HUMANIZED ANTIBODIES AGAINST CXCR3

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to US Provisional Application No. 60/898,709 filed February 1, 2007, the disclosure of which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] The interaction between chemokines and their receptors is an important step in the control of leukocyte migration. Chemokines also mediate a variety of effects independent of chemotaxis, including induction and enhancement of cell-associated cytokine responses.

[0003] The human cell surface protein CD183 is a G protein-coupled receptor with selectivity for three chemokines including IP10 (interferon-g-inducible 10 kDa protein), Mig (monokine induced by interferon-g) and I-TAC (interferon-inducible T cell a-chemoattractant). These three chemokines belong to the structural subfamily of "CXC" chemokines, in which a single amino acid residue separates the first two of four highly conserved Cys residues. Historically, CD183 is the third CXC chemokine receptor discovered and, therefore, CD183 is commonly designated as "CXCR3." Binding of chemokines to CXCR3 induces cellular responses that are involved in leukocyte traffic, most notably integrin activation, cytoskeletal changes and chemotactic migration. CXCR3 is expressed on effector/memory T cells and/or in T cells present in many types of inflamed tissues (*e.g.*, T-helper 1 cells or Th1 cells and $CD8^+$ Tc1 cells). In addition, IP10, Mig and I-TAC are commonly produced by local cells in inflammatory lesions, suggesting that CXCR3 and its chemokines participate in the recruitment of white blood cells to sites of inflammation. Therefore, CXCR3 is a target for the development of antibodies and antagonists, which may be used in the treatment and diagnosis of diverse inflammatory and immune diseases and disorders, such as rheumatoid arthritis, multiple schlerosis, Crohn's disease, inflammatory

bowel disease, chronic obstructive pulmonary disease, psoriasis, type 1 diabetes and transplant rejection. Because CXCR3 is expressed on a subset of B-cell lymphomas, CXCR3 may also be a target for treating and diagnosing lymphomas and leukemias.

SUMMARY

[0004] Disclosed are antigen-binding polypeptide molecules that bind specifically to the chemokine receptor CXCR3 (*see* GenBank gi:4504099). The polypeptides include a humanized heavy chain variable region and a humanized light chain variable region. For example, the polypeptides may include the framework (FR) regions of the light and heavy chain variable regions of a human antibody, while retaining substantially the antigen-binding specificity of a parental monoclonal antibody. The humanized heavy chain variable region and/or the humanized light chain variable region are at least about 90% humanized (preferably at least about 95% humanized, more preferably at least about 98% humanized, and even more preferably at least about 100% humanized), excluding the CDRs. The antigen-binding polypeptides molecules may be derived from monoclonal antibody donors (*e.g.*, mouse monoclonal antibody donors) and may include CDRs from the monoclonal antibodies (*e.g.*, mouse monoclonal CDRs). The polypeptides may function as antagonists for the CXCR3 receptor.

[0005] In some embodiments, the antigen-binding polypeptide binds specifically to CXCR3, and includes: (a) a humanized antibody heavy chain variable region comprising: (1) a CDR-H1 comprising an amino acid sequence of (NYMAS); (2) a CDR-H2 comprising an amino acid sequence of (TISSGGGYTYYPDSLKG); and (3) a CDR-H3 comprising an amino acid sequence of (HGAPMTTVITYAPYYF{D,Y}Y); and (b) a humanized antibody light chain variable region comprising: (1) a CDR-L1 comprising an amino acid sequence of (RASSSVKYMY); (2) a CDR-L2 comprising an amino acid sequence of (YTSNLAP); and (3) a CDR-L3 comprising an amino acid sequence of (QQFTTSPYT). The polypeptide may include a CDR-H3 comprising an amino acid sequence of (HGAPMTTVITYAPYYFYY).

[0006] In some embodiments of the polypeptides, (1) the CDR-H1 consists of the amino acid sequence of (NYMAS); (2) the CDR-H2 consists of the amino acid sequence of (TISSGGGYTYYPDSLKG); (3) the CDR-H3 consists of the amino acid sequence of (HGAPMTTVITYAPYYF{D,Y}Y); (4) the CDR-L1 consists of the amino acid sequence of (RASSSVKYMY); (5) the CDR-L2 consists of the amino acid sequence of (YTSNLAP); and (6) the CDR-L3 consists of the amino acid sequence of (QQFTTSPYT). For example, the CDR-H3 may consist of the amino acid sequence of (HGAPMTTVITYAPYYFYY).

[0007] In some embodiments, the polypeptide comprises a humanized antibody heavy chain variable region of ({E,D}{I,N,V}V{L,M}TQSPA{T,F,I}{L,M}S{L,A,V}{S,T}{L,P}GE{R,K}{A,V}T{L,M,I}{S,T,N}CRASSSVKYMYWYQQK{S,P}{G,D}{Q,A}{A,S}P{R,K}L{L,W}I{Y,K}YTSNLAPG{I,V}P{A,S}RFSGSGSG{T,N}{D,S}{F,Y}{T,S}{L,F}TISS{M,L}E{A,G,P}ED{F,A}A{V,T}YYC{Q,Y}QFTT{S,Y}PYTFGGGTKLEIKR). For example, the polypeptide may comprise a humanized antibody heavy chain variable region of (EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPKGLEWVSTISSGGGYTYYPDSLKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKHGAPMTTVITYAPYYFYYWGQGTTVTVSS). In some embodiments, the polypeptide comprises a humanized antibody light chain variable region of (E{I,N}VLTQSPA{T,F,I}{L,M}S{L,A,V}{S,T}{L,P}GE{R,K}{A,V}T{L,M,I}{S,T,N}CRASSSVKYMYWYQQK{S,P}{G,D}{Q,A}{A,S}P{R,K}L{L,W}IYYTSNLAPG{I,V}P{A,S}RFSGSGSG{T,N}{D,S}{F,Y}{T,S}{L,F}TISS{M,L}E{A,G}ED{F,A}A{V,T}YYCQQFTTSPYTFGGGTKLEIKR). For example, the polypeptide may comprise a humanized antibody light chain variable region of (EIVLTQSPATLSLSLGERATLSCRASSSVKYMYWYQQKSGQAPRLLIYYTSNLAPGIPARFSGSGSGTDFTLTISSMEAEDFAVYYCQQFTTSPYTFGGGTKLEIKR); or (ENVLTQSPAFLSVTPGEKVTITCRASSSVKYMYWYQQKPDQAPKLWIYYTSNLAPGVPSRFSGSGSGNDYTFTISSLEAEDAATYYCQQFTTSPYTFGGGTKLEIKR).

[0008] Also disclosed are humanized antibody heavy chain variable regions. The humanized antibody heavy chain region may comprise: (1) a CDR-H1 comprising an amino acid sequence of ({N,S,Y}YAMS); (2) a CDR-H2 comprising an amino acid sequence of ({T,A,Y}I{S,Y}{S,G,T,Y}{G,S}{G,Y}G{F,S,Y}TYY{P,A}DS{L,Y,V}KG); and (3) a CDR-H3 comprising an amino acid sequence of {H,Y}{G,Y}{A,Y}PM{T,Y}T{V,Y}ITY{A,Y}PYYFYY). For example, the humanized antibody heavy chain variable region may comprise an amino acid sequence of (EVQLLESGGGLVQPGGSLRLSCAASGFTFS{N,S,Y}YAMSWVRQAPGKGLEWVS{T,A,Y}I{S,Y}{S,G,T,Y}{G,S}{G,Y}G{F,S,Y}TYY{P,A}DS{L,Y,V}KGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK{H,Y}{G,Y}{A,Y}PM{T,Y}T{V,Y}ITY{A,Y}PYYFYYWGQGTTVTVSS).

[0009] In another example, a humanized antibody heavy chain variable region comprises: (1)a CDR-H1 comprising an amino acid sequence of (NYAIS); (2) a CDR-H2 comprising an amino acid sequence of (TYSSGGVYTYYRDSLKG); and (3) a CDR-H3 comprising an amino acid sequence of (HGAAMTTVITYAPFYFYY). For example, the humanized antibody heavy chain variable region may comprise an amino acid sequence of (EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYAISWVRQAPGKGLEWVSTYSSGGVYTYYRDSLKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKHGAAMTTVITYAPFYFYYWGQGTTVTVSS).

[0010] In another example, a humanized antibody heavy chain variable region comprises: (1)a CDR-H1 comprising an amino acid sequence of (YYAMS); (2) a CDR-H2 comprising an amino acid sequence of (TIYSGGSYTFYPDSLEG); and (3) a CDR-H3 comprising an amino acid sequence of (HGAPMSTEITYAPYYFYY). For example, the humanized antibody heavy chain variable region may comprise an amino acid sequence of (EVQLLESGGGLVQPGGSLRLSCAASGFTFSYYAMSWVRQAPGKGLEWVSTI

YSGGSYTFYPDSLEGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKHGAPM STEITYAPYYFYYWGQGTTVTVSS).

[0011] In another example, a humanized antibody heavy chain variable region comprises: (1) a CDR-H1 comprising an amino acid sequence of (NYAMS); (2) a CDR-H2 comprising an amino acid sequence of (TIYSGGGYTFYLDSLKG); and (3) a CDR-H3 comprising an amino acid sequence of (HSYPMTTVITYAPYYFYY). For example, the humanized antibody heavy chain variable region may comprise an amino acid sequence of (EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYYMSWVRQAPGKGLEWVSTI YSGGGYTFYLDSLKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKHSYP MTTVITYAPYYFYYWGQGTTVTVSS).

[0012] In another example, a humanized antibody heavy chain variable region comprises: (1)a CDR-H1 comprising an amino acid sequence of (NYAMS); (2) a CDR-H2 comprising an amino acid sequence of (TISSGGGYTYYPDSLKG); and (3) a CDR-H3 comprising an amino acid sequence of (HGAPMTTVITYAPYYFYY). For example, thehumanized antibody heavy chain variable region may comprise an amino acid sequence of (EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSTI SSGGGYTYYPDSLKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKHGAP MTTVITYAPYYFYYWGQGTTVTVSS).

[0013] In another example, a humanized antibody heavy chain variable region comprises:(1) a CDR-H1 comprising an amino acid sequence of (NYAMS); (2) a CDR-H2 comprising an amino acid sequence of (TISSGGGYTYYPDSLKG); and (3) a CDR-H3 comprising an amino acid sequence of (HGAPMTTVITYAPYYFYY). For example, the humanized antibody heavy chain variable region may comprise an amino acid sequence (EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSTI SSGGGYTYYPDSLKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKHGAP MTTVITYAPYYFYYWGQGTTVTVSS).

[0014] Also disclosed are humanized antibody light chain variable regions. The humanized antibody light chain region may comprise: (1) a CDR-L1 comprising an amino acid sequence of (RASSSVKYMY); (2) a CDR-L2 comprising an amino acid sequence of (YTSNLAP); and (3) a CDR-L3 comprising an amino acid sequence of (QQFTTSPYT). For example, the humanized antibody light chain variable region may comprise an amino acid sequence of (EIVLTQSPATLSLSLGERATLSCRASSSVKYMYWYQQKSGQAPRLLIYYTSNLAPGIPARFSGSGSGTDFTLTISSMEAEDFAVYYCQQFTTSPYTFGGGTKLEIKR).

[0015] In another example, a humanized antibody light chain variable region comprises: (1) a CDR-L1 comprising an amino acid sequence of (RASSSVKYMY); (2) a CDR-L2 comprising an amino acid sequence of (YTSNLAP); and (3) a CDR-L3 comprising an amino acid sequence of (QQFTTSPYT). For example, the humanized antibody light chain variable region may comprise an amino acid sequence of (EIVLTQSPATLSLSPGERATLSCRASSSVKYMYWYQQKPGQAPRLLIYYTSNLAPGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQFTTSPYTFGGGTKLEIKR).

[0016] In another example, a humanized antibody light chain variable region comprises: (1) a CDR-L1 comprising an amino acid sequence of (RASSSVKYMY); (2) a CDR-L2 comprising an amino acid sequence of (YTSNLAP); and (3) a CDR-L3 comprising an amino acid sequence of (YQFTTSPYT). For example, the humanized antibody light chain variable region may comprise an amino acid sequence of (EIVLTQSPATLSLSPGERATLSCRASSSVKYMYWYQQKPGQAPRLLIYYTSNLAPGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCYQFTTSPYTFGGGTKLEIKR).

[0017] In another example, the humanized antibody light chain variable region comprises: (1) a CDR-L1 comprising an amino acid sequence of (RASSSVKYMY); (2) a CDR-L2 comprising an amino acid sequence of (YTSNLAP); and (3) a CDR-L3 comprising an amino acid sequence of (QQYTTSPYT). For example, the humanized antibody light chain variable region may comprise an amino acid sequence of

(EIVLTQSPATLSLSPGERATLSCRASSSVKYMYWYQQKPGQAPRLLIYYTSNL APGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQYTTSPYTFGGGTKLEIKR).

[0018] In another example, the humanized antibody light chain variable region comprises: (1)a CDR-L1 comprising an amino acid sequence of (RASSSVKYMY); (2) a CDR-L2 comprising an amino acid sequence of (YTSNLAP); and (3) a CDR-L3 comprising an amino acid sequence of (QQFTTYPYT). For example, the humanized antibody light chain variable region may comprise an amino acid sequence of (EIVLTQSPATLSLSPGERATLSCRASSSVKYMYWYQQKPGQAPRLLIYYTSNL APGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQFTTYPYTFGGGTKLEIKR).

[0019] In another example, the humanized antibody light chain variable region comprises: (1) a CDR-L1 comprising an amino acid sequence of (RAS{S,Q}SV{K,S}SY{M,L}{Y,A}); (2) a CDR-L2 comprising an amino acid sequence of ({Y,D}{T,A}SN{L,R}A{P,T}); and (3) a CDR-L3 comprising an amino acid sequence of (Q,Y}Q{F,Y}TT{S,Y}PYT). For example, the humanized antibody light chain variable region may comprise an amino acid sequence of (EIVLTQSPATLSLSPGERATLSCRAS{S,Q}SV{K,S}SY{M,L}{Y,A}WYQQKPG QAPRLLIY{Y,D}{T,A}SN{L,R}A{P,T}GIPARFSGSGSGTDFTLTISSLEPEDFA VYYC{Q,Y}Q{F,Y}TT{S,Y}PYTFGGGTKLEIKR).

[0020] In another example, the humanized antibody light chain variable region comprises: (1)a CDR-L1 comprising an amino acid sequence of (RASSSVKYMY); (2) a CDR-L2 comprising an amino acid sequence of (YTSNLAP); and (3) a CDR-L3 comprising an amino acid sequence of (QQFTTSPYT). For example, the humanized antibody light chain variable region may comprise an amino acid sequence of (ENVLTQSPAFLSVTPGEKVTITCRASSSVKYMYWYQQKPDQAPKLWIYYTS NLAPGVPSRFSGSGSGNDYTFTISSLEAEDAATYYCQQFTTSPYTFGGGTKLEI KR).

[0021] In another example, the humanized antibody light chain variable region comprises: (1)a CDR-L1 comprising an amino acid sequence of (RAS{S,Q}SV{K,S}SY{M,L}{Y,A}); (2) a CDR-L2 comprising an amino acid

sequence of ({Y,D}{T,A}SN{L,R}A{P,T}); and (3) a CDR-L3 comprising an amino acid sequence of (Q,Y}Q{F,Y}TT{S,Y}PYT). For example, the humanized antibody light chain variable region may comprise an amino acid sequence of ((E,D)(N,V)V(L,M)TQSPAFLSVTPGEKVTITCRASSSVKYMYWYQQKPDQAPK L(W,L)I(Y,K)YTSNLAPGVPSRFSGSGSG(N,T)D(Y,F)TFTISSLEAEDAATYYC(Q,Y)Q(F,Y)TT(S,Y)PYTFGGGTKLEIKR).

[0022] The aforementioned humanized heavy chains and humanized light chains may be present in the antigen binding polypeptides that binds specifically to CXCR3.

[0023] The antigen-binding polypeptide may be selected from the group consisting of an antibody molecule, a Fab fragment, a Fab' fragment, a $F(ab')_2$ fragment, and an scFv molecule. In some embodiments, polypeptide is an antibody molecule. Antibody molecules may include chimeric antibodies that include a human heavy chain constant region and a human light chain constant region. For example, the antibody molecule may be an IgG molecule (*e.g.*, a IgG1 or an IgG4 molecule), where the polypeptide includes the heavy chain and light chain constant domains of an IgG molecule. The polypeptide may be an scFv molecule. For example, the scFv may have a formula selected from the group consisting of NH_2-L-VH-X-VK-COOH and NH_2-L-VK-X-VH-COOH; wherein L is a leader sequence; VH is the humanized antibody heavy chain variable region; X is a linking polypeptide; and VK is the humanized antibody light chain variable region.

[0024] The antigen-binding polypeptide further may be conjugated or fused to a therapeutic or diagnostic agent. For example, therapeutic agents may be selected from the group consisting of a cytotoxic agent, a radioactive label, an immunomodulator, a hormone, an enzyme, an oligonucleotide, a photoactive therapeutic agent or a combination thereof. Examples of diagnostic agents may include a radioactive label, a photoactive diagnostic agent, an ultrasound-enhancing agent or a non-radioactive label.

[0025] The antigen-binding polypeptide may be an antagonist of CXCR3. Typically, the polypeptide is not an agonist of CXCR3.

[0026] The antigen-binding polypeptide binds to the CXCR3 receptor with specificity and high affinity. Typically, the polypeptide binds to CXCR3 with an affinity constant of at least about 10^6M^{-1} (preferably at least about 10^7M^{-1}, more preferably at least about 10^8M^{-1}, even more preferably at least about 10^9M^{-1}).

[0027] Also disclosed are pharmaceutical compositions comprising the aforementioned antigen-binding polypeptides and a carrier (*e.g.*, a diluent or excipient). The pharmaceutical may further comprise an additional therapeutic or diagnostic agent as disclosed herein.

[0028] Also disclosed are methods of treating or diagnosing a disease or condition that comprise administering the disclosed pharmaceutical compositions to a patient in need thereof. For example, the pharmaceutical compositions may be administered to treat or diagnose an inflammatory, immune, and/or malignant disease or condition. Examples of diseases and conditions may include autoimmune disease (*e.g.*, lupus), inflammatory bowel disease (IBD), chronic obstructive pulmonary disease (COPD), arthritis (*e.g.*, rheumatoid arthritis), multiple sclerosis, transplant rejection, central nervous system injury, Crohn's disease, psoriasis, type 1 diabetes and leukemia or lymphoma (*e.g.*, chronic lymphocytic leukemia (CLL)).

[0029] Also disclosed are polynucleotides that encode the aforementioned polypeptides. The polynucleotides may be operably linked to a promoter for expressing the encoded polypeptides in a suitable host cell. As such, methods of producing the polypeptide encoded by the recombinant polynucleotide may include: a) culturing a cell transformed with the recombinant polynucleotide to express the encoded polypeptide; and b) recovering the polypeptide so expressed.

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] **Figure 1** illustrates inhibition of 125I-IP-10 binding to Th1 cells by murine anti-CXCR3 mAb. Ab#1 – 5D4A; Ab#2 – 8A5A; Ab#3 – 19G2; Ab#4 – V36E5A; Ab#5 – V44D7A;

[0031] Ab#6 – 37B5A; Ab#7 – 21A4A; Ab#8 – V15F4A; Ab#9 – V3G6A; Ab#10 – 23E12A; Ab#11 – 35C4; Ab#12 – 39E10.

[0032] **Figure 2** illustrates inhibition of IP-10-induced Th1 cell migration by murine anti-CXCR3 mAb. Ab#1 – 5D4A; Ab#2 – 8A5A; Ab#3 – 19G2; Ab#4 – V36E5A; Ab#5 – V44D7A; Ab#6 – 37B5A; Ab#7 – 21A4A; Ab#8 – V15F4A; Ab#9 – V3G6A; Ab#10 – 23E12A.

[0033] **Figure 3** illustrates a FACs analysis of murine anti-CXCR3 mAb binding to Th1 cells (top panel), CXCR3+/NSO cells (middle panel), and CXCR-/NSO cells (bottom panel).

[0034] **Figure 4** illustrates inhibition of chemokine binding to CXCR3 by murine anti-CXCR3 mAb and humanized anti-CXCR3 mAb.

[0035] **Figure 5** illustrates inhibition of chemokine mediated chemotaxis by murine anti-CXCR3 mAb and humanized anti-CXCR3 mAb.

[0036] **Figure 6** illustrates an alignment of the VH Domains of 5 anti-CXCR3 Clones.

[0037] **Figure 7** illustrates an alignment of the VK Domains of 5 anti-CXCR3 Clones.

[0038] **Figure 8** illustrates an alignment of the VH Domain of anti-CXCR3 Clone V44D7 with the closest expressed human IgG and germline VH.

[0039] **Figure 9** illustrates the risk assessment of amino acid changes required for complete humanization of the VH domain of anti-CXCR3 clone V44D7. The required amino acid changes are indicated below the main sequence and were derived

from an alignment to human VH3-23. The germline gene and an expressed antibody are described in GenBank accession no. AAD53829.

[0040] **Figure 10** illustrates the risk assessment of amino acid changes required for complete humanization of the VH domain of anti-CXCR3 clone V44D7. The required amino acid changes are indicated below the main sequence and were derived from an alignment to human VH3-23. The germline gene and an expressed antibody are described in GenBank accession no. AAD53829.

[0041] **Figure 11** illustrates an alignment of the VK domain of anti-CXCR3 clone V3G6 with the closest expressed human IgG and germiline VK.

[0042] **Figure 12** illustrates the risk assessment of amino acid changes required for complete humanization of the VK domain of anti-CXCR3 clone V3G6.

[0043] **Figure 13** illustrates inhibition of mouse CXCR3mAb binding to $CXCR3^+$ NSO cells by commercial CXCR3mAbs. Approximately 0.5nM Eu-CXCR3mAb was incubated with CXCR3 transfected NSO cells in the presence of various concentrations of unlabeled commercial CXCRmAbs. A dose-dependent inhibition of Eu-CXCR3mAb binding to $CXCR3^+$ NSO cells was observed.

[0044] **Figure 14** illustrates expression of CXCR3 on Th1 cells. Th1 and Th2 cells were generated from cord blood and CXCR3 and CCR4 expression were determined by FACS. CXCR3 was present only Th1 cells.

[0045] **Figure 15** illustrates an ^{125}I-CXCL10 binding assay. Th1 cells were incubated in a 96 well plate with ^{125}I-CXCL10 in the absence or presence of various concentrations CXCRmAbs. Cell bound ^{125}I-CXCL10 was separated from free radioactivity by an oil column and counted using a gamma counter. IC_{50} values were calculated using Prizm software. Lead candidates were highlighted in green.

[0046] **Figure 16** illustrates an ^{125}I-CXCL11 binding assay. Th1 cells were incubated in a 96 well plate with ^{125}I-CXCL11 in the absence or presence of various concentrations CXCRmAbs. Cell bound ^{125}I-CXCL11 was separated from free

radioactivity by an oil column and counted using a gamma counter. IC_{50} values were calculated using Prizm software. Lead candidates were highlighted in green.

[0047] **Figure 17** illustrates Eu-CXCR3mAb binding to Th1 cells. Th1 cells were incubated with increasing concentrations of Eu-CXCR3mAb in the absence or presence 10-fold excess of unlabeled CXCR3mAb. After incubation (1 hr at RT), cell bound Eu-CXCR3mAb was separated from free Europium by washing three times and the plate was read using Vctor2 fluorometer.

[0048] **Figure 18** illustrates inhibition of ^{125}I-CXCL11 binding to Th1 by CXCR3mAb hybridoma supernatants. Th1 cells were incubated in a 96 well plate with ^{125}I-CXCL11 in the absence or presence of various CXCRmAb hybridoma supernatants for 1 hr at RT. Cell bound ^{125}I-ligands were separated from free radioactivity by an oil column and counted using a gamma counter. Seven hybridoma supernatants that inhibited CXCL11 binding to Th1 cells were selected for further development.

[0049] **Figure 19** illustrates inhibition of ^{125}I-CXCL10 binding to Th1 by CXCR3mAb hybridoma supernatants. Th1 cells were incubated in a 96 well plate with ^{125}I-CXCL10 in the absence or presence of various CXCRmAb hybridoma supernatants for 1 hr at RT. Cell bound ^{125}I-ligands were separated from free radioactivity by an oil column and counted using a gamma counter. Seven hybridoma supernatants that inhibited CXCL10 binding to Th1 cells were selected for further development.

[0050] **Figure 20** illustrates that Mouse CXCR3mAb does not cross react with rat Th1 cells. FACS analysis was performed to determine reactivity of mouse CXCR3mAb to polarized rat Th1 cells. Only rabbit anti-mouse CXCR3 Ab bound to rat Th1 cells. Mouse anti-human CXCR3Ab did not bind to rat Th1 cells. As a control, mouse anti-CXCR3mAb binding to human Th1 cells is also shown (bottom panel).

[0051] **Figure 21** illustrates inhibition Eu-CXCR3mAb by humanized CXCR3Abs. Th1 cells were incubated in a 96 well plate with Eu-CXCR3mAb in the absence or presence of various concentrations humanized CXCRmAbs. After incubation (1 hr at RT), cell bound Eu-CXCR3mAb was separated from free Europium by washing three times and the plate was read using Vctor2 fluorometer. IC_{50} values were calculated using Prizm software. Ab1 has a heavy chain sequence of humanized anti-CXCR3 V44D7 VH Lead #5 amino acid and a light chain sequence of humanized anti-CXCR3 V44D7 VK Lead #1 (see Informal Sequence Listing) in an IgG1 backbone. Ab2 has a heavy chain sequence of humanized anti-CXCR3 V44D7 VH Lead #5 and a light chain sequence of humanized anti-CXCR3 V44D7 VK Lead #7 (see Informal Sequence Listing) in an IgG1 backbone. Humanized Ab (IgG4) has a heavy chain sequence of humanized anti-CXCR3 V44D7 VH Lead #5 and a light chain sequence of the original mouse anti-CXCR3 V44D7 VK in an IgG4 backbone.

[0052] **Figure 22** illustrates inhibition Eu-CXC10 by humanized CXCR3Abs. Th1 cells were incubated in a 96 well plate with Eu-CXCL10 in the absence or presence of various concentrations humanized CXCRmAbs. After incubation (1 hr at RT), cell bound Eu-CXCL10 was separated from free Europium by washing three times and the plate was read using Vctor2 fluorometer. IC_{50} values were calculated using Prizm software.

[0053] **Figure 23** illustrates inhibition CXCL10-induced Th1 cell chemotaxis by humanized CXCR3Abs. Chemotaxis assay was performed in a ChemoTx 96-well plate (Neuro Probe, Inc). Approximately 29 μL of CXCL10 or buffer control was added to the bottom wells. 25 μL of Th1 cell suspension in the absence or presence of various concentrations of humanized antibodies was added directly on the wells of the filter. After 2 hr incubation at 37 °C, cells migrated to the bottom wells were determined by cell titer glo method (Promega).

[0054] **Figure 24** illustrates an analysis of Ca^{++} flux in Th1 cells. Th1 cells were loaded with Fluo-4,AM (Molecular Probes) and stimulated with various mouse

CXCR3mAb antibodies as indicated. Increase in intracellular Ca^{++} was determined FLIPR.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0055] Definitions

[0056] An antibody, as described herein, refers to a full-length (*i.e.*, naturally occurring or formed by normal immunoglobulin gene fragment recombinatorial processes) immunoglobulin molecule (*e.g.*, an IgG antibody) or an immunologically active (*i.e.*, specifically binding) portion of an immunoglobulin molecule, like an antibody fragment.

[0057] An antibody fragment is a portion of an antibody such as $F(ab')_2$, $F(ab)_2$, Fab', Fab, Fv, scFv and the like. Regardless of structure, an antibody fragment binds with the same antigen that is recognized by the intact antibody. The term "antibody fragment" also includes any synthetic or genetically engineered protein that acts like an antibody by binding to a specific antigen to form a complex. For example, antibody fragments include isolated fragments consisting of the variable regions, such as the "Fv" fragments consisting of the variable regions of the heavy and light chains, recombinant single chain polypeptide molecules in which light and heavy 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.

[0058] A humanized antibody is a recombinant protein in which the CDRs from an antibody from one species, *e.g.*, a rodent antibody, are transferred from the heavy and light variable chains of the rodent antibody into human heavy and light variable domains or heavy and light variable domains that have been mutagenized to include at least a portion of the amino acid sequence of the human heavy and light variable domains (as represented by "percent humanization"). The constant domains of the antibody molecule may be derived from those of a human antibody.

[0059] As used herein, "percent humanization" is calculated by determining the number of framework amino acid differences (*i.e.*, non-CDR difference) between the humanized domain and the germline domain, subtracting that number from the total number of amino acids, and then dividing that by the total number of amino acids and multiplying by 100.

[0060] As used herein, "CDR" means a "complementarity determining region" that is present in a variable domain of an antibody heavy chain (VH) or a variable domain of an antibody light chain (VL or VK). Each variable domain includes three CDRs which are designated CDR-H1, CDR-H2, and CDR-H3, for those present in the heavy chain variable domain, and CDR-L1, CDR-L2, and CDR-L3 for those present in the light chain variable domain. The Kabat numbering system is used herein. As such, CDR-H1 begins at approximately amino acid 31 (*i.e.*, approximately 9 residues after the first cysteine residue), includes approximately 5-7 amino acids, and ends at the next tyrosine residue. CDR-H2 begins at the fifteenth residue after the end of CDR-H1, includes approximately 16-19 amino acids, and ends at the next arginine or lysine residue. CDR-H3 begins at approximately the thirty third amino acid residue after the end of CDR-H2; includes 3-25 amino acids; and ends at the sequence W-G-X-G, where X is any amino acid. CDR-L1 begins at approximately residue 24 (*i.e.*, following a cysteine residue); includes approximately 10-17 residues; and ends at the next tyrosine residue. CDR-L2 begins at approximately the sixteenth residue after the end of CDR-L1 and includes approximately 7 residues. CDR-L3 begins at approximately the thirty third residue after the end of CDR-L2; includes approximately 7-11 residues and ends at the sequence F-G-X-G, where X is any amino acid.

[0061] The antigen-binding polypeptides disclosed herein may be conjugated or fused to a therapeutic agent, which may include radioactive labels, an immunomodulator, a hormone, a photoactive therapeutic agent, a cytotoxic agent, which may be a drug or a toxin, and a combination thereof. Drugs may include those drugs that possess the pharmaceutical property selected from the group consisting of antimitotic, antikinase, alkylating, antimetabolite, antibiotic, alkaloid, antiangiogenic,

apoptotic agents and combinations thereof. More specifically, these drugs are selected from the group consisting of nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas, triazenes, folic acid analogs, COX-2 inhibitors, pyrimidine analogs, purine analogs, antibiotics, enzymes, epipodophyllotoxins, platinum coordination complexes, vinca alkaloids, substituted ureas, methyl hydrazine derivatives, adrenocortical suppressants, antagonists, endostatin, taxols, camptothecins, anthracyclines, taxanes, and their analogs, and a combination thereof. The toxins encompassed by the present invention may be selected from the group consisting of ricin, abrin, alpha toxin, saporin, ribonuclease (RNase), *e.g.*, onconase, DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtherin toxin, Pseudomonas exotoxin, and Pseudomonas endotoxin.

[0062] Immunomodulators may be selected from the group consisting of a cytokine, a stem cell growth factor, a lymphotoxin, a hematopoietic factor, a colony stimulating factor (CSF), an interferon (IFN), erythropoietin, thrombopoietin and a combination thereof. Specifically useful are lymphotoxins such as tumor necrosis factor (TNF), hematopoietic factors, such as interleukin (IL), colony stimulating factor, such as granulocyte-colony stimulating factor (G-CSF) or granulocyte macrophage-colony stimulating factor (GM-CSF)), interferon, such as interferons-alpha, -beta, or -gamma, and stem cell growth factor, such as designated "S1 factor". More specifically, immunomodulators may include IL-1, IL-2, IL-3, IL-6, IL-10, IL-12, IL-18, IL-21 interferon-gamma, TNF-alpha or a combination thereof.

[0063] The antigen-binding polypeptides disclosed herein may be conjugated or fused to a diagnostic agent. Diagnostic agents may include photoactive diagnostic agents or radiolabels having an energy between 60 and 4,000 keV, or a non-radioactive label. The radioactive label is preferably a gamma-, beta-, and positron-emitting isotope and is selected from the group consisting of ^{125}I, ^{131}I, ^{123}I, ^{124}I, ^{86}Y, ^{186}Re, ^{188}Re, ^{62}Cu, ^{64}Cu, ^{111}In, ^{67}Ga, ^{68}Ga, ^{99m}Tc, ^{94m}Tc, ^{18}F, ^{11}C, ^{13}N, ^{15}O, ^{76}Br and combinations thereof. Diagnostic agents may include contrast agents, for example, such as manganese, iron or gadolinium.

EXAMPLES

Isolation of murine IgG_1,k CXCR-3 binding antibody using the hybridoma technology

[0064] BALB/c mice were immunized with CXCR-3 expressing NSO cells. In a typical procedure $5X10^6$ cells in 50ul of RIBI adjuvant (Sigma) were injected into rear footpads (25 ul per pad). Two additional injections in RIBI adjuvant were given at 2 week intervals followed by a final boost in PBS. Three days later mice were sacrificed, their poplietal lymph nodes were harvested and lymphocytes isolated for fusion. Lymphocytes were fused with P3X63Ag8.653 plasmacytoma cells at 5:1 ratio using PEG/DMSO (Sigma) as a fusion agent. After fusion cells were resuspended in selective HAT media and seeded at 10^6 cells per well in 96 well plates. The supernatants from hybridomas that survived HAT selection were screened by ELISA for the presence of mouse IgG. The IgG producing hybridomas were identified and their supernatants were further screened by FACS analysis for antibodies binding to CXCR3 expressing NSO cells ($CXCR3^+$NSO). The hybridomas identified as positives for $CXCR3^+$NSO cell binding were then screened for differential binding to $CXCR3^+$NSO and PC-NSO (vector control) cells in order to identify CXCR3 specific clones. The CXCR3 specific hybridomas were subcloned twice by limiting dilutions. Hybridoma subclones were expanded in serum-free medium, the antibodies were purified on Protein-A column and further characterized in order to pick the lead candidate.

Humanization Strategy

[0065] One goal in humanizing the anti-CXCR3 antibodies was to obtain 60-80% humanized VH and VK domains that retain 90-100% of original binding affinity and specificity. Site-directed mutagenesis of individual high risk positions in VH and VK was used to further humanize the antibodies while maintaining binding affinity and specificity.

[0066] Humanization was performed by CDR grafting and structure based analysis and variable region resurfacing. (*See* Jones *et al.*, NATURE (1986) May 29-Jun. 4;321(6069):522-5; Roguska *et al.*, PROTEIN ENGINEERING, 1996, 9(10):895-904; and Xoma, Humanizing Mouse Antibody Frameworks While Preserving 3–D Structure. PROTEIN ENGINEERING, 1994, Vol.7, pg 805). The primary antibody sequence and 3-D structure data were utilized to identify key framework residues required to maintain the binding affinity and specificity. The 3-D structures of nine (9) different Fab and IgG molecules were analyzed (human and mouse, with or without ligand). After aligning the mouse anti-CXCR3 V44 VH and VK to the nearest human germline genes, the amino acid at every position was evaluated for potential influence on binding and immunogenicity. This information was used to assign a low, moderate, or high risk value for mutation at each position. In general, only the low and moderate risk positions were mutated while avoiding the high risk positions.

[0067] The heavy chain was 98% humanized relative to the mouse heavy chain (excluding CDR's) after this process. An affinity maturation strategy was then performed by incorporating tyrosines pair wise at each position in CDR3, including a Y115D substitution, which gave on average a 2-fold increase in affinity. The heavy chain that was used in the 2 lead candidates included 2 additional mutations at positions 97 and 98 making it 100% human, excluding the CDR's. Following the same strategy for the light chain, the VK was aligned to the A14 germline gene and low and moderate risk positions were mutated. After determining that this germline gene appears to be rarely expressed in normal humans, the process was repeated using the L6 germline as template

[0068] The "Blast for Ig sequences" website sponsored by the NCBI was used to identify the closest match to the mouse VH and VK region used in the study. The V-base website at the MRC was used to confirm the human germline sequences.

[0069] Human germline VH and VK genes were chosen as the best matches to the mouse sequence VH and VK sequences. For the mouse VH sequence, the human germline sequence VH3-23 (as designated in V-base) was identified as the best match: VH3-23 germline (EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEQVSAIS GSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK). For the mouse VK sequence, the human germline sequence A14 and L6 (as designated in V-base) were identified as the best matches: L6 Germline (EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASN RATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWP); and A14 Germline (DVVMTQSPAFLSVTPGEKVTITCQASEGIGNYLYWYQQKPDQAPKLLIKYAS QSISGVPSRFSGSGSGTDFTFTISSLEAEDAATYYCQQGNKHP).

Cloning and Sequencing of Murine anti-CXCR3 VH and VK domains from Hybridoma Cell Lines

[0070] Hybridoma cells were pelleted,washed 3X with PBS and RNA extracted using Trizol reagent (Invitrogen, Cat. No. 15596-026) following the manufacturers protocol. Total RNA was converted to cDNA using a 5' RACE kit (Rapid Amplification of cDNA Ends, Invitrogen, Cat. No. 18374-058)following the manufacturers protocol. Briefly, RNA was ligated to random hexamer primer, Random N6, and 1st strand cDNA generated using superscript II RNAase H negative reverse transcriptase. The cDNA was purified using a GlassMax spin cartridge provided with the kit and then reacted with TdT (terminal deoxynucleotidyl transferase) in the presence of dCTP to append the cDNA with C basepairs at the 5'end. The dC-tailed cDNA was PCR amplified using an anchor primer specific for the dC tail and a gene specific primer that hybridizes to highly conserved DNA sequence in the mouse constant heavy 1 (CH1) for VH and constant kappa (CK) for

VK. The resulting PCR product was analyzed by gel electrophoresis for correct size corresponding to intact VH or VK domain then purified and ligated in to a Topo TA vector (Invitrogen Cat. No. 45-0071) following manufacturers protocol. After transformation in to bacteria DNA was prepared from clones containing correct size insert and the DNA sequence determined using a Big Dye terminator sequencing reaction mix (Applied Biosystems, Part No. 4336699) and a 3700 ABI/Prism DNA analyzer following manufacturers protocol.

Humanizing Murine anti-CXCR3 Antibodies

[0071] First, a single lead murine anti-CXCR3 antibody, V44D7, was identified based on binding data and sequence data generated as described above. The amino acid sequence of the VH and VK domains from this antibody were aligned to all known human germline VH and VK domains using currently available public databases (*i.e.*, Blast for IgG at the NCBI and V-base at the MRC). By focusing on alignment within the framework regions a highly homologous human germline VH domain, VH3-23, and 2 different human germline VK domains, A14 and L6, were identified. At those positions in the framework where the mouse sequence differed from the human germline, an iterative process was used to convert or mutate the mouse framework so it matched the corresponding human germline framework. In addition, certain residues in CDR3 of both the VH and VK were mutated by replacement with tyrosine (*i.e.*, affinity matured) to potentially help compensate for any losses in affinity due to the framework residues changes. The affinity matured and humanized mouse VH and VK domains were generated by a polymerase chain reaction process using a panel of overlapping synthetic DNA oligonucleotides. As part of the synthetic gene design process a codon optimization strategy was used, that is to say the triplet code for each amino acid that is preferentially utilized by mammalian cells for gene expression was incorporated at each position. The synthetic VH and VK domains were cloned in to specialized mammalian expression vectors that allowed the corresponding domains to be expressed in the context of a fully human IgG1, G4 or Kappa antibody backbone. Small-scale production of the humanized antibodies was achieved by co-tranfection of an IgG1 or G4 construct with

the Kappa construct in to 293F cells with lipofectamine (Invitrogen) following manufactures protocol. Supernatants from the transient transfections were passed through Protein A or G resin and the IgG purified to homogeneity for testing in cell based assays.

Epitope competition studies

[0072] Various commercial CXR3mAbs were tested in a competitive binding assay using Europium (Eu) labeled -mouse CXCR3mAb. CXCR3mAbs from various commercial sources inhibited Eu-CXCR3mAb binding to CXCR3. This data indicated that mouse CXCR3mAb and commercial antibodies bind to overlapping epitopes on CXCR3 (Figure 13).

Antibody Affinities

[0073] Binding affinity and activity of mouse and humanized CXCR3mAbs were determined by various competitive binding assays using ^{125}I- and Eu-labeled chemokines and Eu-labeled CXCR3mAb and Th1 chemotaxis assays including: ^{125}I-CXCL10 binding assay; ^{125}I-CXCL11 binding assay; Eu-CXCL10 binding assay; Th1 chemotaxis assay; and Eu-mouse CXCR3mAb binding assay.

Th1 cells

[0074] Primary Th1 cells generated from cord blood were used for all binding assays. As described in the literature, CXCR3 expression was observed only on Th1 cells but not on Th2 cells as determined by FACS analysis (Figure 14). Th2 cells specifically expressed CCR4.

^{125}I-CXCL10 and ^{125}I-CXCL11 Binding Assays

[0075] The binding affinity of mouse CXCR3mAb antibodies was determined based on their ability to inhibit radiolabeled CXCL10 and CXCL11 binding to Th1 cells (Figures 15, 16, 18, and 19 and Table 1). Based on these binding studies and the chemotaxis assay, three mouse CXCRmAbs were selected for further study.

Table 1. Characterization of Anti-CXCR3 mAbs

	Binding to Th1 cells, FACS	Inhibition of IP-10-induced Th1 chemo taxis, IC_{50} (ng/mL)	Displacement of ^{125}I-IP-10 binding to Th1 cells (IC_{50}, nM)	Displacement of ^{125}I-I-Tac binding to Th1 cells by (IC_{50}, nM)
IgG2 a	4.02	N/A	N/A	N/A
Ab#1	1498	123	0.38	0.44
Ab#2	1215	575	0.41	0.47
Ab#3	681	N/A	2.6	5.7
Ab#4	1262	49	0.12	0.13
Ab#5	1119	35	0.13	0.10
Ab#6	831	172	0.94	0.76
Ab#7	4.20	N/A	N/A	-
Ab#8	1096	264	0.68	0.57
Ab#9	1348	39	0.20	0.14
Ab#10	66.84	N/A	N/A	-
Ab#11	4.80	N/A		-
Ab#12	5.77	N/A	N/A	-
R&D mAb	N/D	351	0.61	0.6

Ab#1 – 5D4A; Ab#2 – 8A5A; Ab#3 – 19G2; Ab#4 – V36E5A; Ab#5 – V44D7A;

[0076] Ab#6 – 37B5A; Ab#7 – 21A4A; Ab#8 – V15F4A; Ab#9 – V3G6A; Ab#10 – 23E12A; Ab#11 – 35C4; Ab#12 – 39E10.

Eu-CXCRmAb saturation binding assay

[0077] Binding affinity of mouse CXCR3 antibodies to CXCR3 was determined by direct saturation binding assay using Europium labeled mouse CXCR3 antibodies. An example of this assay using one mouse CXCR3mAb is shown in Figure 17. Data from this study indicated that Eu-CXCR3mAb binding to CXCR3 was specific and saturable with binding affinity of subnanomolar Kd (0.47 nM).

Antibody Specificity

[0078] Mouse antibody hybridomas (20000) were screened by a differential screening assay with $CXCR3^+$ and CXCR3-NSO membranes using a Eu-secondary antibody (DELFIA). Antibodies (~2000) that bound to $CXCR3^+$ membranes were further tested by FACS using $CXCR3^+$/$CXCR3^-$ NSO and Th1 cells. An example for specific binding of CXCR3mAb to CXCR3 expressing cells is shown in Figure 3.

Species Cross Reactivity

[0079] The parental mouse CXCR3mAb was tested for binding to polarized rat Th1 cells by FACS. Only rabbit anti-mouse CXCR3 Ab (positive control) bound to rat Th1 cells. The mouse parental CXCR3mAb did not bind to rat Th1 cells as shown in Figure 20.

Biological Activity

[0080] Binding assay: CXCR3mAb specifically inhibited 125I-labeled IP-10 and –I-Tac binding to primary Th1 cells. Due to unavailability of labeled Mig, it was not tested in binding assays. Chemotaxis assay: CXCR3mAb inhibited Th1 cell migration mediated by CXCR3 chemokines.

[0081] Humanized antibodies were tested by binding and chemotaxis assays. The results of an Eu-CXCRmAb competitive binding assay are shown in Figure 21. The results of the Eu-CXCL10 binding assay are shown in Figure 22. The results of the Th1 chemotaxis assay are shown in Figure 23.

Mouse antibody tested for agonism of the CXCR3 receptor

[0082] Mouse CXCR3mAbs were tested for agonistic activity in inducing ca mobilization in Th1 cells. As shown in Figure 24, none of the antibodies s showed any agonistic activity in inducing Ca^{++} flux.

Table 2. IC_{50} Values of Different Humanized Antibodies As Determined From Binding and Chemotaxis Assays

Antibody ID	Binding Assay, IC_{50} (nM)		Chemotaxis IC_{50} (nM)	Percent Humanization	
	Eu-IP-10	Eu-V44		H	L
Mouse anti-CXCR3 V44D7	0.14	0.29	0.09	88	79
Humanized Ab1 H5K1	0.8	2.6	0.31	100	96
Humanized Ab2 H5K7	0.35	1.82	0.28	100	94
Humanized Ab3 H5K2	1.25	1.86	N.D.	100	100
Humanized Ab4 H5K3	0.38	0.56	N.D.	100	100
Humanized Ab5 H5K4	0.69	1.49	N.D.	100	100
Humanized Ab6 H5K5	0.42	0.65	N.D.	100	100

N.D. = not determined

[0083] In Table 2, Ab1 has a heavy chain sequence of humanized anti-CXCR3 V44D7 VH Lead #5 amino acid and a light chain sequence of humanized anti-CXCR3 V44D7 VK Lead #1 (see Informal Sequence Listing) in an IgG1 backbone. Ab2 has a heavy chain sequence of humanized anti-CXCR3 V44D7 VH Lead #5 and

a light chain sequence of humanized anti-CXCR3 V44D7 VK Lead #7 (see Informal Sequence Listing) in an IgG1 backbone. Ab3 has a heavy chain sequence of humanized anti-CXCR3 V44D7 VH Lead #5 amino acid and a light chain sequence of humanized anti-CXCR3 V44D7 VK Lead #2 (see Informal Sequence Listing) in an IgG1 backbone. Ab4 has a heavy chain sequence of humanized anti-CXCR3 V44D7 VH Lead #5 and a light chain sequence of humanized anti-CXCR3 V44D7 VK Lead #3 (see Informal Sequence Listing) in an IgG1 backbone. Ab5 has a heavy chain sequence of humanized anti-CXCR3 V44D7 VH Lead #5 amino acid and a light chain sequence of humanized anti-CXCR3 V44D7 VK Lead #4 (see Informal Sequence Listing) in an IgG1 backbone. Ab6 has a heavy chain sequence of humanized anti-CXCR3 V44D7 VH Lead #5 and a light chain sequence of humanized anti-CXCR3 V44D7 VK Lead #5 (see Informal Sequence Listing) in an IgG1 backbone.

WHAT IS CLAIMED IS:

1. An antigen-binding polypeptide that binds specifically to CXCR3, comprising:

(a) a humanized antibody heavy chain variable region comprising:

(1) a CDR-H1 comprising an amino acid sequence of (NYMAS);

(2) a CDR-H2 comprising an amino acid sequence of (TISSGGGYTYYPDSLKG); and

(3) a CDR-H3 comprising an amino acid sequence of (HGAPMTTVITYAPYYF{D,Y}Y); and

(b) a humanized antibody light chain variable region comprising:

(1) a CDR-L1 comprising an amino acid sequence of (RASSSVKYMY);

(2) a CDR-L2 comprising an amino acid sequence of (YTSNLAP); and

(3) a CDR-L3 comprising an amino acid sequence of (QQFTTSPYT).

2. The polypeptide of claim 1, wherein CDR-H3 comprises an amino acid sequence of (HGAPMTTVITYAPYYFYY).

3. The polypeptide of claim 1 or 2, wherein CDR-H3 comprises an amino acid sequence of (HGAPMTTVITYAPYYFDY).

4. The polypeptide of any of claims 1-3, wherein:

(1) the CDR-H1 consists of the amino acid sequence of (NYMAS);

(2) the CDR-H2 consists of the amino acid sequence of (TISSGGGYTYYPDSLKG);

(3) the CDR-H3 consists of the amino acid sequence of (HGAPMTTVITYAPYYF{D,Y}Y);

(4) the CDR-L1 consists of the amino acid sequence of (RASSSVKYMY);

(5) the CDR-L2 consists of the amino acid sequence of (YTSNLAP); and

(6) the CDR-L3 consists of the amino acid sequence of (QQFTTSPYT).

5. The polypeptide of any of claims 1-4, wherein the CDR-H3 consists of the amino acid sequence of (HGAPMTTVITYAPYYFYY).

6. The polypeptide of any of claims 1-5, wherein the humanized antibody heavy chain variable region comprises an amino acid sequence of (EV{M,Q}L{V,L}ESGGGLV{K,Q}PGGSL{K,R}LSCAASGFTFSNYAMSWVRQ{T,A}P{E,G}K{R,G}LEWV{A,S}TISSGGGYTYYPDSLKGRFTISRDN{A,S}KNTL{F,Y}LQM{S,N}SLR{S,A}EDTAVYYC{V,A}{R,K}HGAPMTTVITYAPYYF{D,Y}YWGQGTT{L,V}TVSS).

7. The polypeptide of any of claim 6, wherein the humanized antibody heavy chain variable region comprises an amino acid sequence of (EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPKGLEWVSTISSGGGYTYYPDSLKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKHGAPMTTVITYAPYYFYYWGQGTTVTVSS).

8. The polypeptide of any of claims 1-7, wherein the humanized antibody light chain variable region comprises an amino acid sequence of ({E,D}{I,N,V}V{L,M}TQSPA{T,F,I}{L,M}S{L,A,V}{S,T}{L,P}GE{R,K}{A,V}T{L,M,I}{S,T,N}CRASSSVKYMYWYQQK{S,P}{G,D}{Q,A}{A,S}P{R,K}L{L,W}I{Y,K}YTSNLAPG{I,V}P{A,S}RFSGSGSG{T,N}{D,S}{F,Y}{T,S}{L,F}TISS{M,L}E{A,G,P}ED{F,A}A{V,T}YYC{Q,Y}QFTT{S,Y}PYTFGGGTKLEIKR).

9. The polypeptide of claim 8 wherein the humanized antibody light chain variable region comprises an amino acid sequence of (EIVLTQSPATLSLSLGERATLSCRASSSVKYMYWYQQKSGQAPRLLIYYTSNLAPGIPARFSGSGSGTDFTLTISSMEAEDFAVYYCQQFTTSPYTFGGGTKLEIKR).

10. The polypeptide of claim 8 wherein the humanized antibody light chain variable region comprises an amino acid sequence of (ENVLTQSPAFLSVTPGEKVTITCRASSSVKYMYWYQQKPDQAPKLWIYYTSNLAPGVPSRFSGSGSGNDYTFTISSLEAEDAATYYCQQFTTSPYTFGGGTKLEIKR).

11. The polypeptide of any of claims 1-10, wherein the polypeptide is selected from the group consisting of an antibody molecule, a Fab fragment, a Fab' fragment, a $F(ab')_2$ fragment, and an scFv molecule.

12. The polypeptide of claim 11, wherein the molecule is an antibody molecule.

13. The polypeptide of claim 12, wherein the antibody is a chimeric antibody comprising a human heavy chain constant region and a human light chain constant region

14. The polypeptide of claim 12 or 13, wherein the antibody is an IgG molecule (*e.g.*, a IgG1 or an IgG4 molecule).

15. The polypeptide of claim 1, wherein the polypeptide is an scFv molecule.

16. The polypeptide of claim 15, wherein the scFv has a formula selected from the group consisting of NH_2-L-VH-X-VK-COOH and NH_2-L-VK-X-VH-COOH; wherein L is a leader sequence; VH is the humanized antibody heavy chain variable region; X is a linking polypeptide; and VK is the humanized antibody light chain variable region.

17. The polypeptide of any of claims 1-16, conjugated to a therapeutic or diagnostic agent.

18. The polypeptide of claim 17, wherein the therapeutic agent is selected from the group consisting of a cytotoxic agent, a radioactive label, an immunomodulator, a hormone, an enzyme, an oligonucleotide, a photoactive therapeutic agent or a combination thereof.

19. The polypeptide of claim 18, wherein the diagnostic agent is selected from the group consisting of a radioactive label, a photoactive diagnostic agent, an ultrasound-enhancing agent or a non-radioactive label.

20. The polypeptide of any of claims 1-19, wherein the polypeptide is an antagonist of CXCR3.

21. The polypeptide of any of claims 1-20, wherein the polypeptide is not an agonist of CXCR3.

22. The polypeptide of any of claims 1-21, wherein the polypeptide binds to CXCR3 with an affinity constant of at least about 10^6M^{-1} (preferably at least about 10^7M^{-1}, more preferably at least about 10^8M^{-1}, even more preferably at least about 10^9M^{-1}).

23. A pharmaceutical composition comprising any of the polypeptides of claims 1-22 and a carrier.

24. The pharmaceutical composition of claim 23, further comprising an additional therapeutic or diagnostic agent.

25. A method of treating or diagnosing an inflammatory disease or condition comprising administering the composition of any of claim 23 or 24 to a patient in need thereof.

26. A method of treating or diagnosing an immune disease or condition comprising administering the composition of any of claim 23 or 24 to a patient in need thereof.

27. A method of treating or diagnosing a malignant disease or condition comprising administering the composition of any of claim 23 or 24 to a patient in need thereof.

28. The methods of any of claims 24-27, wherein the disease or condition is selected from the group consisting of autoimmune disease (*e.g.*, lupus), inflammatory bowel disease (IBD), chronic obstructive pulmonary disease (COPD), arthritis (*e.g.*, rheumatoid arthritis), multiple sclerosis, transplant rejection, central nervous system injury, Crohn's disease, psoriasis, and leukemia or lymphoma (*e.g.*, chronic lymphocytic leukemia (CLL)).

29. A polynucleotide encoding any of the polypeptides of claims 1-22.

30. A polynucleotide encoding a polypeptide comprising a humanized antibody heavy chain variable region comprising: (1) a CDR-H1 comprising an amino acid sequence of (NYMAS); (2) a CDR-H2 comprising an amino acid sequence of (TISSGGGYTYYPDSLKG); and (3) a CDR-H3 comprising an amino acid sequence of (HGAPMTTVITYAPYYF{D,Y}Y).

31. The polynucleotide of claim 30, wherein the humanized antibody heavy chain variable region comprises an amino acid sequence of (EV{M,Q}L{V,L}ESGGGLV{K,Q}PGGSL{K,R}LSCAASGFTFSNYAMSWVRQ {T,A}P{E,G}K{R,G}LEWV{A,S}TISSGGGYTYYPDSLKGRFTISRDN{A,S}KNT L{F,Y}LQM{S,N}SLR{S,A}EDTAVYYC{V,A}{R,K}HGAPMTTVITYAPYYF{D ,Y}YWGQGTT{L,V}TVSS).

32. The polynucleotide of claim 31, wherein the humanized antibody heavy chain variable region comprises an amino acid sequence of (EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPKGLEWVSTISS GGGYTYYPDSLKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKHGAPMT TVITYAPYYFYYWGQGTTVTVSS).

33. A polynucleotide encoding a polypeptide comprising a humanized antibody light chain variable region comprising: (1) a CDR-L1 comprising an amino acid sequence of (RASSSVKYMY); (2) a CDR-L2 comprising an amino acid sequence of (YTSNLAP); and (3) a CDR-L3 comprising an amino acid sequence of (QQFTTSPYT).

34. The polynucleotide of claim 33, wherein the humanized antibody light chain variable region comprises an amino acid sequence of ({E,D}{I,N,V}V{L,M}TQSPA{T,F,I}{L,M}S{L,A,V}{S,T}{L,P}GE{R,K}{A,V}T {L,M,I}{S,T,N}CRASSSVKYMYWYQQK{S,P}{G,D}{Q,A}{A,S}P{R,K}L{L,W }I{Y,K}YTSNLAPG{I,V}P{A,S}RFSGSGSG{T,N}{D,S}{F,Y}{T,S}{L,F}TISS{ M,L}E{A,G,P}ED{F,A}A{V,T}YYC{Q,Y}QFTT{S,Y}PYTFGGGTKLEIKR).

35. The polynucleotide of claim 34, wherein the humanized antibody light chain variable region comprises an amino acid sequence of (EINVLTQSPATLSLSLGERATLSCRASSSVKYMYWYQQKSGQAPRLLIYYTS NLAPGIPARFSGSGSGTDFTLTISSMEAEDFAVYYCQQFTTSPYTFGGGTKLEI KR).

36. The polynucleotide of claim 34, wherein the humanized antibody light chain variable region comprises an amino acid sequence of (ENVLTQSPAFLSVTPGEKVTITCRASSSVKYMYWYQQKPDQAPKLWIYYTS NLAPGVPSRFSGSGSGNDYTFTISSLEAEDAATYYCQQFTTSPYTFGGGTKLEI KR).

37. A recombinant polynucleotide comprising a promoter sequence operably linked to any of the polynucleotides of claims 29-36.

38. An isolated cell transformed with the polynucleotide of claim 37.

39. A method of producing the polypeptide encoded by the recombinant polynucleotide of claim 37, the method comprising:

a) culturing a cell transformed with the recombinant polynucleotide to express the encoded polypeptide; and

b) recovering the polypeptide so expressed.

40. A humanized antibody heavy chain variable region comprising:

(1) a CDR-H1 comprising an amino acid sequence of ({N,S,Y}MAS);

(2) a CDR-H2 comprising an amino acid sequence of ({T,A,Y}I{S,Y}{S,G,T,Y}{G,S}{G,Y}G{F,S,Y}TYY{P ,A}DS{L,Y,V}KG and

(3) a CDR-H3 comprising an amino acid sequence of {H,Y}{G,Y}{A,Y}PM{T,Y}T{V,Y}ITY{A,Y}PYYFY Y.

41. The humanized antibody heavy chain variable region of claim 40, comprising an amino acid sequence of (EVQLLESGGGLVQPGGSLRLSCAASGFTFS{N,S,Y}YAMSWVRQAPGKGLE WVS{T,A,Y}I{S,Y}{S,G,T,Y}{G,S}{G,Y}G{F,S,Y}TYY{P,A}DS{L,Y,V}KGRF TISRDNSKNTLYLQMNSLRAEDTAVYYCAK{H,Y}{G,Y}{A,Y}PM{T,Y}T{V, Y}ITY{A,Y}PYYFYYWGQGTTVTVSS).

42. An humanized antibody heavy chain variable region comprising:

(1) a CDR-H1 comprising an amino acid sequence of (NYAIS);

(2) a CDR-H2 comprising an amino acid sequence of (TYSSGGVYTYYRDSLKG); and

(3) a CDR-H3 comprising an amino acid sequence of (HGAAMTTVITYAPFYFYY).

43. The humanized antibody heavy chain variable region of claim 42, comprising an amino acid sequence of (EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYAISWVRQAPGKGLEWVSTYSSGGVYTYYRDSLKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKHGAAMTTVITYAPFYFYYWGQGTTVTVSS).

44. An humanized antibody heavy chain variable region comprising:

(1) a CDR-H1 comprising an amino acid sequence of (YYAMS);

(2) a CDR-H2 comprising an amino acid sequence of (TIYSGGSYTFYPDSLEG); and

(3) a CDR-H3 comprising an amino acid sequence of (HGAPMSTEITYAPYYFYY).

45. The humanized antibody heavy chain variable region of claim 44, comprising an amino acid sequence of (EVQLLESGGGLVQPGGSLRLSCAASGFTFSYYAMSWVRQAPGKGLEWVSTIYSGGSYTFYPDSLEGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKHGAPMSTEITYAPYYFYYWGQGTTVTVSS).

46. A humanized antibody heavy chain variable region comprising:

(1) a CDR-H1 comprising an amino acid sequence of (NYAMS);

(2) a CDR-H2 comprising an amino acid sequence of (TIYSGGGYTFYLDSLKG); and

(3) a CDR-H3 comprising an amino acid sequence of (HSYPMTTVITYAPYYFYY).

47. The humanized antibody heavy chain variable region of claim 46, comprising an amino acid sequence of (EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYYMSWVRQAPGKGLEWVSTIYSGGGYTFYLDSLKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKHSYPMTTVITYAPYYFYYWGQGTTVTVSS).

48. A humanized antibody heavy chain variable region comprising:

(1) a CDR-H1 comprising an amino acid sequence of (NYAMS);

(2) a CDR-H2 comprising an amino acid sequence of (TISSGGGYTYYPDSLKG); and

(3) a CDR-H3 comprising an amino acid sequence of (HGAPMTTVITYAPYYFYY).

49. The humanized antibody heavy chain variable region of claim 48, comprising an amino acid sequence of (EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSTISSGGGYTYYPDSLKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKHGAPMTTVITYAPYYFYYWGQGTTVTVSS).

50. A humanized antibody heavy chain variable region comprising:

(1) a CDR-H1 comprising an amino acid sequence of (NYAMS);

(2) a CDR-H2 comprising an amino acid sequence of (TISSGGGYTYYPDSLKG); and

(3) a CDR-H3 comprising an amino acid sequence of (HGAPMTTVITYAPYYFYY).

51. The humanized antibody heavy chain variable region of claim 50, comprising an amino acid sequence

(EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSTI SSGGGYTYYPDSLKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKHGAP MTTVITYAPYYFYYWGQGTTVTVSS).

52. A humanized antibody light chain variable region comprising:

(1) a CDR-L1 comprising an amino acid sequence of (RASSSVKYMY);

(2) a CDR-L2 comprising an amino acid sequence of (YTSNLAP); and

(3) a CDR-L3 comprising an amino acid sequence of (QQFTTSPYT).

53. The humanized antibody light chain variable region of claim 52, comprising an amino acid sequence of (EIVLTQSPATLSLSLGERATLSCRASSSVKYMYWYQQKSGQAPRLLIYYTSNL APGIPARFSGSGSGTDFTLTISSMEAEDFAVYYCQQFTTSPYTFGGGTKLEIKR) .

54. A humanized antibody light chain variable region comprising:

(1) a CDR-L1 comprising an amino acid sequence of (RASSSVKYMY);

(2) a CDR-L2 comprising an amino acid sequence of (YTSNLAP); and

(3) a CDR-L3 comprising an amino acid sequence of (QQFTTSPYT).

55. The humanized antibody light chain variable region of claim 54, comprising an amino acid sequence of (EIVLTQSPATLSLSPGERATLSCRASSSVKYMYWYQQKPGQAPRLLIYYTSNL APGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQFTTSPYTFGGGTKLEIKR).

56. A humanized antibody light chain variable region comprising:

(1) a CDR-L1 comprising an amino acid sequence of (RASSSVKYMY);

(2) a CDR-L2 comprising an amino acid sequence of (YTSNLAP); and

(3) a CDR-L3 comprising an amino acid sequence of (YQFTTSPYT).

57. The humanized antibody light chain variable region of claim 56, comprising an amino acid sequence of (EIVLTQSPATLSLSPGERATLSCRASSSVKYMYWYQQKPGQAPRLLIYYTSNLAPGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCYQFTTSPYTFGGGTKLEIKR).

58. A humanized antibody light chain variable region, comprising:

(1) a CDR-L1 comprising an amino acid sequence of (RASSSVKYMY);

(2) a CDR-L2 comprising an amino acid sequence of (YTSNLAP); and

(3) a CDR-L3 comprising an amino acid sequence of (QQYTTSPYT).

59. The humanized antibody light chain variable region of claim 58, comprising an amino acid sequence of (EIVLTQSPATLSLSPGERATLSCRASSSVKYMYWYQQKPGQAPRLLIYYTSNLAPGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQYTTSPYTFGGGTKLEIKR).

60. A humanized antibody light chain variable region comprising:

(1) a CDR-L1 comprising an amino acid sequence of (RASSSVKYMY);

(2) a CDR-L2 comprising an amino acid sequence of (YTSNLAP); and

(3) a CDR-L3 comprising an amino acid sequence of (QQFTTYPYT).

61. The humanized antibody light chain variable region of claim 60, comprising an amino acid sequence of (EIVLTQSPATLSLSPGERATLSCRASSSVKYMYWYQQKPGQAPRLLIYYTSNLAPGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQFTTYPYTFGGGTKLEIKR).

62. A humanized antibody light chain variable region comprising:

(1) a CDR-L1 comprising an amino acid sequence of (RAS{S,Q}SV{K,S}SY{M,L}{Y,A});

(2) a CDR-L2 comprising an amino acid sequence of ({Y,D}{T,A}SN{L,R}A{P,T}); and

(3) a CDR-L3 comprising an amino acid sequence of (Q,Y}Q{F,Y}TT{S,Y}PYT).

63. The humanized antibody light chain variable region of claim 62, comprising an amino acid sequence of (EIVLTQSPATLSLSPGERATLSCRAS{S,Q}SV{K,S}SY{M,L}{Y,A}WYQQKPGQAPRLLIY{Y,D}{T,A}SN{L,R}A{P,T}GIPARFSGSGSGTDFTLTISSLEPEDFAVYYC{Q,Y}Q{F,Y}TT{S,Y}PYTFGGGTKLEIKR).

64. A humanized antibody light chain variable region comprising:

(1) a CDR-L1 comprising an amino acid sequence of (RASSSVKYMY);

(2) a CDR-L2 comprising an amino acid sequence of (YTSNLAP); and

(3) a CDR-L3 comprising an amino acid sequence of (QQFTTSPYT).

65. The humanized antibody light chain variable region of claim 64, comprising an amino acid sequence of (ENVLTQSPAFLSVTPGEKVTITCRASSSVKYMYWYQQKPDQAPKLWIYYTSNLAPGVPSRFSGSGSGNDYTFTISSLEAEDAATYYCQQFTTSPYTFGGGTKLEIKR).

66. A humanized antibody light chain variable region comprising:

(1) a CDR-L1 comprising an amino acid sequence of (RAS{S,Q}SV{K,S}SY{M,L}{Y,A});

(2) a CDR-L2 comprising an amino acid sequence of ({Y,D}{T,A}SN{L,R}A{P,T}); and

(3) a CDR-L3 comprising an amino acid sequence of (Q,Y}Q{F,Y}TT{S,Y}PYT).

67. The humanized antibody light chain variable region of claim 66, comprising an amino acid sequence of ({E,D}{N,V}V{L,M}TQSPAFLSVTPGEKVTITCRAS{S,Q}SV{K,S}SY{M,L}{Y,A} WYQQKPDQAPKL{W,L}I{Y,K}{Y,D}{T,A}SN{L,R}A{P,T}GVPSRFSGSGSG{N,T}D{Y,F}TFTISSLEAEDAATYYC{Q,Y}Q{F,Y}TT{S,Y}PYTFGGGTKLEIKR).

68. An antigen binding polypeptide that binds specifically to CXCR3, comprising a humanized heavy chain of one of claims 40-51 and a humanized light chain of one of claims 52-67.

69. The polypeptide of claim 68, comprising the humanized heavy chain of claim 48 or 49 and the humanized light chain of claim 52 or 53.

70. The polypeptide of claim 68, comprising the humanized heavy chain of claim 48 or 49 and the humanized light chain of claim 64 or 65.

71. The polypeptide of claim 68, comprising the humanized heavy chain of claim 48 or 49 and the humanized light chain of claim 54 or 55.

72. The polypeptide of claim 68, comprising the humanized heavy chain of claim 48 or 49 and the humanized light chain of claim 56 or 57.

73. The polypeptide of claim 68, comprising the humanized heavy chain of claim 48 or 49 and the humanized light chain of claim 58 or 59.

74. The polypeptide of claim 68, comprising the humanized heavy chain of claim 48 or 49 and the humanized light chain of claim 60 or 61.

75. The polypeptide of any of claims 68-74, wherein the polypeptide is selected from the group consisting of an antibody molecule, a Fab fragment, a Fab' fragment, a $F(ab')_2$ fragment, and an scFv molecule.

76. The polypeptide of claim 75, wherein the molecule is an antibody molecule.

77. The polypeptide of claim 76, wherein the antibody is a chimeric antibody comprising a human heavy chain constant region and a human light chain constant region.

78. The polypeptide of claim 76 or 77, wherein the antibody is an IgG molecule (*e.g.*, an IgG1 or an IgG4 molecule).

79. The polypeptide of claim 75, wherein the molecule is an scFv molecule.

80. The polypeptide of claim 79, wherein the scFv has a formula selected from the group consisting of NH_2-L-VH-X-VK-COOH and NH_2-L-VK-X-VH-COOH; wherein L is a leader sequence; VH is the humanized antibody heavy chain variable region; X is a linking polypeptide; and VK is the humanized antibody light chain variable region.

81. The polypeptide of any of claims 68-80, conjugated to a therapeutic or diagnostic agent.

82. The polypeptide of claim 81, wherein the therapeutic agent is selected from the group consisting of a cytotoxic agent, a radioactive label, an immunomodulator, a hormone, an enzyme, an oligonucleotide, a photoactive therapeutic agent or a combination thereof.

83. The polypeptide of claim 81, wherein the diagnostic agent is selected from the group consisting of a radioactive label, a photoactive diagnostic agent, an ultrasound-enhancing agent or a non-radioactive label.

84. The polypeptide of any of claims 68-83, wherein the polypeptide is an antagonist of CXCR3.

85. The polypeptide of any of claims 68-84, wherein the polypeptide is not an agonist of CXCR3.

86. The polypeptide of any of claims 68-85, wherein the polypeptide binds to CXCR3 with an affinity constant of at least about 10^6M^{-1} (preferably at least about 10^7M^{-1}, more preferably at least about 10^8M^{-1}, even more preferably at least about 10^9M^{-1}).

87. A pharmaceutical composition comprising any of the polypeptides of claims 68-86 and a carrier.

88. The pharmaceutical composition of claim 81, further comprising an additional therapeutic or diagnostic agent.

89. A method of treating or diagnosing an inflammatory disease or condition comprising administering the composition of claim 87 or 88 to a patient in need thereof.

90. A method of treating or diagnosing an immune disease or condition comprising administering the composition of claim 87 or 88 to a patient in need thereof.

91. A method of treating or diagnosing a malignant disease or condition comprising administering the composition of claim 87 or 88 to a patient in need thereof.

92. The methods of any of claims 89-91, wherein the disease or condition is selected from the group consisting of autoimmune disease (*e.g.*, lupus),

inflammatory bowel disease (IBD), chronic obstructive pulmonary disease (COPD), arthritis (*e.g.*, rheumatoid arthritis), multiple sclerosis, transplant rejection, central nervous system injury, Crohn's disease, psoriasis, and leukemia or lymphoma (*e.g.*, chronic lymphocytic leukemia (CLL)).

93. A polynucleotide encoding any of the humanized heavy chain variable regions of claims 40-51.

94. A polynucleotide encoding any of the humanized light chain variable regions of claims 52-67.

95. A polynucleotide encoding any of the polypeptides of claims 68-86.

96. A recombinant polynucleotide comprising a promoter sequence operably linked to any of the polynucleotides of claims 93-95.

97. An isolated cell transformed with the polynucleotide of claim 96.

98. A method of producing the polypeptide encoded by the recombinant polynucleotide of claim 96, the method comprising:

a) culturing a cell transformed with the recombinant polynucleotide to express the encoded polypeptide; and

b) recovering the polypeptide so expressed.