242, 244, 246, and 248, or a sequence substantially identical or

homologous thereto. In yet another embodiment, the binding protein or antigen-binding fragment comprises a heavy chain, a light chain, or an F_v fragment comprising an amino acid sequence encoded by a nucleotide sequence selected from the group consisting of SEQ ID NOs:13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 239, 241, 243, 245, and 247, or a sequence substantially identical or homologous thereto.

[0021] In yet other embodiments, the binding protein comprises at least one complementarity determining region (CDR) of these V_H and V_L domains (e.g., one or more, two or more, three or more, four or more, or five or more of contiguous CDRs (e.g., two or more CDRs separated by framework regions (FR) or a linker) or noncontiguous CDRs (e.g., two or more CDRs separated by at least one other CDR and, e.g., FR(s)). For example, the binding protein can include one, two, three or more CDRs of the V_H domain and/or the V_L domain.

[0022] The disclosure provides nucleic acid sequences from the V_H and V_L domains of AbA-AbZ, H3-H6, L1-L6, L8-L21, and L23-L25. Also contemplated are nucleic acid sequences that comprise at least one CDR from AbA-AbZ, H3-H6, L1-L6, L8-L21, and L23-L25. The disclosure also provides vectors and host cells comprising such nucleic acids.

[0023] The binding proteins of the invention can be full-length (e.g., include at least one complete heavy chain and at least one complete light chain), or can include only an antigen-binding fragment (e.g., a Fab, a Fab', a $F(ab')_2$, an Fv, a single chain Fv fragment, an Fd fragment, a dAb fragment, a CDR, or other fragment of a full-length binding protein that retains the ability to specifically bind to an antigen). The binding protein can include a constant region, or a portion thereof, chosen from any of the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes. For example, heavy chain constant regions of the various isotypes can be used,

including: IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD, and IgE. The light chain constant region can be chosen from kappa or lambda. The binding protein may be an IgG, or it may also be $IgG1_{\kappa}$ or $IgG1\lambda$.

[0024] The anti-IL-21R binding protein described herein can be derivatized or linked to another functional molecule (such as another peptide or protein, e.g., a Fab fragment). For example, a binding protein of the invention can be functionally linked (e.g., by chemical coupling, genetic fusion, noncovalent association or otherwise) to at least one other molecular entity, such as another binding protein (e.g., a bispecific or a multispecific binding protein), toxin, radioisotope, cytotoxic or cytostatic agent, among others.

[0025] In one embodiment of the invention, the association constant of the binding protein or antigen-binding fragment for human IL-21R is at least about 10^5 $M^{-1}s^{-1}$. In another embodiment, the binding protein or antigen-binding fragment inhibits IL-21-mediated proliferation of BaF3 cells with an IC_{50} of about 1.75 nM or less, and the BaF3 cells comprise a human IL-21R. In another embodiment, the binding protein or antigen-binding fragment inhibits IL-21-mediated proliferation of TF1 cells with an IC_{50} of about 14.0 nM or less, and the TF1 cells comprise a human IL-21R. In another embodiment, the binding protein or antigen-binding fragment inhibits IL-21-mediated proliferation of primary human B cells with an IC_{50} of about 1.9 nM or less, and the B cells comprise a human IL-21R. In yet another embodiment, the binding protein or antigen-binding fragment inhibits IL-21-mediated proliferation of primary human $CD4^+$ cells with an IC_{50} of about 1.5 nM or less, and the $CD4^+$ cells comprise a human IL-21R.

[0026] In one embodiment, the present invention provides a binding protein or antigen-binding fragment that specifically binds to an amino acid sequence that is at least about 95% identical to any sequence of at least 100 contiguous amino acids of SEQ ID NO:2. Another embodiment provides a binding protein or antigen-binding fragment that inhibits the binding of IL-21 to IL-21R. In at least one embodiment,

the binding protein or antigen-binding fragment is IgG1. In at least one embodiment, the binding protein or antigen-binding fragment is human.

[0027] In another aspect, the invention features a pharmaceutical composition containing at least one anti-IL-21R binding protein and a pharmaceutically acceptable carrier. The pharmaceutical composition can further include a combination of at least one anti-IL-21R binding protein and at least one other therapeutic agent (e.g., cytokine and growth factor inhibitors, immunosuppressants, anti-inflammatory agents, metabolic inhibitors, enzyme inhibitors, cytotoxic agents, cytostatic agents, or combinations thereof, as described in more detail herein). Combinations of the anti-IL-21R binding protein and therapeutic agent(s) are also within the scope of the invention. The compositions and combinations of the invention can be used to regulate IL-21R-associated immune disorders, e.g., by modulating IL-21R signaling.

[0028] In one embodiment, the binding proteins of the invention are antibodies. In further embodiments, the antibodies are polyclonal, monoclonal, monospecific, polyspecific, nonspecific, humanized, human, single-chain, chimeric, synthetic, recombinant, hybrid, mutated, grafted, *in vitro*-generated and/or multispecific (e.g., bispecific antibodies formed from at least two intact antibodies).

[0029] Other embodiments of the invention include an isolated nucleic acid encoding an anti-IL-21R binding protein, an expression vector comprising the nucleic acid, and a host cell transformed with the vector. The host cell may be a bacteria, a mammalian cell, a yeast cell, a plant cell, or an insect cell.

[0030] The binding protein may be directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound binding protein. Suitable detectable substances include, but are not limited to, various enzymes, prosthetic groups, fluorescent materials, luminescent materials, and radioactive materials.

[0031] In another aspect, the invention provides a method for delivering or targeting an agent, e.g., a therapeutic or a cytotoxic agent, to an IL-21R-expressing cell *in vivo*. The method includes administering an anti-IL-21R binding protein to a subject under

conditions that allow for binding of the binding protein to IL-21R. The binding protein may be coupled to a second therapeutic moiety, e.g., a toxin.

[0032] In another embodiment, the invention provides a diagnostic kit comprising a binding protein or antigen-binding fragment of the present invention.

[0033] Additional aspects of the disclosure will be set forth in part in the description, and in part will be obvious from the description, or may be learned by practicing the invention. The invention is set forth and particularly pointed out in the claims, and the disclosure should not be construed as limiting the scope of the claims. The following detailed description includes exemplary representations of various embodiments of the invention, which are not restrictive of the invention as claimed. The accompanying figures constitute a part of this specification and, together with the description, serve only to illustrate embodiments and not limit the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0034] **FIG. 1(a-c)** depicts the neutralization of proliferation of human IL-21R-BaF3 cells by scFv. Cells were mixed with the indicated scFv and then incubated with 100 pg/ml of human IL-21. Proliferation was measured by CELLTITER-GLO® (Promega Corporation, Madison, WI) after 48 hours.

[0035] **FIG. 2(a-c)** depicts the neutralization of proliferation of human IL-21R-TF1 cells by scFv. Cells were mixed with the indicated scFv and then incubated with 100 pg/ml of human IL-21. Proliferation was measured by CELLTITER-GLO® after 48 hours.

[0036] **FIG. 3(a-c)** depicts the neutralization of proliferation of murine IL-21R-BaF3 cells by scFv. Cells were mixed with the indicated scFv and then incubated with 400 pg/ml of murine IL-21. Proliferation was measured by CELLTITER-GLO® after 48 hours.

[0037] **FIG. 4(a-c)** depicts scFv competition with parental antibody 18A5 IgG for binding to murine IL-21R. The scFv were mixed with biotinylated-murine

IL-21R-H/F, and the mixtures were added to antibody 18A5 immobilized on an ELISA plate. Capture of mIL-21R was detected with HRP-streptavidin, and competition for binding to mIL-21R was indicated by a reduction in the A450 signal.

[0038] FIG. 5 depicts the neutralization of IL-21-dependent proliferation by 21 heavy chain / light chain pairs. Antibodies, as indicated in the figure, were added to cells. IL-21 was subsequently added, and proliferation measured with CELLTITER-GLO® after 48 hours. Assays were conducted on human IL-21R-BaF3 cells with 100 pg/ml of human IL-21 (**FIGs. 5a-c**), human IL-21R-TF1 cells with 100 pg/ml of human IL-21 (**FIGs. 5d-f**), or murine IL-21R-BaF3 cells with 400 pg/ml of murine IL-21 (**FIGs. 5g-i**).

[0039] FIG. 6 depicts the binding of 21 anti-IL-21R IgGs to CHO cells transiently expressing human IL-21R (**FIGs. 6a-c**), rat IL-21R (**FIGs. 6d-f**), cynomolgus monkey IL-21R (**FIGs. 6g-i**), and human gamma common chain (**FIGs. 6j-l**). CHO cells were transiently transfected with IL-21R or the control gamma common chain, and binding was detected with HRP-conjugated anti-human IgG in a cell-based ELISA.

[0040] FIG. 7(a-c) depicts the binding specificity of particular anti-IL-21R antibodies (**FIG. 7a**, AbS; **FIG. 7b**, AbQ, AbT, AbO; **FIG. 7c**, AbR, AbP, and AbU), measured by surface plasmon resonance. The anti-IL-21R antibodies were captured on anti-human IgG, and subsequent binding to either murine IL-21R-H/F, human IL-13-H/F, human IL-2Rβ, or human soluble IL-4R was measured in a BIACORE™ (GE Healthcare, Piscataway, NJ) instrument. **FIG. 7d** shows that human IL-2Rβ and human soluble IL-4R are captured by specific anti-IL-2Rβ and anti-IL-4R antibodies, respectively (control).

[0041] FIG. 8(a-d) depicts the binding of anti-IL-21R antibodies to human and murine IL-21R. The indicated human anti-IL-21R antibodies were captured on anti-human IgG immobilized on a BIACORE™ chip. Varying concentrations of human IL-21R-His/FLAG (**FIGs. 8a-b**) and murine IL-21R-His/FLAG (**FIGs. 8c-d**) were allowed to flow over the chip, and binding and dissociation were monitored.

[0042] FIG. 9 depicts the binding of anti-IL-21R antibodies to human and cynomolgus monkey IL-21R. Human anti-IL-21R antibodies AbS and AbT were captured on anti-human IgG immobilized on a BIACORE™ chip. Varying concentrations of human and cynomolgus monkey IL-21R-His/FLAG were allowed to flow over the chip, and binding and dissociation were monitored. **FIG. 9a** shows cynomolgus monkey IL-21R-His/FLAG binding to AbS. **FIG. 9b** shows human IL-21R-His/FLAG binding to AbS. **FIG. 9c** shows cynomolgus monkey IL-21R-His/FLAG binding to AbT. **FIG. 9d** shows human IL-21R-His/FLAG binding to AbT.

[0043] FIG. 10 depicts an epitope assessment of IL-21R antibodies. In the experiment depicted in **FIG. 10a** (see also illustration at left of Y-axis), murine IL-21R-H/F (His-Flag fusion protein) was captured by anti-IL-21R antibody AbS immobilized on a BIACORE™ chip. Additional anti-IL-21R antibodies (AbS, AbT, D5 (D5-20, a neutralizing anti-murine IL-21R antibody), and 7C2 (a nonneutralizing anti-murine IL-21R control antibody)) were flowed over the chip and their binding to the captured IL-21R-H/F was monitored. In the experiment depicted in **FIG. 10b**, human IL-21R-H/F was captured by anti-IL-21R antibody AbS immobilized on a BIACORE™ chip. Additional anti-IL-21R antibodies (AbS, AbT, and 9D2 (a nonneutralizing anti-human IL-21R control antibody)) were flowed over the chip and their binding to the captured IL-21R-H/F was monitored.

[0044] FIG. 11 depicts the neutralization of proliferation of human IL-21R-BaF3 cells and murine IL-21R-BaF3 cells by the indicated antibodies. Antibodies were added to cells. IL-21 was subsequently added and proliferation measured with CELLTITER-GLO® after 48 hours. Assays were conducted on human IL-21R-BaF3 cells with 100 pg/ml of human IL-21 (**FIG. 11a**), murine IL-21R-BaF3 cells with 200 pg/ml of murine IL-21 (**FIG. 11b**), and human IL-21R-TF1 cells with 100 pg/ml of human IL-21 (**FIG. 11c**).

[0045] FIG. 12 depicts the neutralization of IL-21-dependent proliferation of human primary B cells. The indicated antibodies were added to primary human B cells

along with anti-CD40 antibodies and human IL-21. Incorporation of ^{3}H-thymidine was measured after three days. **FIG. 12a** depicts the comparison between AbQ, AbR, AbS, AbT, AbU, IL-13 triple-mutant, and 18A5 parental antibody; **FIG. 12b** depicts the comparison between AbT, AbV, AbW, AbU, and human IgG1 control (hIg1).

[0046] FIG. 13 depicts the neutralization of IL-21-dependent proliferation of human primary $CD4^{+}$ T cells. The indicated antibodies were added to activated primary human $CD4^{+}$ T cells along with human IL-21, and incorporation of ^{3}H-thymidine was measured after three days.

[0047] FIG. 14 depicts the neutralization of IL-21-dependent proliferation of murine primary $CD8^{+}$ T cells. The indicated antibodies were added to activated primary murine $CD8^{+}$ T cells along with human IL-21, and incorporation of ^{3}H-thymidine was measured after three days.

[0048] FIG. 15 depicts the measurement of antibody-dependent cellular cytotoxicity (ADCC) induced by anti-IL-21R antibodies. PBMC-dependent killing of CFSE-labeled BJAB cells coated with the indicated anti-IL-21R antibodies was measured by incorporation of propidium iodide. The anti-CD20 antibody rituximab (RITUXAN®, Genentech, Inc., South San Francisco, CA) was included as a positive control, and an anti-IL-13 antibody was included as a negative control.

[0049] FIG. 16 depicts complement C1q binding by anti-IL-21R antibodies. The indicated anti-IL-21R antibodies were immobilized on an ELISA plate and, following incubation with human serum, C1q binding was measured with chicken anti-human C1q and an HRP-conjugated anti-chicken IgY antibody. The anti-CD20 antibody rituximab (RITUXAN®) was included as a positive control, and an anti-IL-13 antibody was included as a negative control.

[0050] FIG. 17(a-c) depicts amino acid sequences for AbQ, including V_H and V_L domains, CDRs (H1, H2, H3, L1, L2, and L3), and constant regions.

[0051] FIG. 18(a-c) depicts amino acid sequences for AbR, including V_H and V_L domains, CDRs (H1, H2, H3, L1, L2, and L3), and constant regions.

[0052] FIG. 19(a-c) depicts amino acid sequences for AbW, including V_H and V_L domains, CDRs (H1, H2, H3, L1, L2, and L3), and constant regions.

[0053] FIG. 20(a-c) depicts amino acid sequences for AbS, including V_H and V_L domains, CDRs (H1, H2, H3, L1, L2, and L3), and constant regions.

[0054] FIG. 21(a-c) depicts amino acid sequences for AbT, including V_H and V_L domains, CDRs (H1, H2, H3, L1, L2, and L3), and constant regions.

[0055] FIG. 22(a-c) depicts amino acid sequences for AbO, including V_H and V_L domains, CDRs (H1, H2, H3, L1, L2, and L3), and constant regions.

[0056] FIG. 23(a-c) depicts amino acid sequences for AbP including V_H and V_L domains, CDRs (H1, H2, H3, L1, L2, and L3), and constant regions.

[0057] FIG. 24(a-c) depicts amino acid sequences for AbU, including V_H and V_L domains, CDRs (H1, H2, H3, L1, L2, and L3), and constant regions.

[0058] FIG. 25(a-c) depicts amino acid sequences for AbV, including V_H and V_L domains, CDRs (H1, H2, H3, L1, L2, and L3), and constant regions.

[0059] FIG. 26(a-g) depicts results generated from additional studies that were performed similarly to those performed to generate the results shown in **FIGs. 5, 11, 12, 13,** and **14** (described above).

[0060] FIG. 27 depicts IL-21 cytokine competition with antibody AbT for binding to murine IL-21R. Vehicle or increasing amounts of IL-21 was mixed with biotinylated murine IL-21R-His/FLAG, and the mixtures were added to AbT immobilized on an ELISA plate. Capture of mIL-21R was detected with HRP-streptavidin, and competition for binding to mIL-21R was indicated by a reduction in the A450 signal.

DETAILED DESCRIPTION OF THE INVENTION

[0061] The binding proteins of the present invention were initially derived from parental antibody 18A5, but differ from 18A5 in the amino acid sequences of portions of the heavy chain and/or light chain complementarity determining region 3 (CDR3). Additionally, the present binding proteins show improved potency in

binding to and neutralizing both human and murine IL-21R as compared to 18A5 in the equivalent format (e.g., scFv or IgG). High-potency neutralization of IL-21R from both species (human and mouse) by a single binding protein has not previously been reported. The present binding proteins having a greater neutralization potency than their parental antibody may translate into higher efficacy as compared to agents previously described. In addition, the amino acid sequence of the V_H and V_L framework regions has been altered to match sequences encoded by human genomic sequence, thereby reducing the potential for human anti-human antibody responses in patients treated with the present binding proteins.

Definitions

[0062] 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 and elsewhere in the specification.

[0063] The term "binding protein" as used herein includes any naturally occurring, recombinant, synthetic, or genetically engineered protein, or a combination thereof, that binds an antigen, target protein, or peptide, or a fragment(s) thereof. Binding proteins of the invention can include antibodies, or be derived from at least one antibody fragment. The binding proteins can include naturally occurring proteins and/or proteins that are synthetically engineered. Binding proteins of the invention can bind to an antigen or a fragment thereof to form a complex and elicit a biological response (e.g., agonize or antagonize a particular biological activity). Binding proteins can include isolated antibody fragments, "Fv" fragments consisting of the variable regions of the heavy and light chains of an antibody, recombinant single-chain polypeptide molecules in which light and heavy chain variable regions are connected by a peptide linker ("scFv proteins"), and minimal recognition units consisting of the amino acid residues that mimic the hypervariable region. Binding protein fragments can also include functional fragments of an antibody, such as, for example, Fab, Fab', $F(ab')_2$, Fc, Fd, Fd', Fv, and a single variable domain of an

antibody (dAb). The binding proteins can be double or single chain, and can comprise a single binding domain or multiple binding domains.

[0064] Binding proteins can also include 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., Ledbetter et al., U.S. Patent Publication 2005/0136049, 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. The binding proteins of the invention can be derived from any species including, but not limited to mouse, human, camel, llama, fish, shark, goat, rabbit, chicken, and bovine.

[0065] The term "antibody" as used herein refers to an immunoglobulin that is reactive to a designated protein or peptide or fragment thereof. Suitable antibodies include, but are not limited to, human antibodies, primatized antibodies, chimeric antibodies, monoclonal antibodies, monospecific antibodies, polyclonal antibodies, polyspecific antibodies, nonspecific antibodies, bispecific antibodies, multispecific antibodies, humanized antibodies, synthetic antibodies, recombinant antibodies,

hybrid antibodies, mutated antibodies, grafted conjugated antibodies (i.e., antibodies conjugated or fused to other proteins, radiolabels, cytotoxins), and *in vitro*-generated antibodies. The antibody can be from any class of antibodies including, but not limited to, IgG, IgA, IgM, IgD, and IgE, and from any subclass (e.g., IgG1, IgG2, IgG3, and IgG4) of antibodies. The antibody can have a heavy chain constant region chosen from, e.g., IgG1, IgG2, IgG3, or IgG4. The antibody can also have a light chain chosen from, e.g., kappa (κ) or lambda (λ). The antibodies of the invention can be derived from any species including, but not limited to mouse, human, camel, llama, fish, shark, goat, rabbit, chicken, and bovine. 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., an inflammatory, immune, autoimmune, neurodegenerative, metabolic, and/or malignant disorder.

[0066] The term "single domain binding protein" as used herein includes any single domain binding scaffold that binds to an antigen, protein, or polypeptide. Single domain binding proteins can include any natural, recombinant, synthetic, or genetically engineered protein scaffold, or a combination thereof, that binds an antigen or fragment thereof to form a complex and elicit a biological response (e.g., agonize or antagonize a particular biological activity). Single domain binding proteins may be derived from naturally occurring proteins or antibodies, or they can be synthetically engineered or produced by recombinant techology. Single domain binding proteins may be any in the art or any future single domain binding proteins, and may be derived from any species including, but not limited to mouse, human, camel, llama, fish, shark, goat, rabbit, chicken, and bovine. In some embodiments of the invention, a single domain binding protein scaffold 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 binding scaffolds dervied from a variable region of NAR ("IgNARs") are described in International Application Publication No. WO 03/014161 and Streltsov (2005) *Protein Sci.* 14(11):2901-09.

[0067] In other embodiments, a single domain binding protein is a naturally occurring single domain binding protein which has been described in the art as a heavy chain antibody devoid of light chains. Such single domain binding proteins are disclosed in, e.g., International Application Publication No. WO 94/004678. For clarity reasons, a variable domain binding protein that is 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 families besides *Camelidae* may also be used to produce heavy chain binding proteins naturally devoid of light chains. VHH molecules are approximately ten times smaller than traditional IgG molecules. They are single polypeptides and are very stable, resisting extreme pH and temperature conditions. Moreover, they are resistant to the action of proteases, which is not the case for conventional antibodies. Furthermore, *in vitro* expression of VHHs can produce high-yield, properly folded functional VHHs. In addition, binding proteins generated in Camelids can recognize epitopes other than those recognized by antibodies generated *in vitro* via antibody libraries or via immunization of mammals other than Camelids (see, e.g., International Application Publication Nos. WO 97/049805 and WO 94/004678, both hereby incorporated by reference herein).

[0068] The terms "antigen-binding domain" and "antigen-binding fragment" refer to a part of a binding protein that comprises amino acids responsible for the specific binding between the binding protein and an antigen. The part of the antigen that is specifically recognized and bound by the binding protein is referred to as the "epitope." An antigen-binding domain may comprise a light chain variable region

(V_L) and a heavy chain variable region (V_H) of an antibody; however, it does not have to comprise both. Fd fragments, for example, have two V_H regions and often retain antigen-binding function of the intact antigen-binding domain. Examples of antigen-binding fragments of a binding protein include, but are not limited to: (1) a Fab fragment, a monovalent fragment having V_L, V_H, C_L and C_H1 domains; (2) a $F(ab')_2$ fragment, a bivalent fragment having two Fab fragments linked by a disulfide bridge at the hinge region; (3) an Fd fragment, having two V_H and one C_H1 domains; (4) an Fv fragment, having the V_L and V_H domains of a single arm of an antibody; (5) a dAb fragment (see, e.g., Ward et al. (1989) *Nature* 341:544-46), having a V_H domain; (6) an isolated CDR; and (7) a single chain variable fragment (scFv). Although the two domains of an 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 scFv) (see, e.g., Bird et al. (1988) *Science* 242:423-26; Huston et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-83). These binding domain fragments can be 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 binding proteins such as, for example, antibodies.

[0069] The term "neutralizing" refers to a binding protein or antigen-binding fragment thereof (for example, an antibody) that reduces or blocks the activity of a signaling pathway or an antigen, e.g., IL-21/IL-21R signaling pathway or IL-21R antigen.

[0070] The term "effective amount" refers to a dosage or amount that is sufficient to regulate IL-21R activity to ameliorate or lessen the severity of 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.

[0071] The term "human binding protein" includes binding proteins 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.

(5th ed. 1991) *Sequences of Proteins of Immunological Interest*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242. The human antibodies of the invention 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.

[0072] The phrases "inhibit," "antagonize," "block," or "neutralize" IL-21R activity and its cognates refer to a reduction, inhibition, or otherwise diminution of at least one activity of IL-21R due to binding an anti-IL-21R antibody, wherein the reduction is relative to the activity of IL-21R in the absence of the same antibody. The IL-21R activity can be measured using any technique known in the art. Inhibition or antagonism does not necessarily indicate a total elimination of the IL-21R biological activity. A reduction in activity may be about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more.

[0073] The terms "interleukin-21 receptor" or "IL-21R" or the like refer to a class I cytokine family receptor, also known as MU-1 (see, e.g., U.S. Patent Application No. 09/569,384 and U.S. Application Publication Nos. 2004/0265960; 2006/0159655; 2006/0024268; and 2008/0241098), NILR or zalpha11 (see, e.g., International Application Publication No. WO 01/085792; Parrish-Novak et al. (2000) *supra*; Ozaki et al. (2000) *supra*), that binds to an IL-21 ligand. IL-21R is homologous to the shared β chain of the IL-2 and IL-15 receptors, and IL-4α (Ozaki et al. (2000) *supra*). Upon ligand binding, IL-21R is capable of interacting with a common gamma cytokine receptor chain (γc) and inducing the phosphorylation of STAT1 and STAT3 (Asao et al. (2001) *supra*) or STAT5 (Ozaki et al. (2000) *supra*). IL-21R shows widespread lymphoid tissue distribution. The terms "interleukin-21 receptor" or "IL-21R" or the like also refer to a polypeptide (preferably of mammalian origin, e.g., murine or human IL-21R) or, as context requires, a polynucleotide encoding

such a polypeptide, that is capable of interacting with IL-21 (preferably IL-21 of mammalian origin, e.g., murine or human IL-21) and has at least one of the following features: (1) an amino acid sequence of a naturally occurring mammalian IL-21R polypeptide or a fragment thereof, e.g., an amino acid sequence set forth in SEQ ID NO:2 (human - corresponding to GENBANK® (U.S. Dept. of Health and Human Services, Bethesda, MD) Accession No. NP_068570) or SEQ ID NO:4 (murine - corresponding to GENBANK® Acc. No. NP_068687), or a fragment thereof; (2) an amino acid sequence substantially homologous to, e.g., at least 85%, 90%, 95%, 98%, or 99% homologous to, an amino acid sequence set forth in SEQ ID NO:2 or SEQ ID NO:4, or a fragment thereof; (3) an amino acid sequence that is encoded by a naturally occurring mammalian IL-21R nucleotide sequence or fragment thereof (e.g., SEQ ID NO:1 (human - corresponding to GENBANK® Accession No. NM_021798) or SEQ ID NO:3 (murine - corresponding to GENBANK® Acc. No. NM_021887), or a fragment thereof); (4) an amino acid sequence encoded by a nucleotide sequence which is substantially homologous to, e.g., at least 85%, 90%, 95%, 98%, or 99% homologous to, a nucleotide sequence set forth in SEQ ID NO:1 or SEQ ID NO:3 or a fragment thereof; (5) an amino acid sequence encoded by a nucleotide sequence degenerate to a naturally occurring IL-21R nucleotide sequence or a fragment thereof, e.g., SEQ ID NO:1 or SEQ ID NO:3, 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. In addition, other nonhuman and nonmammalian IL-21Rs are contemplated as useful in the disclosed methods.

[0074] The term "interleukin-21" or "IL-21" refers to a cytokine that shows sequence homology to IL-2, IL-4 and IL-15 (Parrish-Novak et al. (2000) *supra*), and binds to an IL-21R. Such cytokines share a common fold into a "four-helix-bundle" structure that is representative of the family. IL-21 is expressed primarily in activated $CD4^+$ T cells, and has been reported to have effects on NK, B and T cells (Parrish-Novak et al. (2000) *supra*; Kasaian et al. (2002) *supra*). Upon IL-21

binding to IL-21R, activation of IL-21R leads to, e.g., STAT5 or STAT3 signaling (Ozaki et al. (2000) *supra*). The term "interleukin-21" or "IL-21" also refers to a polypeptide (preferably of mammalian origin, e.g., murine or human IL-21), or as context requires, a polynucleotide encoding such a polypeptide, that is capable of interacting with IL-21R (preferably of mammalian origin, e.g., murine or human IL-21R) and has at least one of the following features: (1) an amino acid sequence of a naturally occurring mammalian IL-21 or a fragment thereof, e.g., an amino acid sequence set forth in SEQ ID NO:212 (human), or a fragment thereof; (2) an amino acid sequence substantially homologous to, e.g., at least 85%, 90%, 95%, 98%, or 99% homologous to, an amino acid sequence set forth in SEQ ID NO:212, or a fragment thereof; (3) an amino acid sequence which is encoded by a naturally occurring mammalian IL-21 nucleotide sequence or a fragment thereof (e.g., SEQ ID NO:211 (human), or a fragment thereof); (4) an amino acid sequence encoded by a nucleotide sequence which is substantially homologous to, e.g., at least 85%, 90%, 95%, 98%, or 99% homologous to, a nucleotide sequence set forth in SEQ ID NO:211 or a fragment thereof; (5) an amino acid sequence encoded by a nucleotide sequence degenerate to a naturally occurring IL-21 nucleotide sequence 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.

[0075] The terms "IL-21R activity" and the like (e.g., "activity of IL-21R," "IL-21/IL-21R activity") refer to at least one cellular process initiated or interrupted as a result of IL-21R binding. IL-21R activities include, but are not limited to: (1) interacting with, e.g., binding to, a ligand, e.g., an IL-21 polypeptide; (2) associating with or activating signal transduction (also called "signaling," which refers to the intracellular cascade occurring in response to a particular stimuli) and signal transduction molecules (e.g., gamma chain (γc) and JAK1), and/or stimulating the phosphorylation and/or activation of STAT proteins, e.g., STAT5 and/or STAT3; and (3) modulating the proliferation, differentiation, effector cell function, cytolytic

activity, cytokine secretion, and/or survival of immune cells, e.g., T cells, NK cells, B cells, macrophages, regulatory T cells (Tregs) and megakaryocytes.

[0076] As used herein, "*in vitro*-generated antibody" refers to an antibody where all or part of the variable region (e.g., at least one CDR) is generated in a nonimmune cell selection (e.g., an *in vitro* phage display, protein chip, or any other method in which candidate sequences can be tested for their ability to bind to an antigen).

[0077] 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 is at least 70-80% (w/w) pure, at least 80-90% (w/w) pure, at least 90-95% (w/w) pure, or at least 95%, 96%, 97%, 98%, 99%, or 100% (w/w) pure.

[0078] 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 Search Tool (BLAST) described by Altshul et al. ((1990) *J. Mol. Biol.* 215:403-10); the algorithm of Needleman et al. ((1970) *J. Mol. Biol.* 48:444-53); 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 Meyers and Miller ((1989) *CABIOS* 4:11-17), which has been incorporated into the ALIGN program (version 2.0), using a PAM120 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.

[0079] The term "repertoire" refers to at least one nucleotide sequence derived wholly or partially from at least one sequence encoding at least one immunoglobulin. The sequence(s) may be generated by rearrangement *in vivo* of the V, D, and J

segments of heavy chains, and the V and J segments of light chains. Alternatively, the sequence(s) can be generated from a cell in response to which rearrangement occurs, e.g., *in vitro* stimulation. Alternatively, part or all of the sequence(s) may be obtained by DNA splicing, nucleotide synthesis, mutagenesis, or other methods (see, e.g., U.S. Patent No. 5,565,332). A repertoire may include only one sequence or may include a plurality of sequences, including ones in a genetically diverse collection.

[0080] The terms "specific binding," "specifically binds," and the like refer 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 Ka is higher than about $10^6 M^{-1}s^{-1}$. 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 binding protein, ionic strength of the solution, temperature, time allowed for binding, concentration of a blocking agent (e.g., serum albumin or milk casein), etc., can be improved by a skilled artisan using routine techniques. Illustrative conditions are set forth herein, but other conditions known to the person of ordinary skill in the art fall within the scope of this invention.

[0081] As used herein, the terms "stringent," "stringency," and the like describe conditions for hybridization and washing. The isolated polynucleotides of the present invention can 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. Therefore, polynucleotides isolated in this fashion may be used to produce binding proteins against IL-21R or to identify cells expressing such binding proteins. Hybridization methods for identifying and isolating nucleic acids include polymerase chain reaction (PCR), Southern hybridizations, *in situ* hybridization and Northern hybridization, and are well known to those skilled in the art.

[0082] Hybridization reactions can be performed under conditions of different stringencies. The stringency of a hybridization reaction includes the difficulty with which any two nucleic acid molecules will hybridize to one another and the conditions under which they will remain hybridized. Preferably, each hybridizing polynucleotide hybridizes to its corresponding polynucleotide under reduced stringency conditions, more preferably stringent conditions, and most preferably highly stringent conditions. Stringent conditions are known to those skilled in the art and can be found in, e.g., *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989) 6.3.1-6.3.6. Both aqueous and nonaqueous methods are described in this reference, and either can be used. One example of stringent hybridization conditions is hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by at least one wash in 0.2X SSC / 0.1% SDS at 50°C. Stringent hybridization conditions are also accomplished with wash(es) in, e.g., 0.2X SSC / 0.1% SDS at 55°C, 60°C, or 65°C. Highly stringent conditions include, e.g., hybridization in 0.5M sodium phosphate / 7% SDS at 65°C, followed by at least one wash at 0.2X SSC / 1 % SDS at 65°C. Further 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: Hybridization Conditions

Condition	Hybrid	Hybrid Length (bp)[1]	Hybridization Temperature and Buffer[2]	Wash Temperature and Buffer[2]
A	DNA:DNA	> 50	65°C; 1X SSC -or- 42°C; 1X SSC, 50% formamide	65°C; 0.3X SSC
B	DNA:DNA	< 50	T_B*; 1X SSC	T_B*; 1X SSC
C	DNA:RNA	> 50	67°C; 1X SSC -or- 45°C; 1X SSC, 50% formamide	67°C; 0.3X SSC
D	DNA:RNA	< 50	T_D*; 1X SSC	T_D*; 1X SSC

Condition	Hybrid	Hybrid Length (bp)[1]	Hybridization Temperature and Buffer[2]	Wash Temperature and Buffer[2]
E	RNA:RNA	> 50	70°C; 1X SSC -or- 50°C; 1X SSC, 50% formamide	70°C; 0.3X SSC
F	RNA:RNA	< 50	T_F*; 1X SSC	T_F*; 1X SSC
G	DNA:DNA	> 50	65°C; 4X SSC -or- 42°C; 4X SSC, 50% formamide	65°C; 1X SSC
H	DNA:DNA	< 50	T_H*; 4X SSC	T_H*; 4X SSC
I	DNA:RNA	> 50	67°C; 4X SSC -or- 45°C; 4X SSC, 50% formamide	67°C; 1X SSC
J	DNA:RNA	< 50	T_J*; 4X SSC	T_J*; 4X SSC
K	RNA:RNA	> 50	70°C; 4X SSC -or- 50°C; 4X SSC, 50% formamide	67°C; 1X SSC
L	RNA:RNA	< 50	T_L*; 2X SSC	T_L*; 2X SSC
M	DNA:DNA	>50	50°C; 4X SSC -or- 40°C; 6X SSC, 50% formamide	50°C; 2X SSC
N	DNA:DNA	< 50	T_N*; 6X SSC	T_N*; 6X SSC
O	DNA:RNA	> 50	55°C; 4X SSC -or- 42°C; 6X SSC, 50% formamide	55°C; 2X SSC
P	DNA:RNA	< 50	T_P*; 6X SSC	T_P*; 6X SSC
Q	RNA:RNA	> 50	60°C; 4X SSC -or- 45°C; 6X SSC, 50% formamide	60°C; 2X SSC
R	RNA:RNA	< 50	T_R*; 4X SSC	T_R*; 4X SSC

[1] 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.

[2] SSPE (1xSSPE is 0.15M NaCl, 10mM NaH_2PO_4, and 1.25mM EDTA, pH 7.4) can be substituted for SSC (1xSSC is 0.15M NaCl and 15mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 min 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(# of A + T bases) + 4(# of G + C bases). For hybrids between 18 and 49 base pairs in length, T_m(°C) = 81.5 + 16.6($\log_{10}Na^+$) + 0.41(%G + C) - (600/N), where N is the number of bases in the hybrid, and Na^+ is the concentration of sodium ions in the hybridization buffer (Na^+ for 1X SSC = 0.165 M).

Additional examples of stringency conditions for polynucleotide hybridization are provided in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Chs. 9 & 11, Cold Spring Harbor

Laboratory Press, Cold Spring Harbor, NY (1989), and Ausubel et al. eds., *Current Protocols in Molecular Biology*, Sects. 2.10 & 6.3-6.4, John Wiley & Sons, Inc. (1995), herein incorporated by reference.

[0083] The isolated polynucleotides of the present invention 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 about 90% sequence identity (more preferably, at least about 95% identity; most preferably, at least about 99% identity) with the disclosed polynucleotides. The isolated polynucleotides of the present invention 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. Preferably, polynucleotide homologs have at least about 50% sequence identity (more preferably, at least about 75% identity; most preferably, at least about 90% identity) with the disclosed polynucleotides, whereas polypeptide homologs have at least about 30% sequence identity (more preferably, at least about 45% identity; most preferably, at least about 60% identity) with the disclosed binding proteins / polypeptides. Preferably, homologs of the disclosed polynucleotides and polypeptides are those isolated from mammalian species. The isolated polynucleotides of the present invention may additionally be used as hybridization probes and primers to identify cells and tissues that express the binding proteins of the present invention and the conditions under which they are expressed.

[0084] The phrases "substantially as set out," "substantially identical," and "substantially homologous" mean that the relevant amino acid or nucleotide sequence (e.g., CDR(s), V_H, or V_L domain(s)) will be identical to or have

insubstantial differences (e.g., through conserved amino acid substitutions) in comparison to the sequences which are set out. Insubstantial differences include minor amino acid changes, such as one or two substitutions in a five amino acid sequence of a specified region. In the case of antibodies, the second antibody has the same specificity and has at least about 50% of the affinity of the first antibody.

[0085] Sequences substantially identical or homologous to the sequences disclosed herein are also part of this application. In some embodiments, the sequence identity can be about 85%, 90%, 95%, 96%, 97%, 98%, 99%, or higher. Alternatively, substantial identity or homology 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.

[0086] The term "therapeutic agent" or the like is a substance that treats or assists in treating a medical disorder or symptoms therof. Therapeutic agents may include, but are not limited to, substances that modulate immune cells or immune responses in a manner that complements the use of anti-IL-21R binding proteins. In one embodiment of the invention, a therapeutic agent is a therapeutic antibody, e.g., an anti-IL-21R antibody. In another embodiment of the invention, a therapeutic agent is a therapeutic binding protein, e.g., an anti-IL-21R nanobody. Nonlimiting examples and uses of therapeutic agents are described herein.

[0087] As used herein, a "therapeutically effective amount" of an anti-IL-21R binding protein (e.g., an antibody) refers to an amount of the binding protein that is effective, upon single or multiple dose administration to a subject (such as a human patient) for treating, preventing, curing, delaying, reducing the severity of, and/or ameliorating at least one symptom of a disorder or a recurring disorder, or prolonging the survival of the subject beyond that expected in the absence of such treatment.

Anti-IL-21R Binding Proteins

[0088] The disclosure of the present application provides novel anti-IL-21R binding proteins that comprise novel antigen-binding fragments. Numerous methods known to those skilled in the art are available for obtaining binding proteins or antigen-binding fragments thereof. For example, anti-IL-21R binding proteins that comprise antibodies can be produced using recombinant DNA methods (see, e.g., U.S. Patent No. 4,816,567). Monoclonal antibodies may also be produced by generation of hybridomas in accordance with known methods (see, e.g., Kohler and Milstein (1975) *Nature* 256:495-99). Hybridomas formed in this manner are then screened using standard methods, such as enzyme-linked immunosorbent assays (ELISA) and surface plasmon resonance (BIACORE™) analysis, to identify one or more hybridomas that produce an antibody that specifically binds with a particular 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, and antigenic peptides thereof.

[0089] One exemplary method of making binding proteins that comprise antibodies includes screening protein expression libraries, e.g., phage or ribosome display libraries. Phage display is described, for example, in U.S. Patent No. 5,223,409; Smith (1985) *Science* 228:1315-17; Clackson et al. (1991) *Nature* 352:624-28; Marks et al. (1991) *J. Mol. Biol.* 222:581-97; and International Application Publication Nos. WO 92/018619; WO 91/017271; WO 92/020791; WO 92/015679; WO 93/001288; WO 92/001047; WO 92/009690; and WO 90/002809.

[0090] In addition to the use of display libraries, the specified antigen can be used to immunize a nonhuman animal, e.g., a cynomolgus monkey, a chicken, or a rodent (e.g., a mouse, hamster, or rat). In one embodiment, the nonhuman 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 binding proteins, such as antibodies, derived from the genes with the desired

specificity may be produced and selected (see, e.g., XENOMOUSE™ (Amgen Inc., Thousand Oaks, CA); Green et al. (1994) *Nat. Genet.* 7:13-21; U.S. Patent No. 7,064,244; and International Application Publication Nos. WO 96/034096 and WO 96/033735).

[0091] In one embodiment of the invention, the binding proteins is a monoclonal antibody that is obtained from a nonhuman animal, and then modified (e.g., humanized, deimmunized, or chimeric) 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. (1985) *Proc. Natl. Acad. Sci. USA* 81(21):6851-55; Takeda et al. (1985) *Nature* 314(6010):452-54; U.S. Patent Nos. 4,816,567 and 4,816,397; European Application Publication Nos. EP 0 171 496 and EP 0 173 494; and United Kingdom Patent No. GB 2 177 096). Humanized binding proteins 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 (U.S. Patent No. 5,225,539) describes an exemplary CDR-grafting method that may be used to prepare the humanized binding proteins described herein. All of the CDRs of a particular human binding protein may be replaced with at least a portion of a nonhuman CDR, or only some of the CDRs may be replaced with nonhuman CDRs. It is only necessary to replace the number of CDRs required for binding of the humanized binding protein to a predetermined antigen.

[0092] Humanized binding proteins 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 binding proteins or fragments thereof are provided by, e.g., Morrison (1985) *Science* 229:1202-07; Oi et al. (1986) *BioTechniques* 4:214; and U.S. Patent Nos. 5,585,089; 5,693,761; 5,693,762; 5,859,205; and 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.

[0093] In certain embodiments, a humanized binding protein is improved by the introduction of conservative substitutions, consensus sequence substitutions, germline substitutions and/or backmutations. Such altered immunoglobulin molecules can be made by any of several techniques known in the art, (see, e.g., Teng et al. (1983) *Proc. Natl. Acad. Sci. USA* 80:7308-73; Kozbor et al. (1983) *Immunol. Today* 4:7279; Olsson et al. (1982) *Meth. Enzymol.* 92:3-16); International Application Publication No. WO 92/006193; and European Patent No. EP 0 239 400).

[0094] A binding protein or fragment thereof may also be modified by specific deletion of human T cell epitopes or "deimmunization" by the methods disclosed in, e.g., International Application Publication Nos. WO 98/052976 and WO 00/034317. Briefly, the heavy and light chain variable domains of a binding protein (such as, for example, a binding protein derived from an antibody) can be analyzed for peptides that bind to MHC Class II; these peptides represent potential T cell epitopes (as defined in, e.g., International Application Publication Nos. WO 98/052976 and WO 00/034317). 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 International Application Publication Nos. WO 98/052976 and WO 00/034317. 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 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 are disclosed in, e.g., Tomlinson et al. (1992) *J. Mol. Biol.* 227:776-98; Cook et al. (1995) *Immunol. Today* 16(5):237-42; Chothia et al. (1992) *J. Mol. Biol.* 227:799-817; and Tomlinson et al. (1995) *EMBO J.* 14:4628-38. The V BASE directory provides a comprehensive directory of human immunoglobulin variable region sequences (compiled by Tomlinson 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, as described in, e.g., U.S. Patent No. 6,300,064.

[0095] In certain embodiments, a binding protein can contain an altered immunoglobulin constant or Fc region. For example, binding proteins 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 binding protein such as effector cell activity, lysis, complement-mediated activity, binding protein clearance, and binding protein half-life. Typical Fc receptors that bind to an Fc region of a binding protein (e.g., an IgG antibody) include, but are not limited to, receptors of the FcγRI, FcγRII, and FcRn subclasses, including allelic variants and alternatively spliced forms of these receptors. Fc receptors are reviewed in, e.g., Ravetch and Kinet (1991) *Annu. Rev. Immunol.* 9:457-92; Capel et al. (1994) *Immunomethods* 4:25-34; and de Haas et al. (1995) *J. Lab. Clin. Med.* 126:330-41. For additional binding protein / antibody production techniques, see, e.g., *Antibodies: A Laboratory Manual* (1988) Harlow et al. eds., Cold Spring Harbor Laboratory. The present invention is not necessarily limited to any particular source, method of production, or other special characteristic of a binding protein or an antibody.

[0096] Binding proteins comprising antibodies (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 chains, termed lambda (λ) and kappa (κ), 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, lgG2, IgG3, IgG4, IgA1, and IgA2. Each light chain includes an N-terminal variable (V) domain (V_L) and a constant (C) domain (C_L). Each heavy chain includes an N-terminal V domain (V_H), three or four C domains (C_Hs), and a hinge region. The C_H domain most proximal to V_H is designated as C_H1. The V_H and V_L domains consist of four regions of relatively conserved sequences called framework regions (FR1, FR2, FR3, and FR4) that form a scaffold for three regions of hypervariable sequences, called 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. CDR constituents on the heavy chain are referred to as H1, H2, and H3 (also referred to herein as CDR H1, CDR H2, and CDR H3, respectively), while CDR constituents on the light chain are referred to as L1, L2, and L3 (also referred to herein as CDR L1, CDR L2, and CDR L3, respectively).

[0097] CDR3 is typically the greatest source of molecular diversity within the antigen-binding site. CDR 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 antibody structure, see, e.g., Harlow et al. (1988) *supra.* One of skill in the art will recognize that each subunit structure, e.g., a C_H, V_H, C_L, V_L, CDR, and/or FR structure, comprises active fragments, e.g., the portion of the V_H, V_L, or CDR subunit that binds to the antigen, i.e., the antigen-binding fragment, or, e.g., the portion of the C_H 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 Kabat et al. (1991) *supra*). Another standard for characterizing the antigen binding site is to refer to the hypervariable loops as described in, e.g., Chothia et al. (1992) *supra* and Tomlinson et al. (1995) *supra*. 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* (2001) Duebel and Kontermann eds., 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.

[0098] The Fab fragment consists of V_H-C_H1 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 noncovalently linked. To overcome the tendency of noncovalently linked domains to dissociate, an scFv can be constructed. The scFv 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 $(Gly_4Ser)_3$ peptide, for example, may be used as a linker, but other linkers are known in the art.

[0099] 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. 1995) Jonio et al. eds., Academic Press, San Diego, CA).

[0100] In certain embodiments of the invention, the binding protein is a single domain binding protein. Single domain binding proteins include binding proteins wherein the CDRs are part of a single domain polypeptide. Examples include, but are not limited to, heavy chain binding proteins, binding proteins that are naturally devoid of light chains, single domain binding proteins derived from conventional four-chain antibodies, engineered binding proteins, and single domain protein scaffolds other than those derived from antibodies. Single domain binding proteins include any known in the art, as well as any future-determined or -learned single domain binding proteins.

[0101] Single domain binding proteins may be derived from any species including, but not limited to, mouse, human, camel, llama, fish, shark, goat, rabbit, chicken, and bovine. In one aspect of the invention, the single domain binding protein 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 binding proteins derived from a variable region of NAR (IgNARs) are described in, e.g., International Application Publication No. WO 03/014161 and Streltsov (2005) *Protein Sci.* 14:2901-09. Single domain binding proteins also include naturally occurring single domain binding proteins known in the art as heavy chain antibodies devoid of light chains. This variable domain derived from a heavy chain antibody naturally devoid of a light chain is known herein as a VHH, or a nanobody, to distinguish it from the conventional V_H 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, and is sometimes called a camelid or camelized variable domain (see, e.g., Muyldermans (2001) *J. Biotechnol.* 74(4):277-302, incorporated herein by reference). Other species besides those in the family *Camelidae* may also produce heavy chain binding proteins naturally devoid of light chains. VHH molecules are about ten times smaller than IgG molecules. They are single polypeptides and are very stable, resisting extreme pH and temperature conditions. Moreover, they are resistant to the actions of proteases, which is not the case for conventional antibodies. Furthermore, *in vitro* expression of VHHs can produce high-yield, properly folded functional VHHs. In addition, binding proteins generated in camelids will recognize epitopes other than those recognized by antibodies generated *in vitro* via antibody libraries or via immunization of mammals other than camelids (see, e.g., International Application Publication Nos. WO 97/049805 and WO 94/004678, which are incorporated herein by reference).

[0102] A "bispecific" or "bifunctional" binding protein is an artificial hybrid binding protein having two different heavy/light chain pairs and two different binding sites. Bispecific binding proteins can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments (see, e.g., Songsivilai and Lachmann (1990) *Clin. Exp. Immunol.* 79:315-21; Kostelny et al. (1992) *J. Immunol.*

148:1547-53). In one embodiment, the bispecific binding protein comprises a first binding domain polypeptide, such as a Fab' fragment, linked via an immunoglobulin constant region to a second binding domain polypeptide.

[0103] Another binding protein according to the invention can comprise, for example, a binding domain-immunoglobulin fusion protein that includes 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 C_H1, for example, the C_H2 and C_H3 regions of IgG and IgA1 or the C_H3 and C_H4 regions of IgE (see, e.g., U.S. Application Publication No. 2005/0136049, which is incorporated by reference herein, 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 C_H2 constant region polypeptide (or C_H3 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 C_H3 constant region polypeptide (or C_H4 in the case of a construct derived in whole or in part from IgE) that is fused or otherwise connected to the C_H2 constant region polypeptide (or C_H3 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 (ADCC), complement fixation, and/or binding to a target, for example, a target antigen, such as human IL-21R.

[0104] Binding proteins of the invention can also comprise peptide mimetics. Peptide 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* (1993) Pezzuto et al. eds., Chapman and Hall, New York, incorporated by reference herein in its entirety). 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 between antibody and antigen. A peptide mimetic is expected to permit molecular interactions similar to the natural molecule. These principles may be used to engineer second generation molecules having many of the natural properties of the targeting peptides disclosed herein, but with altered and potentially improved characteristics.

[0105] Other embodiments of binding proteins useful for practing the invention include fusion proteins. These molecules generally have all or a substantial portion of a targeting peptide, for example, IL-21R or an anti IL-21R antibody, linked at the N- or C-terminus, to all or a portion of a second polypeptide or protein. For example, fusion proteins may employ leader (or signal) sequences from other species to permit the recombinant expression of a protein in a heterologous host. For example, amino acid sequences, or nucleic acid sequences encoding amino acid sequences, of the the binding proteins and antigen-binding fragments thereof of the present invention comprising a leader (or signal) sequence may be selected from SEQ ID NOs:87-109 and 239-248. Another useful fusion includes the addition of an immunologically active domain, such as a binding protein 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 the 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, but are not limited to, 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.

[0106] In one embodiment, the targeting peptide, for example, IL-21R, 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, e.g., U.S. Patent Nos. 6,018,026 and 5,750,375, incorporated by reference herein). The Fc region may be a naturally occurring Fc region, or may be altered to improve certain qualities, e.g., therapeutic qualities, circulation time, reduced aggregation. Peptides and proteins fused to an Fc region typically exhibit a greater half-life *in vivo* than the unfused counterpart does. In addition, a fusion to an Fc region permits dimerization / multimerization of the fusion polypeptide.

[0107] One aspect of the present invention comprises binding proteins and antigen-binding fragments thereof that bind IL-21R. The disclosure provides novel CDRs that have been derived from human immunoglobulin gene libraries. The protein structure that is generally used to carry a CDR is an antibody heavy or light chain or a portion thereof, wherein the CDR is localized to a region associated with a naturally occurring CDR. The structures and locations of variable domains may be determined as described in Kabat et al. ((1991) *supra*).

[0108] Illustrative embodiments of the binding proteins (and antigen-binding fragments thereof) of the invention are identified as AbA-AbZ, H3-H6, L1-L6, L8-L21, and L23-L25. DNA and amino acid sequences of the nonlimiting illustrative embodiments of the anti-IL-21R binding proteins of the invention are set forth in SEQ ID NOs:5-195, 213-229, and 239-248. DNA and amino acid sequences of some illustrative embodiments of the anti-IL-21R binding proteins of the invention, including their scFv fragments, V_H and V_L domains, and CDRs, as well as their present codes and previous designations, are set forth in **FIGs. 17-25**, and **Tables 2A** and **2B**.

Table 2A: Correlation of Present Antibody Codes and Previous Designations

Present Code	Previous Designation
AbA	VHP/VL2
AbB	VHP/VL3
AbC	VHP/VL11
AbD	VHP/VL13
AbE	VHP/VL14
AbF	VHP/VL17
AbG	VHP/VL18
AbH	VHP/VL19
AbI	VHP/VL24
AbJ	VH3/VLP
AbK	VH3/VL3
AbL	VH3/VL13
AbM	VH6/VL13
AbN	VH6/VL24
AbO	VHP/VL16; VHPTM/VL16
AbP	VHP/VL20; VHPTM/VL20
AbQ	VH3/VL2; VH3DM/VL2
AbR	VH3/VL18; VH3DM/VL18
AbS	VHP/VL6; VHPTM/VL6; VL6
AbT	VHP/VL9; VHPTM/VL9; VL9
AbU	VHP/VL25; VHPTM/VL25
AbV	VH3TM/VL2
AbW	VH3TM/VL18
AbX	VHPDM/VL9
AbY	VHPg4/VL9
AbZ	VHPWT/VL9

Table 2B: Amino Acid and Nucleotide Sequences of V_H and V_L Domains, scFv, and CDRs of Illustrative Binding Proteins of the Invention

REGION	TYPE	H3 SEQ ID	H4 SEQ ID	H5 SEQ ID	H6 SEQ ID	L1 SEQ ID
V_H	AA	NO:14	NO:16	NO:18	NO:20	NO:6
V_L	AA	NO:10	NO:10	NO:10	NO:10	NO:22
scFv	AA	NO:110	NO:112	NO:114	NO:116	NO:118
CDR H1	AA	NO:163	NO:163	NO:163	NO:163	NO:163
CDR H2	AA	NO:164	NO:164	NO:164	NO:164	NO:164
CDR H3	AA	NO:165	NO:166	NO:167	NO:168	NO:169
CDR L1	AA	NO:194	NO:194	NO:194	NO:194	NO:194
CDR L2	AA	NO:195	NO:195	NO:195	NO:195	NO:195
CDR L3	AA	NO:170	NO:170	NO:170	NO:170	NO:171
V_H	DNA	NO:13	NO:15	NO:17	NO:19	NO:5
V_L	DNA	NO:9	NO:9	NO:9	NO:9	NO:21
scFv	DNA	NO:109	NO:111	NO:113	NO:115	NO:117

Table 2B (continued)

REGION	TYPE	L2 SEQ ID	L3 SEQ ID	L4 SEQ ID	L5 SEQ ID	L6 SEQ ID
V_H	AA	NO:6	NO:6	NO:6	NO:6	NO:6
V_L	AA	NO:24	NO:26	NO:28	NO:30	NO:32
scFv	AA	NO:120	NO:122	NO:124	NO:126	NO:128
CDR H1	AA	NO:163	NO:163	NO:163	NO:163	NO:163
CDR H2	AA	NO:164	NO:164	NO:164	NO:164	NO:164
CDR H3	AA	NO:169	NO:169	NO:169	NO:169	NO:169
CDR L1	AA	NO:194	NO:194	NO:194	NO:194	NO:194
CDR L2	AA	NO:195	NO:195	NO:195	NO:195	NO:195
CDR L3	AA	NO:172	NO:173	NO:174	NO:175	NO:176
V_H	DNA	NO:5	NO:5	NO:5	NO:5	NO:5
V_L	DNA	NO:23	NO:25	NO:27	NO:29	NO:31
scFv	DNA	NO:119	NO:121	NO:123	NO:125	NO:127

Table 2B (continued)

REGION	TYPE	L8 SEQ ID	L9 SEQ ID	L10 SEQ ID	L11 SEQ ID	L12 SEQ ID
V_H	AA	NO:6	NO:6	NO6	NO:6	NO:6
V_L	AA	NO:34	NO:36	NO:38	NO:40	NO:42
scFv	AA	NO:130	NO:132	NO:134	NO:136	NO:138
CDR H1	AA	NO:163	NO:163	NO:163	NO:163	NO:163
CDR H2	AA	NO:164	NO:164	NO:164	NO:164	NO:164
CDR H3	AA	NO:169	NO:169	NO:169	NO:169	NO:169
CDR L1	AA	NO:194	NO:194	NO:194	NO:194	NO:194
CDR L2	AA	NO:195	NO:195	NO:195	NO:195	NO:195
CDR L3	AA	NO:177	NO:178	NO:179	NO:180	NO:181
V_H	DNA	NO:5	NO:5	NO:5	NO:5	NO:5
V_L	DNA	NO:33	NO:35	NO:37	NO:39	NO:41
scFv	DNA	NO:129	NO:131	NO:133	NO:135	NO:137

Table 2B (continued)

REGION	TYPE	L13 SEQ ID	L14 SEQ ID	L15 SEQ ID	L16 SEQ ID	L17 SEQ ID
V_H	AA	NO:6	NO:6	NO:6	NO:6	NO:6
V_L	AA	NO:44	NO:46	NO:48	NO:50	NO:52
scFv	AA	NO:140	NO:142	NO:144	NO:146	NO:148
CDR H1	AA	NO:163	NO:163	NO:163	NO:163	NO:163
CDR H2	AA	NO:164	NO:164	NO:164	NO:164	NO:164
CDR H3	AA	NO:169	NO:169	NO:169	NO:169	NO:169
CDR L1	AA	NO:194	NO:194	NO:194	NO:194	NO:194
CDR L2	AA	NO:195	NO:195	NO:195	NO:195	NO:195
CDR L3	AA	NO:182	NO:183	NO:184	NO:185	NO:186
V_H	DNA	NO:5	NO:5	NO:5	NO:5	NO:5
V_L	DNA	NO:43	NO:45	NO:47	NO:49	NO:51
scFv	DNA	NO:139	NO:141	NO:143	NO:145	NO:147

Table 2B (continued)

REGION	TYPE	L18 SEQ ID	L19 SEQ ID	L20 SEQ ID	L21 SEQ ID	L23 SEQ ID
V_H	AA	NO:6	NO:6	NO:6	NO:6	NO:6
V_L	AA	NO:54	NO:56	NO:58	NO:60	NO:62
scFv	AA	NO:150	NO:152	NO:154	NO:156	NO:158
CDR H1	AA	NO:163	NO:163	NO:163	NO:163	NO:163
CDR H2	AA	NO:164	NO:164	NO:164	NO:164	NO:164
CDR H3	AA	NO:169	NO:169	NO:169	NO:169	NO:169
CDR L1	AA	NO:194	NO:194	NO:194	NO:194	NO:194
CDR L2	AA	NO:195	NO:195	NO:195	NO:195	NO:195
CDR L3	AA	NO:187	NO:188	NO:189	NO:190	NO:191
V_H	DNA	NO:5	NO:5	NO:5	NO:5	NO:5
V_L	DNA	NO:53	NO:55	NO:57	NO:59	NO:61
scFv	DNA	NO:149	NO:151	NO:153	NO:155	NO:157

Table 2B (continued)

REGION	TYPE	L24 SEQ ID	L25 SEQ ID
V_H	AA	NO:6	NO:6
V_L	AA	NO:64	NO:66
scFv	AA	NO:160	NO:162
CDR H1	AA	NO:163	NO:163
CDR H2	AA	NO:164	NO:164
CDR H3	AA	NO:169	NO:169
CDR L1	AA	NO:194	NO:194
CDR L2	AA	NO:195	NO:195
CDR L3	AA	NO:192	NO:193
V_H	DNA	NO:5	NO:5
V_L	DNA	NO:63	NO:65
scFv	DNA	NO:159	NO:161

[0109] Anti-IL-21R binding proteins of the present invention may comprise antibody constant regions or parts thereof. For example, a V_L domain may be attached at its C-terminal end to a light chain constant domain like Cκ or Cλ. Similarly, a V_H domain, or portion thereof, may be attached to all or part of a heavy chain like IgA, IgD, IgE, IgG, and IgM, and any isotype subclass. Constant regions are known in the art (see, e.g., Kabat et al. (1991) *supra*). Therefore, binding proteins within the scope of this invention include V_H and V_L domains, or portions thereof, combined with constant regions known in the art.

[0110] Certain embodiments comprise a V_H domain, a V_L domain, or a combination thereof, of the Fv fragment from AbA-AbZ, H3-H6, L1-L6, L8-L21, and/or L23-L25. Further embodiments comprise one, two, three, four, five or six CDRs from the V_H and V_L domains. Binding proteins whose CDR sequence(s) are the same as, or similar to (i.e., differ insubstantially from), one or more CDR sequence(s) present within the sequences set forth in SEQ ID NOs:5-195, 213-229, and 239-248 are encompassed within the scope of the invention.

[0111] In certain embodiments, the V_H and/or V_L domains may be germlined, i.e., the FR of these domains are mutated using conventional molecular biology

techniques to match those produced by the germline cells. In other embodiments, the FR sequences remain diverged from the consensus germline sequences.

[0112] In one embodiment, mutagenesis is used to make a binding protein more similar to one or more germline sequences. This may be desirable when mutations are introduced into the FR of a binding protein (e.g., 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 FRs of scFvs are mutated in conformity with the closest matches in the VBASE database and the CDR portions are kept intact.

[0113] In certain embodiments, binding proteins of the invention specifically react with an epitope that is the same as the epitope recognized by AbA-AbZ, H3-H6, L1-L6, L8-L21, or L23-L25, such that they competitively inhibit the binding of AbA-AbZ, H3-H6, L1-L6, L8-L21, or L23-L25 to human IL-21R. Such binding proteins can be determined in competitive binding assays. In one embodiment, the association constant (K_A) of these binding proteins for human IL-21R is at least 10^5 $M^{-1}s^{-1}$. The binding affinity may be determined using techniques known in the art, such as ELISA, biosensor technology (such as biospecific interaction analysis) or other techniques, including those described in this application.

[0114] It is contemplated that binding proteins of the invention may bind other proteins, such as, for example, recombinant proteins comprising all or a portion of IL-21R.

[0115] One of ordinary skill in the art will recognize that the disclosed binding proteins may be used to detect, measure, and/or inhibit proteins that differ somewhat from IL-21R. For example, these proteins may be homologs of IL-21R. Anti-IL-21R binding proteins are expected to bind proteins that comprise a sequence which is at

least about 60%, 70%, 80%, 90%, 95%, or more identical to any sequence of at least 100, 80, 60, 40, or 20 contiguous amino acids in the sequence set forth SEQ ID NOs:2 or 4.

[0116] In addition to sequence homology analyses, epitope mapping (see, e.g., *Epitope Mapping Protocols* (1996) Morris ed., Humana Press), and secondary and tertiary structure analyses can be carried out to identify specific 3D structures assumed by the presently disclosed binding proteins and their complexes with antigens. Such methods include, but are not limited to, x-ray crystallography (Engstom (1974) *Biochem. Exp. Biol.* 11:7-13) and computer modeling of virtual representations of the present binding proteins (Fletterick et al. (1986) *Computer Graphics and Molecular Modeling, in Current Communications in Molecular Biology*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

[0117] The disclosure provides a method for obtaining anti-IL-21R binding proteins. The method comprises creating binding proteins with V_H and/or V_L sequence(s) that are altered from those sequences disclosed herein. Such binding proteins may be derived by a skilled artisan using techniques known in the art. For example, amino acid substitutions, deletions, or additions can be introduced in FR and/or CDR regions. FR changes are usually designed to improve the stability and immunogenicity of the binding protein, while CDR changes are typically designed to increase a binding protein's affinity for its antigen. The changes that increase affinity may be tested by altering one or more CDR sequences and measuring the affinity of the binding protein for its target (see, e.g., *Antibody Engineering* (2nd ed. 1995) Borrebaeck ed., Oxford University Press).

[0118] Binding proteins whose CDR sequences differ insubstantially from those set forth in or included within the sequences of SEQ ID NOs:5-195, 213-229, and 239-248 are encompassed within the scope of this invention. Typically, such an insubstantial difference(s) involves substitution of an amino acid with an amino acid having similar charge, hydrophobicity, or stereochemical characteristics. More drastic substitutions in FR regions, in contrast to CDR regions, may also be made as

long as they do not adversely affect (e.g., reduce affinity by more than 50% as compared to the unsubstituted binding protein) the binding properties of the binding protein. Substitutions may also be made to germline the binding protein or stabilize its antigen binding site.

[0119] Conservative modifications will produce molecules having functional and chemical characteristics similar to those of the molecule from which such modifications are made. In contrast, substantial modifications in the functional and/or chemical characteristics of the molecules may be accomplished by selecting substitutions in the amino acid sequence that differ significantly in their effect on maintaining (1) the structure of the molecular backbone in the area of the substitution, for example, as a sheet or helical conformation, (2) the charge or hydrophobicity of the molecule at the target site, and/or (3) the size of the molecule.

[0120] For example, a "conservative amino acid substitution" may involve a substitution of a native amino acid residue with a normative residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position (see, e.g., MacLennan et al. (1998) *Acta Physiol. Scand. Suppl.* 643:55-67; Sasaki et al. (1998) *Adv. Biophys.* 35:1-24).

[0121] Desired amino acid substitutions (whether conservative or nonconservative) can be determined by those skilled in the art at the time such substitutions are desired. For example, amino acid substitutions can be used to identify important residues of the molecule sequence, or to increase or decrease the affinity of the molecules described herein. Exemplary amino acid substitutions include, but are not limited to, those set forth in **Table 3**.

Table 3: Exemplary Amino Acid Substitutions

Original Residues	Exemplary Substitutions	More Conservative Substitutions
Ala (A)	Val, Leu, Ile	Val
Arg (R)	Lys, Gln, Asn	Lys
Asn (N)	Gln	Gln
Asp (D)	Glu	Glu
Cys (C)	Ser, Ala	Ser
Gln (Q)	Asn	Asn
Gly (G)	Pro, Ala	Ala
His (H)	Asn, Gln, Lys, Arg	Arg
Ile (I)	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu (L)	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys (K)	Arg, 1,4-diamino-butyric acid, Gln, Asn	Arg
Met (M)	Leu, Phe, Ile	Leu
Phe (F)	Leu, Val, Ile, Ala, Tyr	Leu
Pro (P)	Ala, Gly	Gly
Ser (S)	Thr, Ala, Cys	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr, Phe	Tyr
Tyr (Y)	Trp, Phe, Thr, Ser	Phe
Val (V)	Ile, Met, Leu, Phe, Ala, Norleucine	Leu

[0122] In certain embodiments, conservative amino acid substitutions also encompass nonnaturally occurring amino acid residues that are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems.

[0123] In one embodiment, the method for making a variant V_H domain comprises adding, deleting, or substituting at least one amino acid in the disclosed V_H domains, or combining the disclosed V_H domains with at least one V_L domain, and testing the variant V_H domain for IL-21R binding or modulation of IL-21R/IL-21 activity.

[0124] An analogous method for making a variant V_L domain comprises adding, deleting, or substituting at least one amino acid in the disclosed V_L domains, or

combining the disclosed V_L domains with at least one V_H domain, and testing the variant V_L domain for IL-21R binding or modulation of IL-21R activity.

[0125] In some alternative embodiments, the anti-IL-21R binding proteins can be linked to a protein (e.g., albumin) by chemical cross-linking or recombinant methods. The disclosed binding proteins may also be linked to a variety of nonproteinaceous polymers (e.g., polyethylene glycol, polypropylene glycol, or polyoxyalkylenes) in manners set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192; and 4,179,337. The binding proteins can be chemically modified by covalent conjugation to a polymer, for example, to increase their half-life in blood circulation. Exemplary polymers and attachment methods are shown in U.S. Patent Nos. 4,766,106; 4,179,337; 4,495,285; and 4,609,546.

[0126] The disclosed binding proteins can be modified to alter their glycosylation; that is, at least one carbohydrate moiety can be deleted or added to the binding protein. 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 binding protein, e.g., antibody (see, e.g., International Application Publication No. 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, e.g., Hakimuddin et al. (1987) *Arch. Biochem. Biophys.* 259:52; Edge et al. (1981) *Anal. Biochem.* 118:131; and Thotakura et al. (1987) *Meth. Enzymol.* 138:350). Modification of carbohydrate structures may be preferable as amino acid changes in the Fc domain may enhance immunogenicity of a pharmaceutical composition (see, e.g., International Application Publication No. WO 2008/052030). For immunoglobulin molecules it has been demonstrated that attachment of N-linked carbohydrate to Asn-297 of the CH2 domain is critical for ADCC activity. Its removal enzymatically or through mutation of the N-linked consensus site results in little to no ADCC activity. In glycoproteins, carbohydrates may attach to the amide

nitrogen atom in the side chain of an asparagine in a tripeptide motif Asn-X-Thr/Ser. This type of glycosylation, termed N-linked glycosylation, commences in the endoplasmic reticulum (ER) with the addition of multiple monosaccharides to a dolichol phosphate to form a 14-residue branched carbohydrate complex. This carbohydrate complex is then transferred to the protein by the oligosaccharyltransferase (OST) complex. Before the glycoprotein leaves the lumen of the ER, three glucose molecules are removed from the 14-residue oligosaccharide. The enzymes ER glucosidase I, ER glucosidase II and ER mannosidase are involved in ER processing. Subsequently, the polypeptides are transported to the Golgi complex, where the N-linked sugar chains are modified in many different ways. In the cis and medial compartments of the Golgi complex, the original 14-saccharide N-linked complex may be trimmed through removal of mannose (Man) residues and elongated through addition of N-acetylglucosamine (GlcNac) and/or fucose (Fuc) residues. The various forms of N-linked carbohydrates generally have in common a pentasaccharide core consisting of three mannose and two N-acetylglucosamine residues. Finally, in the trans Golgi, other GlcNac residues can be added, followed by galactose (Gal) and a terminal sialic acid (Sial). Carbohydrate processing in the Golgi complex is called "terminal glycosylation" to distinguish it from "core glycosylation," which takes place in the ER. The final complex carbohydrate units can take on many forms and structures, some of which have two, three or four branches (termed biantennary, triantennary or tetraantennary). A number of enzymes are involved in Golgi processing, including Golgi mannosidases IA, IB and IC, GlcNAc-transferase I, Golgi mannosidase II, GlcNAc-transferase II, galactosyl transferase and sialyl transferase.

[0127] Methods for altering the constant region of a binding protein (such as, for example, the constant region of an antibody) are known in the art. Binding proteins 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 with a different residue (see, e.g.,

European Application Publication No. EP 0 388 151 and U.S. Patent Nos. 5,624,821 and 5,648,260). Similar types of alterations could be described that, if applied to a murine or other species of binding protein, would reduce or eliminate similar functions.

[0128] For example, it is possible to alter the affinity of an Fc region of a binding protein (e.g., an IgG, such as a human IgG) for FcR (e.g., Fc gamma R1) or C1q. 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 nonpolar residue such as phenylalanine, tyrosine, tryptophan or alanine (see, e.g., U.S. Patent No. 5,624,821).

[0129] For 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 CIq (see, e.g., U.S. Patent No. 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 C1q binding to the Fc region of IgG antibodies (see, e.g., U.S. Patent No. 5,624,821).

[0130] Modified binding proteins can be produced which have a reduced interaction with an Fc receptor. For example, it has been shown that in human IgG_3, 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 a binding protein (e.g., replacing residues 234, 235 and 237 with Ala) can also be used to affect binding protein affinity for the Fc gamma R1 receptor. The numbering of the residues in the heavy chain is based on the EU index (see Kabat et al. (1991) *supra*). Thus, in some embodiments of the invention, the Fc region of the

binding proteins of the invention contains at least one constant region mutation, such as, for example, changing Leu to Ala at position 234 (L234A), changing Leu to Ala at position 235 (L235A), and/or changing Gly to Ala at position 237 (G237A). In one embodiment, the Fc region of the binding protein contains two constant region mutations, L234A and G237A (i.e., "double-mutant" or "DM"). In another embodiment, the Fc region of the binding protein contains three constant region mutations, L234A, L235A, and G237A (i.e., "triple-mutant" or "TM"). For example, a human IgG constant region triple-mutant is set forth in SEQ ID NO:196.

[0131] Additional methods for altering the lytic activity of a binding protein, for example, by altering at least one amino acid in the N-terminal region of the CH2 domain, are described in International Application Publication No. WO 94/029351 and U.S. Patent No. 5,624,821.

[0132] The binding proteins of the invention can be tagged with a detectable or functional label. These labels include radiolabels (e.g., ^{131}I and ^{99}Tc), enzymatic labels (e.g., horseradish peroxidase and alkaline phosphatase), and other chemical moieties (e.g., biotin).

[0133] The invention may also feature an isolated binding protein or antigen-binding fragment thereof that binds to IL-21R, in particular, human IL-21R. In certain embodiments, the anti-IL-21R binding protein may have at least one of the following characteristics: (1) it is a monoclonal or single specificity binding protein; (2) it is a human binding protein; (3) it is an *in vitro* generated binding protein; (4) it is an *in vivo* generated binding protein (e.g., transgenic mouse system); (5) it inhibits the binding of IL-21 to IL-21R; (6) it is an IgG1; (7) it binds to human IL-21R with an association constant of at least about 10^5 $M^{-1}s^{-1}$; (8) it binds to murine IL-21R with an association constant of at least about $5x10^4$ $M^{-1}s^{-1}$; (9) it binds to human IL-21R with a dissociation constant of about 10^{-3} (1/s) or less; (10) it binds to murine IL-21R with a dissociation constant of about 10^{-2} (1/s) or less; (11) it inhibits human IL-21R-mediated proliferation of human IL-21R-expressing BaF3 cells with an IC_{50} of about 1.75 nM or less; (12) it inhibits murine IL-21R-mediated proliferation of murine

IL-21R-expressing BaF3 cells with an IC_{50} of about 0.5 nM or less; (13) it inhibits human IL-21R-mediated proliferation of human IL-21R-expressing TF1 cells with an IC_{50} of about 14.0 nM or less; (14) it inhibits IL-21-mediated proliferation of human primary B cells with an IC_{50} of about 1.9 nM or less; (15) it inhibits IL-21-mediated proliferation of human primary $CD4^+$ T cells with an IC_{50} of about 1.5 nM or less; and (16) it inhibits IL-21-mediated proliferation of murine primary $CD4^+$ T cells with an IC_{50} of about 5.0 nM or less.

[0134] One of skill in the art will appreciate that the modifications described above are not exhaustive, and that many other modifications will be obvious to a skilled artisan in light of the teachings of the present disclosure.

Nucleic Acids, Cloning and Expression Systems

[0135] The disclosure provides isolated nucleic acids encoding the disclosed binding proteins. The nucleic acids may comprise DNA or RNA, and they may be synthetic (completely or partially) or recombinant (completely or partially). Reference to a nucleotide sequence as set out herein encompasses a DNA molecule with the specified sequence, and encompasses an RNA molecule with the specified sequence in which U is substituted for T.

[0136] Also contemplated are nucleic acids that comprise a coding sequence for one, two, or three CDRs, a V_H domain, a V_L domain, or combinations thereof, as disclosed herein, or a sequence substantially identical thereto (e.g., a sequence at least 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or higher identical thereto, or which is capable of hybridizing under stringent conditions to the sequences).

[0137] In one embodiment, the isolated nucleic acids have nucleotide sequences encoding heavy chain and light chain variable regions of an anti-IL-21R binding protein comprising at least one CDR chosen from the amino acid sequences of SEQ ID NOs:163-195, or a sequence encoding a CDR which differs by one or two or three or four amino acids from the sequences described herein.

[0138] The nucleic acid can encode only the light chain or the heavy chain variable region, or can encode a binding protein light or heavy chain constant region, operatively linked to the corresponding variable region. In one embodiment, the light chain variable region is linked to a constant region chosen from a kappa or a lambda constant region. The light chain constant region may also be a human kappa or lambda type. In another embodiment, the heavy chain variable region is linked to a heavy chain constant region of a binding protein isotype chosen from IgG (e.g., IgG1, IgG2, IgG3, and IgG4), IgM, IgA1, IgA2, IgD, and IgE. The heavy chain constant region may be an IgG (e.g., an IgG1) isotype.

[0139] The nucleic acid compositions of the present invention, while often in the native sequence (of cDNA or genomic DNA or mixtures thereof), except for modified restriction sites and the like, can be mutated in accordance with standard techniques to provide gene sequences. For coding sequences, these mutations, may affect amino acid sequences as desired. In particular, nucleotide sequences substantially identical to or derived from native V, D, J, constant, switches and other such sequences described herein are contemplated (where "derived" indicates that a sequence is identical to or modified from another sequence).

[0140] In one embodiment, the nucleic acid differs (e.g., differs by substitution, insertion, or deletion) from that of the sequences provided (e.g., by at least one but less than 10, 20, 30, or 40 nucleotides; at least one but less than 1%, 5%, 10% or 20% of the nucleotides in the subject nucleic acid). If necessary for this analysis the sequences should be aligned for maximum homology. "Looped" out sequences from deletions or insertions, or mismatches, are considered differences. The difference may be at a nucleotide(s) encoding a nonessential residue(s), or the difference may be a conservative substitution(s).

[0141] The disclosure also provides nucleic acid constructs in the form of plasmids, vectors, and transcription or expression cassettes, which comprise at least one nucleic acid as described herein.

[0142] The disclosure further provides a host cell that comprises at least one nucleic acid construct described herein.

[0143] Also provided is a method of making an encoded protein(s) from a nucleic acid(s) comprising the sequence(s) described herein. The method comprises culturing host cells under appropriate conditions so they express the protein from the nucleic acid. Following expression and production, the V_H or V_L domain, or specific binding member, may be isolated and/or purified using any suitable technique, and then used as appropriate. The method can also include the steps of fusing a nucleic acid encoding an scFv with nucleic acids encoding an Fc portion of a binding protein, and expressing the fused nucleic acid in a cell. The method can also include a step of germlining.

[0144] Antigen-binding fragments, V_H and/or V_L domains, and encoding nucleic acid molecules and vectors may be isolated and/or purified from their natural environment, in substantially pure or homogenous form, or, in the case of nucleic acids, free or substantially free of nucleic acids or genes of origin other than the sequence encoding a polypeptide with the require function.

[0145] Systems for cloning and expressing polypeptides in a variety of host cells are known in the art. Cells suitable for producing binding proteins are described in, for example, Fernandez et al. (1999) *Gene Expression Systems*, Academic Press. In brief, suitable host cells include mammalian cells, insect cells, plant cells, yeast cells, or prokaryotic cells, e.g., *E. coli*. Mammalian cells available in the art for heterologous polypeptide expression include lymphocytic cell lines (e.g., NSO), HEK293 cells, Chinese hamster ovary (CHO) cells, COS cells, HeLa cells, baby hamster kidney cells, oocyte cells, and cells from a transgenic animal, e.g., mammary epithelial cells. In other embodiments, the nucleic acids encoding the binding proteins of the invention are placed under the control of a tissue-specific promoter (e.g., a mammary-specific promoter) and the binding proteins are produced in transgenic animals. For example, the binding proteins are secreted into the milk of the transgenic animal, such as a transgenic cow, pig, horse, sheep, goat, or rodent.

[0146] Suitable vectors may be chosen or constructed to contain appropriate regulatory sequences, including promoter sequences, terminator sequences, polyadenylation sequences, enhancer sequences, marker genes, and other sequences. The vectors may also contain a plasmid or viral backbone. For details, see, e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual* (2nd ed. 1989) Cold Spring Harbor Laboratory Press. Many established techniques used with vectors, including the manipulation, preparation, mutagenesis, sequencing, and transfection of DNA, are described, e.g., in Current Protocols in Molecular Biology (2nd ed. 1992) Ausubel et al. eds., John Wiley & Sons.

[0147] A further aspect of the disclosure provides a method of introducing the nucleic acid into a host cell. For eukaryotic cells, suitable transfection techniques may include calcium phosphate, DEAE-Dextran, electroporation, liposome-mediated transfection, and transduction using a retrovirus or other virus(es), e.g., vaccinia or baculovirus. For bacterial cells, suitable techniques may include calcium chloride transformation, electroporation, and transfection using bacteriophage. DNA introduction may be followed by a selection method (e.g., drug resistance) to select cells that contain the nucleic acid.

Uses of Anti-IL-21R Binding Proteins

[0148] Anti-IL-21R binding proteins that act as antagonists to IL-21R can be used to regulate at least one IL-21R-mediated immune response, such as one or more of cell proliferation, cytokine expression or secretion, chemokine secretion, and cytolytic activity, of T cells, B cells, NK cells, macrophages, or synovial cells. Accordingly, the binding proteins of the invention can be used to inhibit the activity (e.g., proliferation, differentiation, and/or survival) of an immune or hematopoietic cell (e.g., a cell of myeloid, lymphoid, or erythroid lineage, or precursor cells thereof), and, thus, can be used to treat a variety of immune disorders and hyperproliferative disorders of the blood. Examples of immune disorders that can be treated include, but are not limited to, transplant rejection, graft-versus-host disease (GVHD),

allergies (for example, atopic allergy), and autoimmune diseases. Autoimmune diseases include diabetes mellitus, arthritic disorders (including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis, and ankylosing spondylitis), spondyloarthropathy, multiple sclerosis, encephalomyelitis, myasthenia gravis, systemic lupus erythematosus, cutaneous lupus erythematosus, autoimmune thyroiditis, dermatitis (including atopic dermatitis and eczematous dermatitis), psoriasis, Sjögren's syndrome, IBD (including Crohn's disease and ulcerative colitis), asthma (including intrinsic asthma and allergic asthma), scleroderma and vasculitis.

Combination Therapy

[0149] In one embodiment, a pharmaceutical composition comprising at least one anti-IL-21R binding protein and at least one therapeutic agent is administered in combination therapy. The therapy is useful for treating pathological conditions or disorders, such as immune and inflammatory disorders. The term "in combination" in this context means that the binding protein composition and the therapeutic agent 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 may still be detectable at effective concentrations at the site of treatment.

[0150] For example, the combination therapy can include at least one anti-IL-21R binding protein, such as, for example, an anti-IL-21R antibody, coformulated with, and/or coadministered with, at least one additional therapeutic agent. The additional agents may include at least one cytokine inhibitor, growth factor inhibitor, immunosuppressant, anti-inflammatory agent, metabolic inhibitor, enzyme inhibitor, cytotoxic agent, and/or cytostatic agent. Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies. Moreover, the therapeutic agents disclosed herein act on pathways

that differ from the IL-21/IL-21R pathway, and thus are expected to enhance and/or synergize with the effects of the anti-IL-21R binding proteins.

[0151] Another aspect of the present invention relates to kits for carrying out the combined administration of anti-IL-21R binding proteins with other therapeutic agents. In one embodiment, the kit comprises at least one anti-IL-21R binding protein formulated in a pharmaceutical carrier, and at least one therapeutic agent, formulated as appropriate in one or more separate pharmaceutical preparations.

Diagnostic Uses

[0152] The binding proteins of the invention may also be used to detect the presence of IL-21R 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, stimulated T cells increase their expression of IL-21R, and an unusually high concentration of IL-21R expressing T cells in joints may indicate joint inflammation and possible arthritis. Illustrative medical conditions that may be diagnosed by the binding proteins of this invention include, but are not limited to, multiple sclerosis, rheumatoid arthritis, and transplant rejection.

[0153] Binding protein-based detection methods, such as those commonly used for antibodies, are well known in the art, and include ELISA, radioimmunoassays, immunoblots, Western blots, flow cytometry, immunofluorescence, immunoprecipitation, and other related techniques. The binding proteins may be provided in a diagnostic kit that incorporates at least one of these procedures to detect IL-21R. The kit may contain other components, packaging, instructions, reagents and/or other material to aid the detection of the protein and use of the kit.

[0154] Binding proteins may be modified with detectable markers, including ligand groups (e.g., biotin), fluorophores, 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.

[0155] Binding proteins can also be functionally linked (e.g., by chemical coupling, genetic fusion, noncovalent association, or otherwise) to at least one other molecular entity, such as another binding protein (e.g., a bispecific or a multispecific binding protein), toxins, radioisotopes, cytotoxic or cytostatic agents, among others. Other permutations and possibilities are apparent to those of ordinary skill in the art, and they are considered equivalents within the scope of this invention.

Pharmaceutical Compositions and Methods of Administration

[0156] Certain embodiments of the invention include compositions comprising the disclosed binding proteins. The compositions may be suitable for pharmaceutical use and administration to patients. The compositions comprise a binding protein of the present invention and a pharmaceutical excipient. As used herein, "pharmaceutical excipient" includes solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, etc., that are compatible with pharmaceutical administration. Use of these agents for pharmaceutically active substances is well known in the art. The compositions may also contain other active compounds providing supplemental, additional, or enhanced therapeutic functions. The pharmaceutical compositions may also be included in a container, pack, or dispenser, together with instructions for administration.

[0157] A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Methods to accomplish the administration are known to those of ordinary skill in the art. Pharmaceutical compositions may be topically or orally administered, or capable of transmission across mucous membranes. Examples of administration of a pharmaceutical composition include oral ingestion or inhalation. Administration may also be

intravenous, intraperitoneal, intramuscular, intracavity, subcutaneous, cutaneous, or transdermal.

[0158] Solutions or suspensions used for intradermal or subcutaneous application typically include at least one of the following components: a sterile diluent such as water, saline solution, fixed oils, polyethylene glycol, glycerine, propylene glycol, or other synthetic solvent; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetate, citrate, or phosphate; and tonicity agents such as sodium chloride or dextrose. The pH can be adjusted with acids or bases by methods known in the art. Such preparations may be enclosed in ampoules, disposable syringes, or multiple dose vials.

[0159] Solutions or suspensions used for intravenous administration include a carrier such as physiological saline, bacteriostatic water, CREMOPHOR EL® (BASF Corp., Ludwigshafen, Germany), ethanol, or polyol. In all cases, the composition must be sterile and fluid for easy syringability. Proper fluidity can often be obtained using lecithin or surfactants. The composition must also be stable under the conditions of manufacture and storage. Prevention of microorganisms can be achieved with antibacterial and antifungal agents, e.g., parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, etc. In many cases, isotonic agents (sugar), polyalcohols (e.g., mannitol and sorbitol), or sodium chloride may be included in the composition. Prolonged absorption of the composition can be accomplished by adding an agent that delays absorption, e.g., aluminum monostearate or gelatin.

[0160] Oral compositions include an inert diluent or edible carrier. For the purpose of oral administration, the binding proteins can be incorporated with excipients and placed, e.g., in tablets, troches, capsules, or gelatin. Pharmaceutically compatible binding agents or adjuvant materials can be included in the composition. The compositions may contain (1) a binder such as microcrystalline cellulose, gum tragacanth or gelatin; (2) an excipient such as starch or lactose, (3) a disintegrating agent such as alginic acid, Primogel, or corn starch; (4) a lubricant such as

magnesium stearate; (5) a glidant such as colloidal silicon dioxide; and/or (6) a sweetening or flavoring agent.

[0161] The composition may also be administered by a transmucosal or transdermal route. For example, binding proteins that comprise an Fc portion (for example, an antibody) may be capable of crossing mucous membranes in the intestine, mouth, or lungs (via Fc receptors). Transmucosal administration can be accomplished by lozenges, nasal sprays, inhalers, or suppositories. Transdermal administration can be accomplished with a composition containing ointments, salves, gels, or creams known in the art. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used. For administration by inhalation, the binding proteins may be delivered in an aerosol spray from a pressured container or dispenser, which contains a propellant (e.g., liquid or gas), or a nebulizer.

[0162] In certain embodiments, the binding proteins of this invention are prepared with carriers to protect the binding proteins against rapid elimination from the body. Biodegradable polymers (e.g., ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid) are often used. Methods for the preparation of such formulations are known by those skilled in the art. Liposomal suspensions can be used as pharmaceutically acceptable carriers also. The liposomes can be prepared according to established methods known in the art (see, e.g., U.S. Patent No. 4,522,811).

[0163] The binding proteins or binding protein compositions of the invention are administered in therapeutically effective amounts as described. Therapeutically effective amounts may vary with the subject's age, condition, sex, and severity of medical condition. Appropriate dosages can be determined by a physician based upon clinical indications. The binding proteins or compositions may be given as a bolus dose to maximize the circulating levels of binding proteins for the greatest length of time. Continuous infusion may also be used.

[0164] As used herein, the term "subject" is intended to include human and nonhuman animals. Subjects may include a human patient having a disorder

characterized by cells that express IL-21R, e.g., a cancer cell or an immune cell. The term "nonhuman animals" of the invention includes all vertebrates, such as nonhuman primates, sheep, dogs, cows, chickens, amphibians, reptiles, etc.

[0165] Examples of dosage ranges that can be administered to a subject can be chosen from: 1 μg/kg to 20 mg/kg, 1 μg/kg to 10 mg/kg, 1 μg/kg to 1 mg/kg, 10 μg/kg to 1 mg/kg, 10 μg/kg to 100 μg/kg, 100 μg/kg to 1 mg/kg, 250 μg/kg to 2 mg/kg, 250 μg/kg to 1 mg/kg, 500 μg/kg to 2 mg/kg, 500 μg/kg to 1 mg/kg, 1 mg/kg to 2 mg/kg, 1 mg/kg to 5 mg/kg, 5 mg/kg to 10 mg/kg, 10 mg/kg to 20 mg/kg, 15 mg/kg to 20 mg/kg, 10 mg/kg to 25 mg/kg, 15 mg/kg to 25 mg/kg, 20 mg/kg to 25 mg/kg, and 20 mg/kg to 30 mg/kg (or higher). These dosages may be administered daily, weekly, biweekly, monthly, or less frequently, for example, biannually, depending on dosage, method of administration, disorder or symptom(s) to be treated, and individual subject characteristics.

[0166] In certain circumstances, it may be advantageous to formulate compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited for the patient. Each dosage unit contains a predetermined quantity of binding protein calculated to produce a therapeutic effect in association with the carrier. The dosage unit depends on the characteristics of the binding protein and the particular therapeutic effect to be achieved.

[0167] Toxicity and therapeutic efficacy of the composition can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., determining the LD_{5O} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{5O}. Binding proteins that exhibit large therapeutic indices may be less toxic and/or more therapeutically effective.

[0168] The data obtained from the cell culture assays and animal studies can be used to formulate a dosage range in humans. The dosage of these compounds may lie

within the range of circulating binding protein concentrations in the blood, which includes an ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage composition form employed and the route of administration. For any binding protein used in the present invention, the therapeutically effective dose can be estimated initially using cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (i.e., the concentration of binding protein that achieves a half-maximal inhibition of symptoms). The effects of any particular dosage can be monitored by a suitable bioassay. Examples of suitable bioassays include DNA replication assays, transcription-based assays, gene expression assays, IL-21/IL-21R binding assays, and other immunological assays.

[0169] The entire contents of all references, patent applications, and patents cited throughout this application are hereby incorporated by reference herein.

EXAMPLES

[0170] The invention will be further illustrated in the following nonlimiting examples. These Examples are set forth to aid in the understanding of the invention but are not intended to, and should not be construed to, limit its scope in any way. The Examples do not include detailed descriptions of conventional methods that would be well known to those of ordinary skill in the art.

Example 1: Generation of Binding Proteins by Phage Display

[0171] The scFv parental clone 18A5, described in U.S. Patent No. 7,495,085 (incorporated by reference herein), was obtained from the CS human scFv library by standard phage display methods, using BaF3 cells expressing human IL-21R as a target in rounds 1 and 3 and biotinylated IL-21R-Fc fusion protein as a target in round 2.

Example 2: Library Construction

[0172] Phage display libraries were based upon the parental 18A5 scFv, using a pCANTAB6 vector in which the scFv was fused at its 3' end to the intact gene III. Various CDR3 sequences were derived using techniques well known in the art.

[0173] Two overlapping blocks of six consecutive codons were randomized in the CDR3 of the V_H and the V_L, producing a total of four libraries: H3B1, H3B2, L3B1, and L3B2. The following identify nucleotide and amino acid sequences, respectively: IL-21R: 18A5 V_HCDR3 [SEQ ID NOs:199 and 200]; H3B1 (library size $1.40x10^9$) [SEQ ID NOs:201 and 202]; H3B2 (library size $1.00x10^9$) [SEQ ID NOs:203 and 204]; IL-21R: 18A5 V_LCDR3 [SEQ ID NOs:205 and 206]; L3B1 (library size $9.00x10^9$) [SEQ ID NOs:207 and 208]; L3B2 (library size $6.40x10^9$) [SEQ ID NOs:209 and 210].

Example 3: Phage Selection

[0174] All derivatives of 18A5 were isolated from the scFv libraries above by selection of phage able to bind in solution phase to biotinylated human IL-21R extracellular domain His-Flag fusion proteins ("biotin-hIL-21R-H/F") and biotinylated murine IL-21R extracellular domain His-Flag fusion proteins ("biotin-mIL-21R-H/F"); all procedures and techniques related to selection are well known to one of skill in the art. A total of twenty-seven anti-IL-21R scFv were isolated by phage selection procedures.

Example 4: Library Screening

[0175] Resulting binding proteins in scFv format were chosen based on their ability to compete with parental 18A5 in human IgG1 format for binding to biotin-hIL-21R-H/F and biotin-mIL-21R-H/F, to prevent the hIL-21-dependent proliferation of genetically engineered cell lines expressing human IL-21R and the mIL-21-dependent proliferation of genetically engineered cell lines expressing murine IL-21R.

Example 4.1: Preparation of Crude Periplasmic Material ("peri-preps") for Use in Screening Assays

[0176] Depending on the growth conditions used, scFv can be expressed in solution in the bacterial periplasmic space. To induce release of scFv into the periplasm, 96-deep-well plates containing 990 µl 2xTY media with 0.1% glucose / 100 µg/ml ampicillin were inoculated from thawed glycerol stocks (one clone per well) using the QPix2 Colony picker (Genetix, New Milton, England) and grown at 37°C (999 rpm) for about 4 hr. Cultures were induced with IPTG at a final concentration of 0.02 mM and grown overnight at 30°C (999 rpm). The contents of the bacterial periplasm (peri-preps) were released by osmotic shock. Briefly, plates were centrifuged and pellets were resuspended in 150 µl TES periplasmic buffer (50 mM Tris / HCl (pH 8.0) / 1 mM EDTA (pH 8.0) / 20% Sucrose), followed by the addition of 150 µl 1:5 TES:water, and incubated on ice for 30 min. Plates were centrifuged and the scFv-containing supernatant was harvested.

Example 4.2: Epitope Competition Assay for Library Screening

[0177] Those scFv able to compete with the parental 18A5 antibody for binding to human or murine IL-21R were identified from selected phage by a homogeneous time-resolved fluorescence (HTRF®) assay. Purified parental 18A5 antibody was covalently modified with cryptate, a derivative of europium, according to the instructions in an HTRF® Cryptate Labeling Kit (Cisbio, Bedford, MA). Peri-preps of scFv were prepared as described above and diluted to 0.25% in PBS / 0.4 M potassium fluoride / 0.1% BSA (HTRF® buffer); then 10 µl of the mixture was transferred to the wells of black 384-shallow-well plates (Nunc, Rochester, NY). Five µl of cryptate-conjugated 18A5 antibody was then added to each well, followed by 5 µl of a mixture of a 1:800 dilution of streptavidin-XL665 conjugate (Cisbio), and either 4.8 nM biotin-hIL-21R-H/F or 40 nM biotin-mIL-21R-H/F. The mixture was incubated for 2 hr at RT, and time-resolved fluorescence measurements were made (340 nm excitation, 615 nm and 665 nm emission). Competition with 18A5

antibody was indicated by a reduction in the background-corrected ratio of emission at 665 nm to emission at 615 nm.

[0178] A total of 8280 independently isolated scFv were screened in the HTRF® assay using human IL-21R-H/F, and 376 clones able to compete with the parental 18A5 antibody for binding to biotin-hIL-21R-H/F were chosen for further analysis.

Example 5: DNA Sequence Analysis of Library-derived scFv - PCR Amplification of scFv Regions for Sequencing Analysis

[0179] The sequences of 287 18A5-derived scFv variants with improved IL-21R binding over that of the parent 18A5 scFv molecule were determined, and the frequencies of amino acids found at each position were determined. Among the V_H clones, only two (1.7%) were derived from a library which mutated the last six amino acids of, e.g., SEQ ID NO:169 (at the C-terminus of V_H CDR3), while the remainder were derived from a library which mutated the first six amino acids of, e.g., SEQ ID NO:169. Among the V_L clones, only one clone (0.6%) was derived from a library in which the last six amino acids of, e.g., SEQ ID NO:170 (at the C-terminus of V_L CDR3) were mutated, while the majority were derived from alterations in the first six amino acids of, e.g., SEQ ID NO:170 (at the N-terminus of V_L CDR3).

[0180] PCR amplification of scFvs was carried out using VENT® DNA Polymerase (New England Biolabs, Ispwich, MA) in HN buffer (Epicentre Biotechnologies, Madison, WI) according to the manufacturer's instructions. Five µl of a 1:10 dilution of a stationary phase bacterial culture was used as the template for a final reaction volume of 20 µl. The cycling conditions used were a 2-min hot start at 94°C, 30 cycles of denaturation at 94°C (1 min), primer annealing at 55°C (2 min) and extension at 72°C (1 min), followed by a final extension at 72°C (5 min). PCR products were verified by agarose gel electrophoresis and cleaned up with ExoI/SAP (shrimp alkaline phosphatase) prior to sequencing with the M13rev primer.

[0181] The SEQ ID NOs for the CDR3 sequences of twenty-seven scFv are listed in **Table 4**. These scFv were chosen for further analysis based on assays described in Example 6.

Table 4: CDR3 SEQ ID NOs of Improved 18A5-derived scFv

scFv	Heavy CDR3	Light CDR3
H3	165	170
H4	166	170
H5	167	170
H6	168	170
L1	169	171
L2	169	172
L3	169	173
L4	169	174
L5	169	175
L6	169	176
L8	169	177
L9	169	178
L10	169	179
L11	169	180
L12	169	181
L13	169	182
L14	169	183
L15	169	184
L16	169	185
L17	169	186
L18	169	187
L19	169	188
L20	169	189
L21	169	190
L23	169	191
L24	169	192
L25	169	193

Example 6: Characterization of Library-derived scFv

Example 6.1: Preparation of Purified scFv for Quantitative Analysis

[0182] Individual scFv clones were purified on a small scale by Ni-NTA purification on PHYTIP® columns (PhyNexus, Inc., San Jose, CA). Single colonies were grown in 20 ml 2xTY medium with 0.1% glucose / 100 μg/ml ampicillin in 50-ml conical tubes to mid-logarithmic phase at 37°C with shaking at 250 rpm. Expression of scFv was induced with IPTG at a final concentration of 0.02 mM, and cultures were grown overnight at 30°C. Cells were harvested by centrifugation and resuspended in 1 ml TES periplasmic buffer, followed by the addition of 1 ml 1:5 TES:water and incubation on ice for 30 min. Lysates were centrifuged at 3200 rpm for 10 min at

4°C, and supernatants were brought to 2 mM $MgCl_2$. scFv were captured on Ni-NTA PHYTIPs® (PhyNexus) by repeated passage of the supernatant over the PHYTIPs® on a Perkin Elmer (Waltham, MA) MINITRAK™ IX liquid handling robot, followed by washing in IMAC wash buffer and elution with 200 mM imidazole, 50 mM Tris, 300 mM NaCl (pH 8.0). The buffer was exchanged to PBS by three cycles of dilution 1:10 into PBS, followed by concentration on a 10,000 molecular weight cutoff filter plate (Millipore MULTISCREEN® ULTRACEL™ 96-well ultrafiltration plate, Millipore, Billerica, MA). Samples were quantitated using a Micro BCA™ kit (Thermo Fisher Scientific Inc., Rockford, IL) using the manufacturer's bovine serum albumin standard.

Example 6.2: Assays for IL-21-dependent Proliferation of Cells Overexpressing Human or Murine IL-21R

[0183] Inhibition assays were performed with 18A5-derived binding proteins (scFv and IgG) to measure their blockade of IL-21-dependent proliferation of cell lines transfected with human or murine IL-21R. BaF3 cells, a murine pre-B cell line, and TF1 cells, a human erythroid cell line, were retrovirally transduced with IL-21R and green fluorescent protein (GFP). Cells were routinely grown in RPMI 1640 with 10% FBS, 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 0.00036% β-mercaptoethanol. Human IL-21R-BaF3 cell cultures were supplemented with 50 ng/ml of human IL-21; murine IL-21R-BaF3 cell cultures were supplemented with 10 U/ml of IL-3; TF1 cell cultures were supplemented with 50 ng/ml of GM-CSF. Prior to assay, cells were washed 3X in assay medium lacking supplemental growth factors, resuspended in assay medium, and incubated at 37°C / 5% CO_2 for 6 hr. To prepare assay plates, 5000 cells were added to the central 60 wells of a 96-well flat-bottomed white tissue culture plate (Thermo Scientific, Waltham, MA) in a volume of 55 µl/well. Test scFv or IgG samples were prepared by diluting the stock sample in assay medium and diluting serially three-fold. Twenty-five µl of the binding protein samples were added to the cells and incubated for 30 min at 37°C / 5% CO_2. Twenty µl of assay medium containing 100-400 pg/ml

of human or murine IL-21 was added to each well, and the cells were incubated for an additional 48 hr. Proliferation was measured by bringing plates to RT, adding 15 µl/well CELLTITER-GLO®, incubating for 10 min at RT, and measuring luminescence with a Perkin Elmer ENVISION™ plate reader. After purification with PhyNexus IMAC tips, 108 scFv were tested for neutralization of IL-21-dependent proliferation of all three cell lines. All showed neutralization of human IL-21R-BaF3 cells, with IC_{50}s lower than or equal to that of the parental 18A5 scFv. A subset showed strong neutralization of proliferation of murine IL-21R-BaF3 cells and human IL-21R-TF1 cells. Data from the 27 most potent clones are shown in **FIGs. 1-3**, and are summarized in **Table 5**.

[0184] **FIGs. 1-3** show the neutralization of proliferation by scFv of human IL-21R-BaF3 cells (**FIGs. 1a-c**); human IL-21R-TF1 cells (**FIGs. 2a-c**); and murine IL-21R-BaF3 cells (**FIGs. 3a-c**). Cells were mixed with the indicated scFv and incubated with 100 pg/ml (**FIGs. 1-2**) or 400 pg/ml (**FIG. 3**) of human IL-21.

Example 6.3: Quantitative Epitope Competition Assay

[0185] Purified scFv were analyzed quantitatively for their ability to compete with the parental 18A5 antibody for binding to murine IL-21R in an enzyme-linked immunosorbent assay (ELISA). Parental 18A5 antibody was coated overnight at 4°C on 96-well Nunc MAXISORP® plates at a concentration of 0.75 µg/ml in PBS. Plates were washed 3X using PBS, and then blocked for 3 hr at RT in PBS / 1% BSA / 0.05% Tween-20. scFv were mixed with 36 nM biotinylated mIL-21R-H/F and incubated for 10 min at RT. Blocked plates were washed 3X with PBS, and 50 µl/well of scFv / IL-21R mixtures were transferred to the appropriate plates and incubated for 1 hr at RT. Plates were washed 5X with PBS prior to the addition of a 1:6000 dilution of horseradish peroxidase-conjugated streptavidin (Southern Biotech, Birmingham, AL) secondary antibody to detect bound biotinylated mIL-21R-H/F. Plates were then incubated for 1 hr at RT and washed 7X with PBS. Signal was developed using 3,3',5,5'-tetramethylbenzidine (TMB), the

reaction stopped with H_2SO_4, and the absorbance read at 450 nm on an ENVISION™ plate reader (Perkin Elmer). 108 scFv purified by PhyNexus IMAC tips were tested in this assay, and most competed with the parental 18A5 antibody for binding to biotinylated murine IL-21R-H/F with IC_{50}s lower than that of the parental 18A5 scFv. Epitope competition data for the 27 clones with the highest potencies in cell-based neutralization assays are shown in **FIGs. 4a-c** and summarized in **Table 5**.

Table 5: Neutralization of Human and Murine IL-21R in Cell-based Assays and Competition with 18A5 Antibody for Murine IL-21R Binding

	IC_{50} (nM) in Human IL-21R-BaF3 Neutralization Assay	**IC_{50} (nM) in Human IL-21R-TF1 Neutralization Assay**	**IC_{50} (nM) in Murine IL-21R-BaF3 Neutralization Assay**	**IC_{50} (nM) in Murine IL-21R Epitope Competition ELISA**
H3	7.7	98.1	25.68	14
H4	3.8	9.3	nd	nd
H5	7.9	178.5	53.66	17
H6	13.8	150 (estimated)	nd	13
L1	3.7	55 (estimated)	28.77	7
L2	3.1	37.5	2.41	5
L3	27.6	7 (estimated)	13.78	100
L4	2.1	60 (estimated)	nd	8
L5	2.1	20 (estimated)	38.52	7
L6	5.9	150 (estimated)	0.29	4
L8	4.1	51.3	715.27	7
L9	2.8	27.7	3.61	7
L10	15.1	7	nd	40
L11	4.2	38.3	10.03	6
L12	2.6	54.9	87.77	8
L13	11.0	257.4	1.25	7
L14	3.2	33.5	6.49	6
L15	3.3	30.3	53.49	14
L16	3.7	67.4	4.71	6
L17	1.6	60.3	2.66	12
L18	3.7	54.4	8.34	8
L19	4.5	35.3	13.59	15
L20	3.1	57.5	15.39	5
L21	9.4	100 (estimated)	162.27	28
L23	1.5	15.3	nd	12
L24	2.4	18.7	3.73	6
L25	3.7	33.1	15.55	9

Example 7: Conversion of Parental 18A5 IgG to Germline Sequence

[0186] The following fifteen scFv with modified V_L regions, along with the germlined parental 18A5 V_L (see below), were chosen for conversion to full-length human IgG lambda: L2, L3, L6, L9, L11, L13, L14, L16, L17, L18, L19, L20, L23, L24, and L25. Four scFv with modified V_H regions, H3, H4, H5, and H6, along with the germlined parental 18A5 V_H (see below) were chosen for conversion to full-length human IgG1.

[0187] The V_H and V_L amino sequences of the parental 18A5 antibody were modified so that the sequences outside the CDR regions matched the closest human germline sequences: DP67/VH4B+ (VBASE_AA:WAP00CEAZ_1) and JH1/JH4/JH5 in the case of the V_H, and DPL16/VL3.1 (VBASE_AA:WAP00CEMI_1) in the case of the V_L. Modifications were done by a combination of gene synthesis at GENEART (Regensburg, Germany) and site-directed changes introduced by PCR. In addition, the sequences were codon-optimized for expression in mammalian cells by GENEART using their proprietary methods. An alignment of the parental 18A5 sequences and the germline-corrected 18A5 sequences is shown below:

18A5 Heavy Chain Comparison

Parental 18A5 V_H

CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGACTTCGGAGACCCTGTCCCTCACCTGCGCT
GTCTCTGGTTACTCCATCAGCAGTGGTTACTACTGGGGCTGGATCCGGCAGCCCCCAGGGAAGGGGTTG
GAGTGGATTGGGAGTATCTCTCATACTGGGAACACCTACTACAACCCGCCCCTCAAGAGTCGCGTCACC
ATATCAGTAGACACGTCCAAGAACCAGTTCTCCCTGAAACTGAGCTCTGTGACCGCCGCAGACACGGCC
GTGTATTACTGTGCGCGAGGTGGGGGGAATTAGCAGGCCGGAGTACTGGGGCAAAGGCACCCTGGTCACC
GTCTCGAGT (SEQ ID NO:5)

Germlined 18A5 V_H

CAGGTGCAGCTGCAGGAGTCTGGCCCTGGCCTGGTGAAGCCTTCCGAGACCCTGTCTCTGACCTGTGCC
GTGTCCGGCTACTCCATCTCCTCCGGCTACTACTGGGGCTGGATCAGACAGCCTCCTGGCAAGGGCCTG
GAGTGGATCGGCTCCATCTCTCACACCGGCAACACCTACTACAACCCCCCTCTGAAGTCCAGAGTGACC
ATCTCCGTGGACACCTCCAAGAACCAGTTCTCCCTGAAGCTGTCCTCTGTGACCGCTGCCGATACCGCC
GTGTACTACTGTGCCAGAGGCGGCGGAATCTCCAGACCTGAGTACTGGGGCCAGGGCACCCTGGTGACC
GTGTCCTCT (SEQ ID NO:7)

Germlined 18A5 V_H x Parental 18A5 V_H

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            .         .         .         .         .
  1 CAGGTGCAGCTGCAGGAGTCTGGCCCTGGCCTGGTGAAGCCTTCCGAGAC 50
    |||||||||||||||||||| ||||| || ||||||||| |||| |||||
  1 CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGACTTCGGAGAC 50
            .         .         .         .         .

 51 CCTGTCTCTGACCTGTGCCGTGTCCGGCTACTCCATCTCCTCCGGCTACT 100
    |||||| || ||||| || || || || |||||||||  |   || ||||
 51 CCTGTCCCTCACcTGCGCTGTCTCTGGTTACTCCATCAGCAGTGGTTACT 100
            .         .         .         .         .

101 ACTGGGGCTGGATCAGACAGCCTCCTGGCAAGGGCCTGGAGTGGATCGGC 150
    |||||||||||||| | ||||| || || |||||  |||||||||| ||
101 ACTGGGGCTGGATCCGGCAGCCCCCAGGGAAGGGGTTGGAGTGGATTGGG 150
            .         .         .         .         .

151 TCCATCTCTCACACCGGCAACACCTACTACAACCCCCCTCTGAAGTCCAG 200
       |||||||| || || |||||||||||||||| || || |||    |
151 AGTATCTCTCATACTGGGAACACCTACTACAACCCGCCCCTCAAGAGTCG 200
            .         .         .         .         .

201 AGTGACCATCTCCGTGGACACCTCCAAGAACCAGTTCTCCCTGAAGCTGT 250
     || ||||| || || ||||| ||||||||||||||||||||||||| |||
201 CGTCACCATATCAGTAGACACGTCCAAGAACCAGTTCTCCCTGAAACTGA 250
            .         .         .         .         .

251 CCTCTGTGACCGCTGCCGATACCGCCGTGTACTACTGTGCCAGAGGCGGC 300
     |||||||||||| || || || |||||||| ||||||||  |||| ||
251 GCTCTGTGACCGCCGCAGACACGGCCGTGTATTACTGTGCGCGAGGTGGG 300
            .         .         .         .         .

301 GGAATCTCCAGACCTGAGTACTGGGGCCAGGGCACCCTGGTGACCGTGTC 350
    |||||   ||| || |||||||||||| | |||||||||| ||||| ||
301 GGAATTAGCAGGCCGGAGTACTGGGGCAAAGGCACCCTGGTCACCGTCTC 350


351 CTCT 354   (SEQ ID NO:7)
       |
351 GAGT 354   (SEQ ID NO:5)
```

18A5 Light Chain Comparison

Parental 18A5 V_L

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TCTTCTGAGCTGACTCAGGACCCTCCTGTGTCTGTGGCCTTGGGACAGACAGTCACGCTCACATGCCAA
GGAGACAGCCTCAGAACCTATTATGCAAGCTGGTACCAGCAGAAGTCAGGACAGGCCCCTATACTTCTC
CTCTATGGTAAACACAAACGGCCCTCAGGGATCCCAGACCGCTTCTCTGGCTCCACCTCAGGAGACACA
GCTTCCTTGACCATCACTGGGGCTCAGGCGGAAGACGAGGCTGACTATTACTGTAACTCCCGGGACTCC
AGTGGCAACCCCCATGTTCTGTTCGGCGGAGGGACCCAGCTCACCGTTTTA  (SEQ ID NO:9)
```

Germlined 18A5 V_L

```
TCCTCTGAGCTGACCCAGGATCCTGCTGTGTCTGTGGCCCTGGGCCAGACCGTCAGGATCACCTGCCAG
GGCGATAGCCTGAGAACCTACTACGCCTCCTGGTATCAGCAGAAGCCTGGACAGGCCCCTGTGCTGGTG
ATCTACGGCAAGCACAAGAGGCCATCCGGCATCCCTGACAGATTCTCCGGCTCCTCCTCTGGCAATACC
GCCTCCCTGACCATCACCGGCGCTCAGGCCGAGGACGAGGCCGACTACTACTGTAACTCCCGGGACTCT
TCCGGCAACCCTCACGTGCTGTTTGGCGGCGGAACCCAGCTGACCGTGCTA  (SEQ ID NO:11)
```

Germlined 18A5 V_L x Parental 18A5 V_L

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                .         .         .         .         .
  1 TCCTCTGAGCTGACCCAGGATCCTGCTGTGTCTGTGGCCCTGGGCCAGAC 50
    || |||||||||||  |||||  |||  ||||||||||||||  ||||  |||||
  1 TCTTCTGAGCTGACTCAGGACCCTCCTGTGTCTGTGGCCTTGGGACAGAC 50
            .         .         .         .         .

 51 CGTCAGGATCACCTGCCAGGGCGATAGCCTGAGAACCTACTACGCCTCCT 100
     ||||  |  ||||  |||||  ||  ||  |||||  ||||||||  ||  ||    ||
 51 AGTCACGCTCACATGCCAAGGAGACAGCCTCAGAACCTATTATGCAAGCT 100
            .         .         .         .         .

101 GGTATCAGCAGAAGCCTGGACAGGCCCCTGTGCTGGTGATCTACGGCAAG 150
    ||||  |||||||||  |  ||||||||||||  |  ||   |   ||||  ||  ||
101 GGTACCAGCAGAAGTCAGGACAGGCCCCTATACTTCTCCTCTATGGTAAA 150
            .         .         .         .         .

151 CACAAGAGGCCATCCGGCATCCCTGACAGATTCTCCGGCTCCTCCTCTGG 200
    |||||  ||||  ||  ||  |||||  |||  |  |||||  ||||||  ||||  ||
151 CACAAACGGCCCTCAGGGATCCCAGACCGCTTCTCTGGCTCCACCTCAGG 200
            .         .         .         .         .

201 CAATACCGCCTCCCTGACCATCACCGGCGCTCAGGCCGAGGACGAGGCCG 250
      |  ||  ||  |||  ||||||||||  ||  ||||||||  ||  ||||||||  |
201 AGACACAGCTTCCTTGACCATCACTGGGGCTCAGGCGGAAGACGAGGCTG 250
            .         .         .         .         .

251 ACTACTACTGTAACTCCCGGGACTCTTCCGGCAACCCTCACGTGCTGTTT 300
    ||||  ||||||||||||||||||||    ||||||||  ||  ||  |||||
251 ACTATTACTGTAACTCCCGGGACTCCAGTGGCAACCCCCATGTTCTGTTC 300
            .         .

301 GGCGGCGGAACCCAGCTGACCGTGCTA 327   (SEQ ID NO:11)
    |||||  ||  ||||||||  |||||  ||
301 GGCGGAGGGACCCAGCTCACCGTTTTA 327   (SEQ ID NO:9)
```

Germline-corrected V_H sequence (changes from parental sequence are bold and underlined):

Parental (SEQ ID NO:6) QVQLQESGPGLVKTSETLSLTCAVSGYSISSGYYWGWIRQPPGKG

Germlined (SEQ ID NO:8) QVQLQESGPGLVK**P**SETLSLTCAVSGYSISSGYYWGWIRQPPGKG

Parental LEWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARGGGISRP

Germlined LEWIGSISHTGNTYYNPPLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARGGGISRP

Parental EYWGKGTLVTVSS

Germlined EYWG**Q**GTLVTVSS

Germline-corrected V_L sequence (changes from parental sequence are bold and underlined):

Parental (SEQ ID NO:10) SSELTQDPPVSVALGQTVTLTCQGDSLRTYYASWYQQKSGQAPIL

Germlined (SEQ ID NO:12) SSELTQDP**A**VSVALGQTV**RI**TCQGDSLRTYYASWYQQK**P**GQAP**V**L

Parental LLYGKHKRPSGIPDRFSGSTSGDTASLTITGAQAEDEADYYCNSRDSSGNPHVLFGGGTQ

Germlined **VI**YGKHKRPSGIPDRFSGS**S**SG**N**TASLTITGAQAEDEADYYCNSRDSSGNPHVLFGGGTQ

Parental LTVL

Germlines LTVL

Example 8: Conversion of Library-Derived scFv to IgG

[0188] The CDR3 regions of the V_L and V_H domains of improved 18A5 scFv derivatives were amplified by PCR and subcloned into the germline-corrected V_L and V_H frameworks of the parental 18A5 by the following method. A PCR fragment encompassing the 5' portion of the germlined 18A5 V_H gene was generated by amplification of the plasmid pSMED2_OP18A5G_huIgG1 with primers BssHII_II_V_H_F (5'-GCTTGGCGCGCACTCTCAGGTGCAGCTGCAGGAG-3') [SEQ ID NO:230] and GV$_H$_R_for_BssHII (5'-TCAGGGAGAACTGGTTCTTGG-3') [SEQ ID NO:231]. A PCR fragment encompassing the 3' portion of the V_H gene from the improved scFv clone VH3 was amplified with the following primers: G_V_H_F_for_SalI (5'-TCCAAGAACCAGTTCTCCCTG-3') [SEQ ID NO:232] and scFv_SalI_V_H_R

(5'-GCGACGTCGACAGGACTCACCACTCGAGACGGTGACCAGGGTGCC-3') [SEQ ID NO:233]. Fragments were gel-purified, and then the two were mixed and amplified with the outside primer sets BssHII_G_V_H_F and SalI_V_H_R to generate a complete V_H gene fragment. This was digested with BssHII and SalI and ligated into a vector containing the constant regions of human IgG1 with a triple-mutant hinge region. The insert was reamplified with BssHII_II_V_H_F and a new primer (Sal_V_H_R_RJ (5'-GCGACGTCGACAGGACTCACCACTCGAGACGG-3')) [SEQ ID NO:234] in order to alter the coding sequence of the V_H J segment to conform to the JH1 germline sequence, and ligated into a human IgG1-triple-mutant constant region vector.

[0189] The V_L genes from improved scFv were subcloned by a similar method. A PCR fragment encompassing the 5' portion of the 18A5 V_L gene was generated by amplification of the plasmid pSMEN2_OP18A5G_hu Lambda with primers BssHII_II_V_L_F (5'-GCTTGGCGCGCACTCTTCCTCTGAGCTGACCCAG-3') [SEQ ID NO:235] and scFv_V_L_R_for_BssHII (5'-GCCTGAGCCCCAGTGATGGTCA-3') [SEQ ID NO:236]. PCR fragments encompassing the 3' portions of the V_L genes from improved scFv clones were amplified with the primers GV_L_F_for_XbaI (5'-ACCGCCTCCCTGACCATCAC-3') [SEQ ID NO:237] and scFv_XbaI_V_L_R (5'-GCGCCGTCTAGAGTTATTCTACTCACCTAAAACGGTGAGCTGGGTCCC TC-3') [SEQ ID NO:238]. Fragments were gel-purified, and then fragments corresponding to the 5' and 3' portions of each gene were mixed and amplified with the outside primer set BssHII_II_V_L_F and scFv_XbaI_V_L_R to generate complete V_L gene fragments. These were digested with BssHII and XbaI, and ligated into a vector containing the constant regions of the human lambda gene.

Example 9: Characterization of Improved IgG In Vitro

Example 9.1: Transient Small-scale Expression of Binding Proteins

[0190] Clones were tested for function in full IgG format following transient expression in cos-7 cells. Each light chain in the set of sixteen test sequences (germlined parental 18A5 V_L and L2, L3, L6, L9, L11, L13, L14, L16, L17, L18, L19, L20, L23, L24 and L25) was paired with each heavy chain in the set of five test sequences (H3, H4, H5, and H6, along with V_H_P, the germlined parental 18A5 V_H domain). Each plasmid in the pair (1.4 μg) was combined with the *TRANS*IT® transfection reagent (Mirus, Madison, WI) according to the manufacturer's instructions, and DNA:*TRANS*IT® reagent complexes were added to monolayers of cos-7 cells growing in Dulbecco's Modified Eagle's medium (DMEM) / 10% heat-inactivated fetal bovine serum / penicillin / streptomycin / 2 mM L-glutamine in 6-well tissue culture plates. After 24 hr, the medium was changed to a serum-free medium (R1CD1), and was then collected 48 hr later. Binding proteins, now comprising full-length antibodies, were quantitated by anti-human IgG ELISA.

Example 9.2: Activity of Anti-IL-21R IgG in Neutralization of Cell Proliferation

[0191] The 80 transiently expressed IgGs in serum-free conditioned medium were tested for activity in IL-21-dependent proliferation assays in three cell lines as described above: (1) human IL-21R-BaF3 cells, (2) murine IL-21R-BaF3 cells, and (3) human IL-21R-TF1 cells. All 80 pairs showed neutralization of proliferation of human IL-21R-expressing BaF3 cells, and all pairs except those involving VH4 showed neutralization of human IL-21R-expressing TF1 cells (data not shown). All 80 pairs also showed neutralization of proliferation of murine IL-21R-expressing BaF3 cells, with the strongest neutralization generally associated with light chains paired with the parental heavy chain and the weakest neutralization generally associated with the VH4 heavy chain (data not shown). Neutralization data from the most potent 21 IgG combinations (AbA-AbU) are shown in **FIG. 5**, and IC_{50} data are summarized in **Table 6**.

[0192] Assays were conducted on human IL-21R-BaF3 cells with 100 pg/ml of human IL-21 (**FIGs. 5a-c**), human IL-21R-TF1 cells with 100 pg/ml of human IL-21 (**FIGs. 5d-f**), or murine IL-21R-BaF3 cells with 400 pg/ml of murine IL-21 (**FIGs. 5g-i**). IL-21 was added to the cells after the indicated antibodies; proliferation was measured with CELLTITER-GLO® after 48 hr. **FIGs. 26a-c** show additional studies demonstrating similar inhibition in the same three cell lines.

Example 9.3: Anti-IL-21R IgG Binding to Transiently Expressed Rat and Cynomolgus Monkey IL-21R

[0193] A subset of binding proteins was tested for binding to rat, cynomolgus monkey, human IL-21R, or human IL-2R-γ common subunit expressed transiently on the surfaces of CHO-PA-Dukx cells. Cells were transfected 48 hr prior to the assay. On the day of the assay, cells were washed gently 5X in PBS containing 0.9 mM $CaCl_2$ and 0.45 mM $MgCl_2$ (PBS / CaMg) on an automated plate washer (Titertek, Huntsville, AL), and blocked for 1 hr at RT in PBS / CaMg / 5% nonfat dry milk. Conditioned media from transiently expressed anti-IL-21R IgGs were serially diluted in blocking buffer and added to the cells in the blocked plates for 1 hr at RT. Cells were washed 5X with PBS / CaMg and then incubated with horseradish peroxidase-conjugated anti-human IgG for 1 hr at RT. Cells were then washed 10X in PBS / CaMg and all of the wash buffer was removed. Cells were incubated with 100 µl TMB until the color reaction reached saturation, stopped with 100 µl of 0.18 M H_2SO_4, and read at A450 on a Perkin Elmer ENVISION™ plate reader.

[0194] All of the twenty-one IgGs bound to CHO cells transiently expressing human (**FIGs. 6a-c**), rat (**FIGs. 6d-f**), or cynomolgus monkey (**FIGs. 6g-i**) IL-21R. Most showed no binding above background to a control protein (human gamma (γ) common chain) transiently expressed on CHO cells, but a subset of IgGs (AbD, AbE, AbF, AbH, AbL, and AbM) bound above background at 13 nM or greater (**FIGs. 6j-l**). Data are summarized in **Table 6**.

Table 6: Summary of Neutralization of Human and Murine IL-21R Activity in Cell-proliferation Assays and Binding to Human, Rat, and Cynomolgus Monkey IL-21R Expressed on CHO Cells

Binding Protein	Human IL-21R-BaF3 Proliferation IC_{50} (nM)	Human IL-21R-TF1 Proliferation IC_{50} (nM)	Murine IL-21R-BaF3 Proliferation IC_{50} (nM)	Human IL-21R Binding (13 nM Ab in Cell ELISA; A450)	Rat IL-21R Binding (13 nM Ab in Cell ELISA; A450)	Cynomolgus Monkey IL-21R Binding (13 nM Ab in Cell ELISA; A450)	Human Gamma Common Binding (13 nM Ab in Cell ELISA; A450)
AbA	0.97	3.80	0.08	1.196	1.124	1.352	0.111
AbB	1.14	3.34	0.421	1.147	1.09	1.333	0.107
AbC	0.82	3.36	0.03	1.218	0.999	1.277	0.137
AbD	0.91	2.67	0.01	1.247	0.874	1.375	0.197
AbE	0.56	2.28	0.04	1.257	1.111	1.423	0.223
AbF	0.54	2.41	0.304	1.347	1.001	1.458	0.433
AbG	0.77	3.84	0.07	1.35	1.112	1.304	0.108
AbH	0.94	3.64	0.327	1.35	1.097	1.324	0.152
AbI	1.00	3.80	0.224	1.237	1.088	1.209	0.107
AbJ	0.65	4.60	0.4	1.217	1.261	1.273	0.126
AbK	0.98	4.00	0.079	1.364	1.175	1.338	0.108
AbL	0.68	4.25	0.227	1.454	1.257	1.514	0.219
AbM	1.08	4.22	0.125	1.197	0.78	1.45	0.224
AbN	0.50	1.59	0.435	1.214	0.702	1.497	0.136
AbO	0.52	2.91	0.065	1.107	1.101	1.358	0.108
AbP	0.75	3.48	0.03	1.308	1.03	1.313	0.112
AbQ	0.68	4.62	0.153	1.255	1.161	1.31	0.125
AbR	0.87	3.94	0.302	1.334	1.108	1.35	0.109
AbS	1.53	5.00	0.04	1.017	1.166	1.224	0.118
AbT	0.67	3.26	0.093	1.078	0.994	1.219	0.102
AbU	0.73	3.13	0.184	1.289	0.927	1.314	0.104

Example 9.4: BIACORE™ Analysis of Selectivity of Anti-IL-21R IgG Binding to Human IL-21R

[0195] The specificity of binding of a subset of transiently expressed anti-IL-21R binding proteins (here antibodies) was tested on a BIACORE™ 2000 surface plasmon resonance instrument. Anti-human-IgG, anti-murine immunoglobulin antibodies, and murine IL-21R-H/F were immobilized onto a research-grade carboxymethyl-dextran chip (CM5) using standard amine coupling. The sensor chip surface was activated with EDC/NHS for 7 min at a flow rate of 20 µl/min. The first flow cell was used as reference surface to correct for bulk refractive index, matrix effects, and nonspecific binding. Capture antibodies (7,150 resonance units (RU) of

anti-human-Fc antibody (Invitrogen Corporation, Carlsbad, CA) on flow cell 2 and 7,500 RU of anti-murine-Fc antibody on flow cell 3) were diluted to 10 μg/ml in sodium acetate buffer (pH 5.0) and injected over the activated surface. Remaining activated groups were blocked with 1.0 M ethanolamine (pH 8.0). The molecular weights of the anti-human IgG and the anti-murine IgG were both 150 kD, and the molecular weight of the IL-21R monomer was 27 kD.

[0196] Conditioned media containing anti-IL-21R antibodies and antibody controls (murine anti-human IL-2Rβ and murine anti-human IL-4R (R&D Systems, Minneapolis, MN); human anti-human IL-13 (Wyeth, Cambridge, MA)) were diluted in HBS / EP buffer supplemented with 0.2% bovine serum and injected onto all four flow cells of the BIACORE™ chip, capturing 500-700 (RU) of antibody on the species-appropriate capture antibody. Following a 5 sec washing period, 50 nM solutions of a positive control protein (murine IL-21R-H/F), two human proteins related to IL-21R (human IL-2Rβ and human sIL-4R (R&D Systems)), or an unrelated His/FLAG-tagged protein (human IL-13-H/F), were injected over the captured antibodies on the chip. The association and dissociation phases were monitored for 120 and 180 sec, respectively, followed by two 5 μl injections of glycine (pH 1.5) to regenerate a fully active capturing surface. All binding experiments were done at 25°C in HBS / EP buffer. Blank and buffer effects were subtracted for each sensorgram using double referencing.

[0197] All of the anti-IL-21R antibodies tested (18A5 antibody and AbA-AbU) showed clear binding to murine IL-21R, but no binding to the IL-21R-related proteins human IL-2Rβ and human soluble IL-4R, or to the unrelated His/FLAG-tagged protein human IL-13-His/FLAG (**FIGs. 7a-c**). Controls indicated that IL-2Rβ and human soluble IL-4R could be captured by specific anti-IL-2Rβ and anti-IL-4R antibodies (**FIG. 7d**).

Example 9.5: Purification of Transiently Expressed Antibodies

[0198] Seven antibodies (human IgG1 triple-mutant versions: AbS, AbT, AbO, AbP, and AbU; and double-mutant versions: AbQ and AbR) were transiently expressed in cos-7 cells and purified for further analysis. In addition, three versions of AbT with human IgG tails expected to have different levels of Fc receptor binding (wild-type IgG1, IgG4, and IgG1 double-mutants) were also prepared. The *TRANS*IT® protocol described above was followed, except that 25 μg of each plasmid was used to transfect cells in each of eight T-175 flasks. Following the first harvest of conditioned medium, fresh R1CD1 was added and then collected after an additional 72 hr. Conditioned media were pooled and filtered on a 0.22 μm filter. Antibodies were loaded onto protein A resin, eluted with 20 mM citric acid / 150 mM sodium chloride (pH 2.5), neutralized with Tris (pH 8.5), and dialyzed into PBS.

Example 9.6: BIACORE™ Analysis of Antibody Binding to Human and Murine IL-21R

[0199] The kinetics of binding of anti-IL-21R antibodies to human and murine IL-21R-H/F was tested on a BIACORE™ surface plasmon resonance instrument. Anti-human IgG antibodies (Invitrogen Corporation) were immobilized onto a research-grade carboxy-methyl-dextran chip (CM5) using standard amine coupling. The surface was activated with EDC/NHS for 7 min at a flow of 20 μl/min. The first flow cell was used as a reference surface to correct for bulk refractive index, matrix effects, and nonspecific binding. The anti-human-Fc antibody was diluted to 20 μg/ml in 10 mM sodium acetate buffer (pH 5.0), and 2950-3405 resonance units (RU) were captured on each of the four flow cells. Remaining activated groups were blocked with 1.0 M ethanolamine-HCl (pH 8.5).

[0200] Anti-IL-21R antibodies were diluted to 0.1-0.2 μg/ml in HBS / EP buffer supplemented with 0.2% bovine serum albumin and loaded onto the BIACORE™ chip. Following a brief washing period, solutions of 0-100 nM human IL-21R-H/F or 10-500 nM murine IL-21R-H/F were injected over the chip at a flow rate of 50 μl/min. The association phase was run for 3 min for human and murine IL-21R

kinetics, and the dissociation phase was monitored for 15 min for hIL-21R and for 5 min for mIL-21R, followed by two 10 µl injections and one 30 µl injection of glycine (pH 1.5), to regenerate a fully active capturing surface. All binding experiments were done at 25°C in HBS / EP buffer, and the sample rack was kept at 15°C. Blank and buffer effects were subtracted for each sensorgram using double referencing. Sensorgrams are shown in **FIGs. 8a-b** (human IL-21R-His/FLAG) and **8c-d** (murine IL-21R-His/FLAG). Binding kinetic parameters are shown in **Table 7A**, and additional kinetic data from a replicate experiment are shown in **Table 7B**.

[0201] In addition, AbS and AbT were tested for binding kinetics to cynomolgus monkey IL-21R-His/FLAG by the above-described protocol. Binding profiles to human and cynomolgus monkey IL-21R-H/F were similar for both AbS and AbT (**FIG. 9**). **FIG. 9** shows cynomolgus monkey IL-21R-His/FLAG binding to AbS (**9a**); and to AbT (**9c**); and human IL-21R-His/FLAG binding to AbS (**9b**); and AbT (**9d**).

Table 7A: Kinetic Parameters of Anti-IL-21R Antibody Binding Human and Murine IL-21R-His/FLAG

	Human IL-21R			Murine IL-21R		
Antibody	**ka (1/Ms)**	**kd (1/s)**	**KD (M)**	**ka (1/Ms)**	**kd (1/s)**	**KD (M)**
18A5	2.43E+05	1.08E-03	4.43E-09	2.12E+05	1.53E-02	7.20E-08
AbO	2.41E+05	1.14E-04	4.75E-10	1.12E+05	5.49E-03	4.92E-08
AbP	1.94E+05	1.19E-04	6.15E-10	9.99E+04	5.08E-03	5.08E-08
AbQ	4.39E+05	9.34E-05	2.13E-10	3.01E+05	2.07E-02	6.88E-08
AbR	1.70E+05	9.61E-05	5.67E-10	7.65E+04	4.93E-03	6.45E-08
AbS	1.44E+05	2.91E-04	2.02E-09	1.99E+05	3.32E-03	1.67E-08
AbT	1.79E+05	6.78E-05	3.79E-10	2.11E+05	3.31E-03	1.57E-08
AbU	1.86E+05	8.18E-05	4.40E-10	9.81E+04	4.34E-03	4.42E-08

Table 7B: Kinetic Parameters of Anti-IL-21R Antibody Binding Human IL-21R-His/FLAG

	Human IL-21R		
Antibody	**ka (1/Ms)**	**kd (1/s)**	**KD (M)**
18A5	3.04E+05	1.34E-03	4.40E-09
AbP	2.33E+05	1.02E-04	4.36E-10
AbQ	4.39E+05	9.34E-05	2.13E-10
AbR	2.48E+05	9.76E-05	3.94E-10
AbS	2.02E+05	3.05E-04	1.51E-09
AbT	2.73E+05	7.42E-05	2.72E-10
AbU	2.38E+05	7.83E-05	3.29E-10

Example 9.7: BIACORE™ Epitope Competition Assay

[0202] Antibodies AbS and AbT and the parental antibody 18A5 were immobilized directly onto a CM5 BIACORE™ chip. Murine IL-21R-H/F (100 nM) was allowed to flow over the chip for 300 sec, followed by a wash (100 sec), and then a 5 μg/ml solution of either AbS, AbT, D5, or a nonneutralizing anti-mIL-21R antibody (7C2) was allowed to flow over the surface. No additional binding was observed with AbS, AbT, and D5, indicating that their binding site on mIL-21R-H/F was blocked by concurrent binding to AbS, AbT, or 18A5 antibody (**FIG. 10a**). In contrast, the nonneutralizing control anti-IL-21R antibody 7C2 was able to bind to mIL-21R-H/F captured on AbS, AbT, or 18A5 antibody, indicating that this control antibody bound at a different epitope from the one bound by the capture antibodies.

[0203] Similarly, AbS and AbT did not bind to human IL-21R-H/F captured by AbS or AbT immobilized on a CM5 BIACORE™ chip, while the control anti-human IL-21R antibody (9D2) was able to bind human IL-21R-H/F captured by AbS or AbT (**FIG. 10b**). This observation suggested that the binding site for AbS is blocked by concurrent binding by AbT, and vice versa.

Example 9.8: Cell-based Proliferation Assays

[0204] Purified IgGs were tested for activity in IL-21-dependent proliferation assays in three cell lines as described above: human IL-21R-BaF3 cells, murine IL-21R-BaF3 cells, and human IL-21R-TF-1 cells. All showed strong inhibition of

both human and murine IL-21R-dependent proliferation with greater potency than that of the parental 18A5 IgG (**FIG. 11, Table 8**). Assays were conducted on human IL-21R-BaF3 cells with 100 pg/ml of human IL-21 (**FIG. 11a**), murine IL-21R-BaF3 cells with 200 pg/ml of murine IL-21 (**FIG. 11b**), and human IL-21R-TF-1 cells with 100 pg/ml of human IL-21 (**FIG. 11c**). **FIG. 26d** depicts the results of an additional study of the effects of these antibodies on human IL-21R-BaF3 cells.

Table 8: Neutralization of Proliferation of Human IL-21R-BaF3 Cells, Murine IL-21R-BaF3 Cells, and Human IL-21R-TF-1 Cells

Antibody	Human IL-21R-BaF3 Neutralization IC_{50} (nM)	Murine IL-21R-BaF3 Neutralization IC_{50} (nM)	Human IL-21R-TF1 Neutralization IC_{50} (nM)
18A5 antibody	1.71	177.23	13.99
AbR	0.56	0.34	1.63
AbS	0.68	0.04	6.67
AbT	0.30	0.05	2.32
AbX	0.54	nd	nd
IL21R-Fc	0.20 (human IL-21R-Fc)	0.04 (mouse IL-21R-Fc)	7.22 (human IL-21R-Fc)

Example 9.9: Primary Human B cell Proliferation Assays

[0205] Anti-IL-21R antibodies were tested for their ability to inhibit IL-21-dependent proliferation of primary human B cells. Buffy coat cells from healthy human donors were obtained from Massachusetts General Hospital (Boston, MA). The cells were incubated with a ROSETTESEP™ B cell enrichment cocktail (StemCell Technologies, Vancouver, Canada), and B cells isolated according to the manufacturer's instructions. The resulting population (60-80% $CD19^+$ B cells) were cultured in RPMI containing 10% FBS, 50 U/ml penicillin, 50 μg/ml streptomycin, and 2 mM L-glutamine at $1x10^5$/well in 96-well flat-bottom plates. B cells were pretreated with serially diluted anti-human IL-21R antibodies in a 37°C incubator adjusted to 5% CO_2 for 30 min. The treated B cells were then stimulated with

0.5 μg/ml anti-CD40 mAb (BD Biosciences, San Jose, CA) and 10 ng/ml IL-21 cytokine for 3 days in a 37°C incubator adjusted to 5% CO_2. On day 3, cultures were pulsed with 0.5 μCi/well ^{3}H-thymidine (Perkin Elmer (NEN)) and harvested 5 hr later onto glass fiber filter mats. ^{3}H-thymidine incorporation was determined by liquid scintillation counting. All of the improved antibodies neutralized IL-21-dependent proliferation with greater potency than the parental 18A5 antibody (**FIGs. 12a-b, Table 9**; also see **FIG. 26e**).

Table 9: Neutralization of Human Primary B Cell Proliferation

Antibody	Neutralization of B cell proliferation IC_{50} (nM)
AbQ	0.16
AbR	0.22
AbS	0.44
AbT	0.14
AbU	0.13
18A5 antibody	1.86

Example 9.10: Primary Human T cell Proliferation Assays

[0206] Anti-IL-21R antibodies were tested for their ability to inhibit IL-21-dependent proliferation of primary human $CD4^+$ T cells. Buffy coat cells from healthy human donors were obtained from Massachusetts General Hospital. $CD4^+$ T cells were isolated by negative selection using ROSETTESEP™ $CD4^+$ T cell enrichment cocktail (StemCell Technologies), according to the manufacturer's instructions. The resulting population was ~80-90% $CD4^+/CD3^+$ T cells. Enriched human $CD4^+$ T cells were activated for 3 days with anti-CD3/anti-CD28-coated microspheres in RPMI containing 10% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, and HEPES in a 37°C incubator adjusted to 5% CO_2. After activation, the microspheres were removed and the cells were washed and rested overnight at approximately $1x10^6$ cells/ml in culture medium. The rested cells were then washed again before addition to the assay plates. Serial dilutions of

anti-human IL-21 receptor antibodies were made in culture medium in flat-bottomed 96-well plates, followed by the sequential addition of human IL-21 (20 ng/ml final concentration) and the activated and rested $CD4^+$ T cells (10^5 cells/well). The plates were then incubated for an additional 3 days and pulsed with 1 μCi/well ^{3}H-thymidine (Perkin Elmer (NEN)) during the final 6 hr of the assay. Cells were harvested onto glass fiber filter mats and ^{3}H-thymidine incorporation was determined by liquid scintillation counting. All of the improved antibodies neutralized IL-21-dependent proliferation with greater potency than the parental 18A5 antibody (**FIG. 13, Table 10A**; also see **FIG. 26f**).

Table 10A: Neutralization of Human Primary T Cell Proliferation

Antibody	Neutralization of T cell Proliferation IC_{50} (nM)
AbO	0.06
AbP	0.02
AbQ	0.08
AbR	0.04
AbS	0.06
AbT	0.03
AbU	0.03
18A5 antibody	1.42

Example 9.11: Primary Murine T cell Proliferation Assays

[0207] Anti-IL-21R antibodies were tested for their ability to inhibit IL-21-dependent proliferation of primary murine $CD8^+$ T cells. Popliteal, axillary, brachial, and inguinal lymph nodes and spleens from 12-week-old female BALB/C mice were collected. A single-cell suspension of the spleen cells was depleted of red blood cells using 0.16 M NH_4Cl in 0.017 M Tris (pH 7.4). The spleen and lymph node cells were pooled and enriched for $CD8^+$ cells using a murine T cell CD8 Subset Column Kit (R&D Systems). Murine $CD8^+$ cells ($3x10^4$; suspended in DMEM containing 10% fetal calf serum and supplemented with 0.05 mM β-mercaptoethanol, 2 mM L-glutamine, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 100 U/ml

penicillin, 100 µg/ml streptomycin and 50 µg/ml gentamicin) were plated in 96-well, anti-mCD3 activation plates (BD Biosciences); mIL-21 (50 ng/ml) was added to all the wells. The test antibodies were titered in triplicate beginning at 20 µg/ml. Cells were grown for 3 days in a 37°C / 10% CO_2 incubator. During the last 5 hr of culture, cells were labeled with 0.5 µCi methyl-^{3}H-thymidine/well (GE Healthcare). The cells were harvested using a Mach III cell harvester (TomTec, Hamden, CT) and counted using a Trilux microbeta counter (Perkin Elmer). Aside from AbP, all of the improved antibodies neutralized IL-21-dependent proliferation with greater potency than the parental 18A5 antibody (**FIG. 14, Table 10B**; also see **FIG. 26g**).

Table 10B: Neutralization of Murine Primary T Cell Proliferation

Antibody	Neutralization of T cell Proliferation IC_{50} (nM)
AbO	4.92
AbP	no inhibition
AbQ	0.85
AbR	0.13
AbS	0.02
AbT	0.61
AbU	1.79
18A5 antibody	>85

Example 9.12: ADCC Assay

[0208] Anti-IL-21R antibodies were tested for their ability to induce antibody-dependent cellular cytotoxicity (ADCC) when bound to target cells. The day before the experiment, PBMC were isolated from buffy coat by diluting the buffy coat 1:1 in PBS, layering it over FICOLL® (GE Healthcare), and centrifuging at 1200 g for 20 min. PBMCs were removed from the top of the FICOLL® layer, washed, and stimulated overnight with 10 ng/ml IL-2 and 10 ng/ml IL-12 (R&D Systems). The day of the experiment, stimulated PBMCs were collected by centrifugation and resuspended in media at $1x10^8$ cells/ml. BJAB cells were labeled with 0.5 µM CFSE (MOLECULAR PROBES®, Invitrogen Corporation) for 10 min at 37°C, and then

washed with fetal bovine serum once and PBS twice. Cells were then plated into a 96-well flat-bottom plate at $2x10^5$ cells/well in 100 µl media. Fifty µl of the 4x antibodies were added to the BJAB cells, followed by $5x10^6$ PBMC in 50 µl, giving a final 1:25 target:effector cell ratio. Cells were incubated at 37°C for 6 hr and stained with propidium iodide (PI) to label dead and dying cells. Killing of target cells ($CFSE^+$) was assessed by measuring PI staining in a FACSCALIBUR™ flow cytometer (BD Biosciences). Only one anti-IL-21R antibody, AbZ, which has a wild-type human IgG1 constant region, showed ADCC above the background level displayed by a control anti-IL-13 antibody that did not bind to the target cells. All antibodies with the same variable domains as AbZ, including forms with human IgG4 (AbY), and those with double-mutant (AbX) and triple-mutant (AbT) forms of human IgG1, showed only background levels of ADCC (**FIG. 15**). All other anti-IL-21R antibodies tested contained the triple-mutant form of human IgG1 and showed background ADCC. A positive control antibody, rituximab (RITUXAN®), induced ADCC in all experiments.

Example 9.13: C1q ELISA

[0209] In order to determine whether cell-surface binding by anti-IL-21R antibodies is likely to lead to complement-dependent cytotoxicity (CDC), the antibodies were tested for their ability to bind to the complement component C1q in an ELISA. IL-21R antibodies and rituximb (RITUXAN®) were diluted in PBS to 5 µg/ml. Diluted antibodies (100 µl) were coated onto a COSTAR® high-binding ELISA plate (Corning Life Sciences, Lowell, MA) overnight at 4°C. Plates were washed 3X with PBS / Tween-20 and blocked with 200 µl of blocking buffer (0.1 M $NaPO_4$, 0.1 M NaCl, 0.1% gelatin, 0.01% Tween) for 1 hr at RT. Human serum previously determined to contain C1q (Quidel, San Diego, CA) was diluted 1:50 in PBS. After 1 hr of blocking, plates were washed and 100 µl of diluted serum was added to each well and incubated for 2 hr at RT on a shaker. Following three washes, 100 µl of 0.1 µg/ml chicken polyclonal anti-human C1q antibody (AbCam, Cambridge, MA)

was added to each well and incubated for 1 hr at RT. Plates were again washed and incubated with 100 µl of a rabbit polyclonal antibody to chicken Ig-Y-HRP diluted 1:4000 (AbCam) for 1 hr at RT. Plates were washed and developed with TMB for 5 min, followed by 50 µl of 1 M H_2SO_4 to stop the reaction, and then read at 450 nm. Only one anti-IL-21R antibody, AbZ, which has a wild-type human IgG1 constant region, showed C1q binding above the background level displayed by a control antibody with a triple-mutant human IgG1 constant region that had previously been shown to lack C1q binding. All antibodies with the same variable domains as AbZ, including forms with human IgG4 (AbY), and those with double-mutant (AbX) and triple-mutant (AbT) forms of human IgG1, showed only background levels of C1q binding (**FIG. 16**). All other anti-IL-21R antibodies tested contained the triple-mutant form of human IgG1 and showed background C1q binding.

Example 9.14: Cytokine competition assay

[0210] In order to demonstrate that antibody AbT binds to the murine IL-21R in a manner that competes with the IL-21 cytokine, a cytokine competition assay was performed. Antibody AbT was coated at 1 µg/ml onto ELISA plates, which were then blocked with 1% BSA in PBS/.05% Tween. Biotinylated murine IL-21R-His/FLAG (1.5 ng/ml) was added to the wells, either alone or in the presence of increasing concentrations of murine IL-21, and the binding of the receptor to the immobilized antibody was detected with HRP-conjugated streptavidin and subsequent incubation with TMB detection reagent. Mouse IL-21 was able to block the binding of mIL-21R to AbT nearly completely above 4 ng/ml, indicating that the antibody and the cytokine compete for binding to murine IL-21R (**FIG. 27**).

Equivalents

[0211] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents of the specific embodiments of the

invention described herein. Such equivalents are intended to be encompassed by the following claims.

WHAT IS CLAIMED IS:

1. An isolated binding protein or antigen-binding fragment thereof that binds to IL-21R, wherein the binding protein or antigen-binding fragment thereof comprises at least one amino acid sequence that is at least about 95% identical to an amino acid sequence(s) selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 165-168, 171-193, 213-229, 240, 242, 244, 246, and 248.

2. The isolated binding protein or antigen-binding fragment of claim 1, wherein the binding protein or antigen-binding fragment is an antibody.

3. The isolated binding protein or antigen-binding fragment of claim 1, wherein the binding protein or antigen-binding fragment is an scFv.

4. The isolated binding protein or antigen-binding fragment of claim 1, wherein the binding protein or antigen-binding fragment is a V_H.

5. The isolated binding protein or antigen-binding fragment of claim 1, wherein the binding protein or antigen-binding fragment is a V_L.

6. The isolated binding protein or antigen-binding fragment of claim 1, wherein the binding protein or antigen-binding fragment is a CDR.

7. An isolated binding protein or antigen-binding fragment thereof that binds to IL-21R, wherein the binding protein or antigen-binding fragment thereof comprises at least one amino acid sequence encoded by a nucleotide sequence that is at least about 95% identical to a nucleotide sequence(s) selected from the group consisting of SEQ ID NOs:13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53,

55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 239, 241, 243, 245, and 247.

8. The isolated binding protein or antigen-binding fragment of claim 7, wherein the binding protein or antigen-binding fragment is an antibody.

9. The isolated binding protein or antigen-binding fragment of claim 7, wherein the binding protein or antigen-binding fragment is an scFv.

10. The isolated binding protein or antigen-binding fragment of claim 7, wherein the binding protein or antigen-binding fragment is a V_H.

11. The isolated binding protein or antigen-binding fragment of claim 7, wherein the binding protein or antigen-binding fragment is a V_L.

12. The isolated binding protein or antigen-binding fragment of claim 7, wherein the binding protein or antigen-binding fragment is a CDR.

13. The binding protein or antigen-binding fragment of claim 1, comprising at least one amino acid sequence selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 165-168, 171-193, 213-229, 240, 242, 244, 246, and 248.

14. The isolated binding protein or antigen-binding fragment of claim 13, wherein the binding protein or antigen-binding fragment is an antibody.

15. The isolated binding protein or antigen-binding fragment of claim 13, wherein the binding protein or antigen-binding fragment is an scFv.

16. The isolated binding protein or antigen-binding fragment of claim 13, wherein the binding protein or antigen-binding fragment is a V_H.

17. The isolated binding protein or antigen-binding fragment of claim 13, wherein the binding protein or antigen-binding fragment is a V_L.

18. The isolated binding protein or antigen-binding fragment of claim 13, wherein the binding protein or antigen-binding fragment is a CDR.

19. An isolated binding protein or antigen-binding fragment thereof that binds to IL-21R, wherein the binding protein or antigen-binding fragment thereof comprises at least one amino acid sequence that is at least about 95% identical to an amino acid sequence(s) selected from the group consisting of SEQ ID NOs:6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162-195, 213-229, 240, 242, 244, 246, and 248, and

wherein, if the binding protein or antigen-binding fragment comprises at least one amino acid sequence that is at least about 95% identical to the sequence(s) selected from the group consisting of SEQ ID NOs:6, 8, 10, 12, 163, 164, 169, 170, 194, and 195, then the binding protein or antigen-binding fragment must also comprise at least one amino acid sequence that is at least about 95% identical to the amino acid sequence(s) selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146,

148, 150, 152, 154, 156, 158, 160, 162, 165-168, 171-193, 213-229, 240, 242, 244, 246, and 248.

20. An isolated binding protein or antigen-binding fragment thereof that binds to IL-21R, wherein the binding protein or antigen-binding fragment thereof comprises at least one amino acid sequence encoded by a nucleotide sequence that is at least about 95% identical to a nucleotide sequence(s) selected from the group consisting of SEQ ID NOs:5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 239, 241, 243, 245, and 247, and

wherein, if the binding protein or antigen-binding fragment comprises at least one amino acid sequence encoded by a nucleotide sequence that is at least about 95% identical to the sequence(s) selected from the group consisting of SEQ ID NOs:5, 7, 9, and 11, then the binding protein or antigen-binding fragment must also comprise at least one amino acid sequence encoded by a nucleotide sequence that is at least about 95% identical to the nucleotide sequence(s) selected from the group consisting of SEQ ID NOs:13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 239, 241, 243, 245, and 247.

21. The binding protein or antigen-binding fragment of claim 19, comprising at least one amino acid sequence selected from the group consisting of SEQ ID NOs:6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134,

136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162-195, 213-229, 240, 242, 244, 246, and 248,

wherein, if the binding protein or antigen-binding fragment comprises at least one amino acid sequence that is at least about 95% identical to the sequence(s) selected from the group consisting of SEQ ID NOs:6, 8, 10, 12, 163, 164, 169, 170, 194, and 195, then the binding protein or antigen-binding fragment must also comprise at least one amino acid sequence that is at least about 95% identical to the amino acid sequence(s) selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 165-168, 171-193, 213-229, 240, 242, 244, 246, and 248.

22. An isolated binding protein or antigen-binding fragment thereof that binds to IL-21R, wherein the binding protein or antigen-binding fragment thereof comprises a light chain and a heavy chain, and wherein the heavy chain comprises at least one amino acid sequence selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 68, 70, 72, 88, 90, 92, 94, 213, 218, 219, 240, and 242.

23. An isolated binding protein or antigen-binding fragment thereof that binds to IL-21R, wherein the binding protein or antigen-binding fragment thereof comprises a light chain and a heavy chain, and wherein the light chain comprises at least one amino acid sequence selected from the group consisting of SEQ ID NOs:22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 74, 76, 78, 80, 82, 84, 86, 96, 98, 100, 102, 104, 106, 108, 214-217, 220-229, 244, 246, and 248.

24. The binding protein or antigen-binding fragment of claim 22, wherein the binding protein or antigen-binding fragment comprises a V_L domain and a V_H domain,

and wherein the V_H domain comprises at least one amino acid sequence selected from the group consisting of SEQ ID NOs:14, 16, 18, and 20.

25. The binding protein or antigen-binding fragment of claim 23, wherein the binding protein or antigen-binding fragment comprises a V_L domain and a V_H domain, and wherein the V_L domain comprises at least one amino acid sequence selected from the group consisting of SEQ ID NOs:22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 215, 217, 221, 223, 225, 227, and 229.

26. The binding protein or antigen-binding fragment of claim 22 or 23, wherein the heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:88, 90, 92, 94, 213, 218, 219, 240, and 242, and the light chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:96, 98, 100, 102, 104, 106, 108, 214, 216, 220, 222, 224, 226, 228, 244, 246, and 248.

27. An isolated binding protein or antigen-binding fragment thereof that binds to an IL-21R epitope that is recognized by a binding protein selected from the group consisting of AbA-AbW, H3-H6, L1-L6, L8-L21, and L23-L25, wherein the binding protein or antigen-binding fragment competitively inhibits the binding of a binding protein selected from the group consisting of AbA-AbW, H3-H6, L1-L6, L8-L21, and L23-L25 to human IL-21R.

28. The binding protein or antigen-binding fragment of claim 27, comprising a heavy chain, a light chain, or an F_v fragment comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 165-168, 171-193, 213-229, 240, 242, 244, 246, and 248.

29. The binding protein or antigen-binding fragment of claim 27, comprising a heavy chain, a light chain, or an F_v fragment comprising an amino acid sequence encoded by a nucleotide sequence selected from the group consisting of SEQ ID NOs:13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 239, 241, 243, 245, and 247.

30. The binding protein or antigen-binding fragment of any one of claims 1, 2, 19, or 20, wherein the association constant of the binding protein or antigen-binding fragment for human IL-21R is at least about 10^5 $M^{-1}s^{-1}$.

31. The binding protein or antigen-binding fragment of any one of claims 1, 2, 19, or 20, wherein the binding protein or antigen-binding fragment inhibits IL-21-mediated proliferation of BaF3 cells with an IC_{50} of about 1.75 nM or less, and wherein the BaF3 cells comprise a human IL-21R.

32. The binding protein or antigen-binding fragment of any one of claims 1, 2, 19, or 20, wherein the binding protein or antigen-binding fragment inhibits IL-21-mediated proliferation of TF1 cells with an IC_{50} of about 14.0 nM or less, and wherein the TF1 cells comprise a human IL-21R.

33. The binding protein or antigen-binding fragment of any one of claims 1, 2, 19, or 20, wherein the binding protein or antigen-binding fragment inhibits IL-21-mediated proliferation of primary human B cells with an IC_{50} of about 1.9 nM or less, and wherein the B cells comprise a human IL-21R.

34. The binding protein or antigen-binding fragment of any one of claims 1, 2, 19, or 20, wherein the binding protein or antigen-binding fragment inhibits IL-21-

mediated proliferation of primary human $CD4^+$ cells with an IC_{50} of about 1.5 nM or less, and wherein the $CD4^+$ cells comprise a human IL-21R.

35. The binding protein or antigen-binding fragment of any one of claims 1, 2, 19, or 20, wherein the binding protein or antigen-binding fragment specifically binds to an amino acid sequence that is at least about 95% identical to any sequence of at least 100 contiguous amino acids of SEQ ID NO:2.

36. The binding protein or antigen-binding fragment of any one of claims 1, 2, 19, or 20, wherein the binding protein or antigen-binding fragment inhibits the binding of IL-21 to IL-21R.

37. The binding protein or antigen-binding fragment of any one of claims 1, 2, 19, or 20, wherein the binding protein or antigen-binding fragment is IgG1.

38. The binding protein or antigen-binding fragment of any one of claims 1, 2, 19, or 20, wherein the binding protein or antigen-binding fragment is human.

39. A pharmaceutical composition comprising the binding protein or antigen-binding fragment of any one of claims 1, 2, 19, or 20.

40. An isolated nucleic acid encoding the binding protein or antigen-binding fragment of any one of claims 1, 2, 19, or 20.

41. An expression vector comprising the nucleic acid of claim 40.

42. A host cell transformed with the vector of claim 41.

43. The host cell of claim 42, wherein the host cell is a bacteria, mammalian cell, yeast cell, plant cell, or insect cell.

44. A diagnostic kit comprising the binding protein or antigen-binding fragment of any one of claims 1, 2, 19, or 20.

45. The isolated binding protein or antigen-binding fragment of any one of claims 19-38, wherein the binding protein or antigen-binding fragment is an antibody.

46. The isolated binding protein or antigen-binding fragment of any one of claims 19-38, wherein the binding protein or antigen-binding fragment is an scFv.

47. The isolated binding protein or antigen-binding fragment of any one of claims 19-38, wherein the binding protein or antigen-binding fragment is a V_H.

48. The isolated binding protein or antigen-binding fragment of any one of claims 19-38, wherein the binding protein or antigen-binding fragment is a V_L.

49. The isolated binding protein or antigen-binding fragment of any one of claims 19-38, wherein the binding protein or antigen-binding fragment is a CDR.