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(54) **Title**: ANTIBODIES AND USES THEREOF

(57) **Abstract**: Provided herein are, *inter alia*, antibodies that specifically bind to epitopes within the inflammatory chemokine receptor CXCR3, and methods of using the antibodies. In exemplary embodiments, provided herein are antibodies and antigen-binding fragments thereof that specifically bind to an epitope within amino acid residues 23 to 44 of the human CXCR3 set forth in SEQ ID NO:4, or within corresponding amino acid residues in another CXCR3 polypeptide.

ANTIBODIES AND USES THEREOF

FIELD OF THE INVENTION

[0001] The present invention relates generally to antibodies that specifically bind an epitope within CXCR3, and methods of using the antibodies. The present invention is also related to an epitope within CXCR3 and methods of using the epitope.

BACKGROUND OF THE INVENTION

[0002] CXCR3 is an inflammatory chemokine receptor whose expression is associated with $CD4^+$ Type-1 helper (Th1) and $CD8^+$ cytotoxic lymphocytes (CTLs). In addition to CXCR3 expression on effector $CD4^+$ and $CD8^+$ T cells, CXCR3 is also highly expressed on innate lymphocytes, such as natural killer (NK) cells and NKT cells, and on plasmacytoid DCs and subsets of B cells. CXCR3 binds three chemokines: CXCL9 (also known as monokine induced by gammainterferon, MIG), CXCL10 (interferon-induced protein of 10 kDa, IP-10) and CXCL11 (interferon-inducible T-cell alpha chemoattractant, I-TAC). The binding of CXCR3 to these ligands, which themselves are induced by inflammatory cytokines, mediates cell migration, thereby coordinating inflammation in the periphery.

[0003] Because of the expression of CXCR3 on effector cells, and its role in cell migration and inflammation, there is a need for improved molecules specific for CXCR3 that can be used to inhibit cell migration and/or inflammation. For example, migration of activated T-cells to the graft following transplantation results in donor organ damage and is a hallmark of acute and chronic rejection. A wide variety of immunosuppressive agents are available and routinely used in clinics to reduce the rejection episodes and to improve both short and long-term graft survival. However, their mode of action often results in a complete shut down of the immune system and their use relies on indefinite uptake. As such, they are typically associated with side effects such as opportunistic infections, lymphoproliferative disorders or cardiovascular diseases. Accordingly, the development of more specific drugs able to induce transplantation tolerance and thus eliminate the constraint of high doses of continuous immunosuppressive agents is a major goal in the transplantation field.

SUMMARY OF THE INVENTION

[0004] The present invention relates generally to antibodies that specifically bind an epitope within CXCR3, and methods of making and using the antibodies. The present invention is also related to an epitope within CXCR3 and methods of using the epitope.

[0005] In one aspect, the present invention is directed to an isolated antibody or antigen-binding fragment thereof that specifically binds to an epitope within amino acid residues 23 to 44 of the human CXCR3 set forth in SEQ ID NO:4. In one embodiment, the epitope is within amino acid residues 23 to 43; 23 to 42; 23 to 41; 23 to 40; 23 to 39; 23 to 38; 23 to 37; 23 to 36; 23 to 35; 23 to 34; 23 to 33; 23 to 32; 23 to 31; 23 to 30; 23 to 29; 24 to 44; 24 to 43; 24 to 42; 24 to 41; 24 to 40; 24 to 39; 24 to 38; 24 to 37; 24 to 36; 24 to 35; 24 to 34; 24 to 33; 24 to 32; 24 to 31; 24 to 30; 24 to 29; 25 to 44; 25 to 43; 25 to 42; 25 to 41; 25 to 40; 25 to 39; 25 to 38; 25 to 37; 25 to 36; 25 to 35; 25 to 34; 25 to 33; 25 to 32; 25 to 31; 25 to 30; 26 to 44; 26 to 43; 26 to 42; 26 to 41; 26 to 40; 26 to 39; 26 to 38; 26 to 37; 26 to 36; 26 to 35; 26 to 34; 26 to 33; 26 to 32; 26 to 31; 27 to 44; 27 to 43; 27 to 42; 27 to 41; 27 to 40; 27 to 39; 27 to 38; 27 to 37; 27 to 36; 27 to 35; 27 to 34; 27 to 33; 27 to 32; 28 to 44; 28 to 43; 28 to 42; 28 to 41; 28 to 40; 28 to 39; 28 to 38; 28 to 37; 28 to 36; 28 to 35; 28 to 34; 28 to 33; 28 to 32; 29 to 44; 29 to 43; 29 to 42; 29 to 41; 29 to 40; 29 to 39; 29 to 38; 29 to 37; 29 to 36; 29 to 35; 29 to 34; 30 to 44; 30 to 43; 30 to 42; 30 to 41; 30 to 40; 30 to 39; 30 to 38; 30 to 37; 30 to 36; and/or 30 to 35 of the human CXCR3 set forth in SEQ ID NO:4. In a further embodiment, the epitope consists of amino acid residues 25 to 35; 25 to 34; 25 to 33; 25 to 32; 25 to 31; 25 to 30; 26 to 35; 26 to 34; 26 to 33; 26 to 32; 26 to 31; 27 to 35; 27 to 34; 27 to 33; 27 to 32; 28 to 35; 28 to 34; 28 to 33; 29 to 35; 29 to 34 or 30 to 35 of the human CXCR3 set forth in SEQ ID NO:4.

[0006] In another aspect, the present invention provides, an isolated antibody or antigen-binding fragment thereof that specifically binds to a polypeptide consisting of amino acid residues 23 to 44 of SEQ ID NO:4. In one embodiment, the polypeptide consists of amino acid residues 23 to 43; 23 to 42; 23 to 41; 23 to 40; 23 to 39; 23 to 38; 23 to 37; 23 to 36; 23 to 35; 23 to 34; 23 to 33; 23 to 32; 23 to 31; 23 to 30; 23 to 29; 24 to 44; 24 to 43; 24 to 42; 24 to 41; 24 to 40; 24 to 39; 24 to 38; 24 to 37; 24 to 36; 24 to 35; 24 to 34; 24 to 33; 24 to 32; 24 to 31; 24 to 30; 24 to 29; 25 to 44; 25 to 43; 25 to 42; 25 to 41;

25 to 40; 25 to 39; 25 to 38; 25 to 37; 25 to 36; 25 to 35; 25 to 34; 25 to 33; 25 to 32; 25 to 31; 25 to 30; 26 to 44; 26 to 43; 26 to 42; 26 to 41; 26 to 40; 26 to 39; 26 to 38; 26 to 37; 26 to 36; 26 to 35; 26 to 34; 26 to 33; 26 to 32; 26 to 31; 27 to 44; 27 to 43; 27 to 42; 27 to 41; 27 to 40; 27 to 39; 27 to 38; 27 to 37; 27 to 36; 27 to 35; 27 to 34; 27 to 33; 27 to 32; 28 to 44; 28 to 43; 28 to 42; 28 to 41; 28 to 40; 28 to 39; 28 to 38; 28 to 37; 28 to 36; 28 to 35; 28 to 34; 28 to 33; 28 to 32, 29 to 44; 29 to 43; 29 to 42; 29 to 41; 29 to 40; 29 to 39; 29 to 38; 29 to 37; 29 to 36; 29 to 35; 29 to 34; 30 to 44; 30 to 43; 30 to 42; 30 to 41; 30 to 40; 30 to 39; 30 to 38; 30 to 37; 30 to 36; and/or 30 to 35 of the human CXCR3 set forth in SEQ ID NO:4. In another embodiment, the polypeptide consists of amino acid residues 25 to 35; 25 to 34; 25 to 33; 25 to 32; 25 to 31; 25 to 30; 26 to 35; 26 to 34; 26 to 33; 26 to 32; 26 to 31; 27 to 35; 27 to 34; 27 to 33; 27 to 32; 28 to 35; 28 to 34; 28 to 33; 29 to 35; 29 to 34 or 30 to 35 of the human CXCR3 set forth in SEQ ID NO:4.

[0007] The antibodies and antigen-binding fragments of the invention that specifically bind to an epitope within amino acid residues 23 to 44 of the human CXCR3 set forth in SEQ ID NO:4 may comprise a CDR-H1 comprising the amino acid sequence set forth in SEQ ID NO:20 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:20; a CDR-H2 comprising the amino acid sequence set forth in SEQ ID NO:21 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:21; a CDR-H3 comprising the amino acid sequence set forth in SEQ ID NO:22 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:22; a CDR-L1: comprising the amino acid sequence set forth in SEQ ID NO:23 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:23; a CDR-L2 comprising the amino acid sequence set forth in SEQ ID NO:24 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:24; and a CDR-L3 comprising the amino acid sequence set forth in SEQ ID NO:25 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:25. In particular examples, the antibodies or

antigen-binding fragments comprise a CDR-H1 with a sequence of amino acids set forth in SEQ ID NO:20; a CDR-H2 with a sequence of amino acids set forth in SEQ ID NO:21; a CDR-H3 with a sequence of amino acids set forth in SEQ ID NO:22; a CDR-L1 with a sequence of amino acids set forth in SEQ ID NO:23; a CDR-L2 with a sequence of amino acids set forth in SEQ ID NO:24; and a CDR-L3 with a sequence of amino acids set forth in SEQ ID NO:25.

[0008] In some embodiments, the antibodies and antigen-binding fragments of the invention that specifically bind to an epitope within amino acid residues 23 to 44 of the human CXCR3 set forth in SEQ ID NO:4 comprise a variable heavy chain comprising sequence of amino acids set forth in SEQ ID NO:13 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:13, and a variable light chain comprising a sequence of amino acids set forth in SEQ ID NO:15 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:15. In other embodiments, the antibodies and antigen-binding fragments comprise a variable heavy chain comprising sequence of amino acids set forth in SEQ ID NO:41 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:41, and a variable light chain comprising a sequence of amino acids set forth in SEQ ID NO:42 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:42.

[0009] In another aspect, the invention provides an isolated antibody or antigen binding fragment thereof that binds to CXCR3, comprising a CDR-H1 comprising the amino acid sequence set forth in SEQ ID NO:20 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:20; a CDR-H2 comprising the amino acid sequence set forth in SEQ ID NO:21 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:21; a CDR-H3 comprising the amino acid sequence set forth in SEQ ID NO:22 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity

to SEQ ID NO:22; a CDR-L1: comprising the amino acid sequence set forth in SEQ ID NO:23 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:23; a CDR-L2 comprising the amino acid sequence set forth in SEQ ID NO:24 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:24; and a CDR-L3 comprising the amino acid sequence set forth in SEQ ID NO:25 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:25.

[0010] In one embodiment, the antibody or antigen-binding fragment comprises a CDR-H1 with a sequence of amino acids set forth in SEQ ID NO:20; a CDR-H2 with a sequence of amino acids set forth in SEQ ID NO:21; a CDR-H3 with a sequence of amino acids set forth in SEQ ID NO:22; a CDR-L1 with a sequence of amino acids set forth in SEQ ID NO:23; a CDR-L2 with a sequence of amino acids set forth in SEQ ID NO:24; and a CDR-L3 with a sequence of amino acids set forth in SEQ ID NO:25. For example, the antibody or antigen-binding fragment may comprise a variable heavy chain comprising sequence of amino acids set forth in SEQ ID NO:13 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:13, and a variable light chain comprising a sequence of amino acids set forth in SEQ ID NO:15 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:15. In other examples, the antibody or antigen-binding fragment comprises a variable heavy chain comprising sequence of amino acids set forth in SEQ ID NO:41 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:41, and a variable light chain comprising a sequence of amino acids set forth in SEQ ID NO:42 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:42.

[0011] In some instances, the antibodies and antigen-binding fragments of the invention are monoclonal antibodies or antigen-binding fragments of monoclonal antibodies. In one embodiment, the antibody or antigen-binding fragment is multivalent. In further embodiments, the antibody or antigen-binding fragment is a chimeric or

humanized antibody or antigen-binding fragment. In particular embodiments, an antigen-binding fragment of the invention is a Fab fragment, Fab' fragment, $F(ab')_2$ fragment, Fv fragment, disulfide-linked Fv (dsFv), Fd fragment, Fd' fragment, single-chain Fv (scFv), single-chain Fab (scFab), domain antibody, diabody, triabody or tetrabody .

[0012] The antibodies and antigen-binding fragments of the invention may exhibit antibody-dependent cellular cytotoxicity (ADCC) activity and/or complement dependent cytotoxicity (CDC) activity.

[0013] In some embodiments, antibodies and antigen-binding fragments of the invention are linked to one or more other moieties, such as, for example, a polymer, small molecule, peptide or polypeptide. In some instances, the moiety is a cytotoxic agent.

[0014] The present invention also provides isolated nucleic acid molecules encoding the antibodies and antigen-binding fragments of the invention.

[0015] In one aspect, the invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides set forth in SEQ ID NO: 26 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:26; a sequence of nucleotides set forth in SEQ ID NO: 27 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:27; and a sequence of nucleotides set forth in SEQ ID NO: 28 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:28. In some examples, the nucleic acid molecule comprises a sequence of nucleotides set forth in SEQ ID NO:12 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:12.

[0016] In another aspect, the invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides set forth in SEQ ID NO: 29 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:29; a sequence of nucleotides set forth in SEQ ID NO: 30 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more

sequence identity to SEQ ID NO:30; and a sequence of nucleotides set forth in SEQ ID NO: 31 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:31. In one embodiment, the nucleic acid molecule comprises a sequence of nucleotides set forth in SEQ ID NO:14 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:14.

[0017] The invention also provides an isolated nucleic acid molecule comprising a sequence of nucleotides set forth in SEQ ID NO:45 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:45; a sequence of nucleotides set forth in SEQ ID NO:46 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:46; and a sequence of nucleotides set forth in SEQ ID NO:47 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:47. In some examples, the nucleic acid molecule comprises a sequence of nucleotides set forth in SEQ ID NO:43 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:43.

[0018] In another aspect, the invention provides an isolated nucleic acid molecule, comprising a sequence of nucleotides set forth in SEQ ID NO:48 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:48; a sequence of nucleotides set forth in SEQ ID NO:49or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:49; and a sequence of nucleotides set forth in SEQ ID NO:50 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:50. In one embodiment, the isolated nucleic acid molecule comprises a sequence of nucleotides set forth in SEQ ID NO:44 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:44.

[0019] The present invention also provides vectors comprising one or more nucleic acid molecules described herein, cells comprising one or more nucleic acid molecules or vectors described herein, and recombinant cells that produce any one or more antibodies or antigen-binding fragments described herein. The invention also provides compositions comprising one or more of the antibodies or antigen-binding fragments, nucleic acid molecules and/or vectors described herein.

[0020] In a further aspect, the invention is directed to a method for depleting $CXCR3^+$ cells in a subject, comprising administering to the subject an effective amount of an antibody or antigen-binding fragment of the invention. In another aspect, the invention provides a method for inhibiting the recruitment or migration of $CXCR3^+$ cells to an inflammation site in a subject, comprising administering to the subject an effective amount of the antibody or antigen-binding fragment of the invention. In some embodiments, the cells are $CXCR3^+/CD4^-$ T cells, $CXCR3^+/CD8^-$ T cells and/or $CXCR3^+/B220^+$ B cells. In further embodiments, the subject has received, is receiving or is to receive a graft or transplant.

[0021] In another aspect of the invention, provides is a method for inhibiting graft or transplant rejection in a subject that has received, is receiving or is to receive a graft or transplant, comprising administering to the subject an effective amount of an antibody or antigen-binding fragment described herein.

[0022] In some embodiments of the methods of the invention, the graft is an allograft or xenograft, or the transplant is an allotransplant or xenotransplant. In particular examples, the graft or transplant is selected from among a heart, kidney, lung, liver, pancreas, pancreatic islets, brain tissue, stomach, large intestine, small intestine, cornea, skin, trachea, bone, bone marrow, muscle and bladder graft or transplant. In some instances, the antibody or antigen-binding fragment is administered to the subject before, at the same time and/or after the subject has received the graft or transplant.

[0023] In one embodiment of the methods of the invention, the antibody or antigen-binding fragment is administered to the subject two or more times. In some examples, the methods further comprise administering to the subject one or more other therapeutic agents, such as a cytokine (*e.g.* interleukin 2), chemokine or antibody. In particular embodiments, the therapeutic agent is an immunosuppressive agent, such as, for example,

a glucocorticoid, cyclosporine, rapamycin, voclosporin, sirolimus, everolimus, tacrolimus, mycophenolate mofetil, mycophenolic acid, mizoribine, an S1P-R agonists, a malononitrilamide, an anti-CD3 antibody, an anti -CD25 antibody, an anti-CD52 antibody, an anti-CD20 antibody or an anti-tumor necrosis factor antibody.

[0024] In a further aspect of the invention, provided is a method for measuring or detecting CXCR3 in a sample, comprising contacting the sample with an antibody or antigen-binding fragment described herein and measuring or detecting binding of the antibody or antigen-binding fragment to the CXCR3. The sample may comprise one or more cells expressing CXCR3. In some examples, the sample comprises blood, cells or tissue.

[0025] Also provided are uses of an antibody or antigen-binding fragment of the invention for the preparation of a medicament for depleting $CXCR3^+$ cells; the preparation of a medicament for inhibiting the recruitment or migration of $CXCR3^+$ cells to an inflammation site; and/or the preparation of a medicament for inhibiting graft rejection in a subject that has received, is receiving or is to receive a graft.

[0026] A further aspect of the invention provides an isolated peptide consisting of amino acid residues 23 to 44; 23 to 43; 23 to 42; 23 to 41; 23 to 40; 23 to 39; 23 to 38; 23 to 37; 23 to 36; 23 to 35; 23 to 34; 23 to 33; 23 to 32; 23 to 31; 23 to 30; 23 to 29; 24 to 44; 24 to 43; 24 to 42; 24 to 41; 24 to 40; 24 to 39; 24 to 38; 24 to 37; 24 to 36; 24 to 35; 24 to 34; 24 to 33; 24 to 32; 24 to 31; 24 to 30; 24 to 29; 25 to 44; 25 to 43; 25 to 42; 25 to 41; 25 to 40; 25 to 39; 25 to 38; 25 to 37; 25 to 36; 25 to 35; 25 to 34; 25 to 33; 25 to 32; 25 to 31; 25 to 30; 26 to 44; 26 to 43; 26 to 42; 26 to 41; 26 to 40; 26 to 39; 26 to 38; 26 to 37; 26 to 36; 26 to 35; 26 to 34; 26 to 33; 26 to 32; 26 to 31; 27 to 44; 27 to 43; 27 to 42; 27 to 41; 27 to 40; 27 to 39; 27 to 38; 27 to 37; 27 to 36; 27 to 35; 27 to 34; 27 to 33; 27 to 32; 28 to 44; 28 to 43; 28 to 42; 28 to 41; 28 to 40; 28 to 39; 28 to 38; 28 to 37; 28 to 36; 28 to 35; 28 to 34; 28 to 33; 28 to 32; 29 to 44; 29 to 43; 29 to 42; 29 to 41; 29 to 40; 29 to 39; 29 to 38; 29 to 37; 29 to 36; 29 to 35; 29 to 34; 30 to 44; 30 to 43; 30 to 42; 30 to 41; 30 to 40; 30 to 39; 30 to 38; 30 to 37; 30 to 36; or 30 to 35 of SEQ ID NO:4, or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 9%, 95%, 96%, 97%, 98% or 99% sequence identity thereto. In some embodiments, the peptide is linked to a protein, peptide, synthetic polymer, adjuvant, or small molecule.

[0027] In another aspect, the invention provides a method for inducing antibodies that specifically and/or selectively bind to CXCR3, comprising administering to a subject one or more peptides of the invention.

[0028] In a further aspect, the invention provides a method for selecting antibodies or antigen-binding fragments thereof that specifically and/or selectively bind to CXCR3, comprising contacting a sample comprising antibodies or antigen-binding fragments with the peptide of the invention and selecting those antibodies and antigen-binding fragments that specifically bind to the peptide.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] Embodiments of the invention are described herein, by way of non-limiting example only, with reference to the following figures.

[0030] **Figure 1** represents flow cytometry analysis of the binding properties of the 9C5 mAb. **(A)** 9C5 mAb recognizes L1.2 cells transfected with mouse CXCR3 (mCXCR3) or human CXCR3 (hCXCR3) but not untransfected L1.2 cells or mCXCR2 transfected cells. **(B)** 9C5 mAb binds mouse splenocytes and human peripheral blood mononuclear cells (PBMC). **(C)** 9C5 mAb binds marmoset splenocytes.

[0031] **Figure 2** represents the results of an ELISA measuring binding of various anti-CXCR3 antibodies to Peptides 1-4.

[0032] **Figure 3** shows an alignment of the N-terminal region of CXCR3 from human, rhesus macaque, marmoset, pig, dog, mouse and rat, and the regions spanned by Peptides 1-4. Peptide 3 spans a highly conserved region (boxed).

[0033] **Figure 4** represents flow cytometry analysis of splenocytes from mice intravenously injected with the 9C5 mAb at various concentrations. Splenocytes were isolated after 4 days and stained with anti-CD4, anti-CD8, anti-B220 and anti-mCXCR3 antibodies and analysed to assess *in vivo* depletion of $CD8^+/CXCR3^+$; $CD4^+/CXCR3^+$ and $B220^+/CXCR3^+$ cells.

[0034] **Figure 5** represents flow cytometry analysis of splenocytes from mice intravenously injected with 5 mg/kg of the 9C5 mAb. Splenocytes were isolated after 24 or 36 days and stained with anti-CD4, anti-CD8, anti-B220 and anti-mCXCR3 antibodies

and analysed to assess *in vivo* depletion of $CD8^+/CXCR3^+$; $CD4^+/CXCR3^+$ and $B220^+/CXCR3^+$ cells.

[0035] **Figure 6** represents flow cytometry analysis of splenocytes from mice intravenously injected with 5 mg/kg of the 9C5 mAb or an isotype control. Splenocytes were isolated after 4 days and stained with anti-CD44 and anti-CD62L antibodies, anti-B220 and anti-mCXCR3 antibodies. The left panel shows the typical localization of $CXCR3^+$ cells; the middle panel shows cells from isotype control-treated mice and the right panel shows cells from 9C5-treated mice.

[0036] **Figure 7** represents flow cytometry analysis of splenocytes from mice intravenously injected with saline, or 2 mg/kg or 5 mg/kg of the Fc-engineered chimeric 9C5-3MFc or 9C5-Fc-KO mAbs. After 4 days, spleens were harvested and $CD4^+/CXCR3^+$ lymphocytes were analyzed by flow cytometry.

[0037] **Figure 8** represents the results of pancreatic islet allograft transplantations in mice. BALB/c $H2^d$ islets were transplanted under the kidney capsule of streptozotocin-induced diabetic C57BL/6 $H2^b$ mice. Graft rejection was assessed by measuring blood glucose level following or during various therapies as described below. Graft rejection was defined as an increase of blood glucose to > 16 mmol/liter after a period of euglycemia. **A)** Mice were administered a single intravenous dose of 2 mg/kg 9C5 mAb or isotype control 4-5 hr prior to transplantation, or multiple doses on day 0, 7, 14, and 21. **B)** Recipient mice were intravenously administered 0, 0.35, 0.1 or 1 mg/kg rapamycin on the day of transplantation and every day thereafter for 7 days. **C)** Mice were intravenously administered 0.1 mg/kg dose of rapamycin on days 0, 1, 2, 3, 4, 5, 6 and 7, and 2 mg/kg 9C5 mAb or isotype control on day 0, 7, 14, and 21.

[0038] **Figure 9** represents the results of flow cytometry analysis of the binding properties of the humanized 9C5 mAb. (h9C5 mAb) and the parental mouse 9C5 antibody. Human CXCR3-transfected cells (L1.2 cells) were stained with the h9C5 antibody (0.25 μg/ml), the 9C5 antibody (0.25 μg/ml) or an isotype control (1 μg/ml). Staining was detected with an anti-human Fc specific or an anti-mouse Fc specific PE conjugated antibody, followed by flow cytometry analysis.

[0039] **Figure 10** Mean survival time of islet grafts. BALB/c $H2^d$ islets were transplanted under the kidney capsule of streptozotocin-induced diabetic C57BL/6 $H2^b$

mice. Mice were administered with a single dose of humanized 9C5 3MFc mAb (M), humanized 9C5 Fc-KO mAb (S) or isotype control antibody 4-5 h prior to transplantation. Humanized 9C5 3MFc mAb increased the mean survival time of islet grafts.

BRIEF DESCRIPTION OF THE SEQUENCES

Table 1

Sequence ID Number	Sequence description
SEQ ID NO: 1	Mouse CXCR3 (nucleic acid)
SEQ ID NO: 2	Mouse CXCR3 (amino acid)
SEQ ID NO: 3	Human CXCR3 (nucleic acid)
SEQ ID NO: 4	Human CXCR3 (amino acid)
SEQ ID NO: 5	Human CXCR3 R292Q variant (amino acid)
SEQ ID NO: 6	Human CXCR3 A363T variant (amino acid)
SEQ ID NO: 7	Rat CXCR3 (amino acid)
SEQ ID NO: 8	Pig CXCR3 (amino acid)
SEQ ID NO: 9	Cow CXCR3 (amino acid)
SEQ ID NO: 10	Rhesus macaque CXCR3 (amino acid)
SEQ ID NO: 11	Dog CXCR3 (amino acid)
SEQ ID NO: 12	9C5 mAb V_H (nucleic acid)
SEQ ID NO: 13	9C5 mAb V_H (amino acid)
SEQ ID NO: 14	9C5 mAb V_L (nucleic acid)
SEQ ID NO: 15	9C5 mAb V_L (amino acid)
SEQ ID NO: 16	Peptide 1 (amino acid)
SEQ ID NO: 17	Peptide 2 (amino acid)
SEQ ID NO: 18	Peptide 3 (amino acid)

Sequence ID Number	Sequence description
SEQ ID NO: 19	Peptide 4 (amino acid)
SEQ ID NO: 20	9C5 mAb CDR-H1 (amino acid)
SEQ ID NO: 21	9C5 mAb CDR-H2 (amino acid)
SEQ ID NO: 22	9C5 mAb CDR-H3 (amino acid)
SEQ ID NO: 23	9C5 mAb CDR-L1 (amino acid)
SEQ ID NO: 24	9C5 mAb CDR-L2 (amino acid)
SEQ ID NO: 25	9C5 mAb CDR-L3 (amino acid)
SEQ ID NO: 26	9C5 mAb CDR-H1 (nucleic acid)
SEQ ID NO: 27	9C5 mAb CDR-H2 (nucleic acid)
SEQ ID NO: 28	9C5 mAb CDR-H3 (nucleic acid)
SEQ ID NO: 29	9C5 mAb CDR-L1 (nucleic acid)
SEQ ID NO: 30	9C5 mAb CDR-L2 (nucleic acid)
SEQ ID NO: 31	9C5 mAb CDR-L3 (nucleic acid)
SEQ ID NO: 32	Human IgG1 C_H (amino acid)
SEQ ID NO: 33	Human IgG1 C_H (nucleic acid)
SEQ ID NO: 34	Human kappa C_L (amino acid)
SEQ ID NO: 35	Human kappa C_L (nucleic acid)
SEQ ID NO: 36	Human IgG1 C_H S239D/A330L/I332E (amino acid)
SEQ ID NO: 37	Human IgG1 C_H S239D/A330L/I332E (nucleic acid)
SEQ ID NO: 38	Human IgG1 C_H L234F/L235E/P331S (amino acid)
SEQ ID NO: 39	Human IgG1 C_H L234F/L235E/P331S (nucleic acid)
SEQ ID NO: 40	Human CXCR3 cDNA
SEQ ID NO: 41	Humanized 9C5 V_H (amino acid)

Sequence ID Number	Sequence description
SEQ ID NO: 42	Humanized 9C5 V_L(amino acid)
SEQ ID NO: 43	Humanized 9C5 V_H (nucleic acid)
SEQ ID NO: 44	Humanized 9C5 V_L(nucleic acid)
SEQ ID NO: 45	Humanized 9C5 CDR-H1 (nucleic acid)
SEQ ID NO: 46	Humanized 9C5 CDR-H2 (nucleic acid)
SEQ ID NO: 47	Humanized 9C5 CDR-H3 (nucleic acid)
SEQ ID NO: 48	Humanized 9C5 CDR-L1 (nucleic acid)
SEQ ID NO: 49	Humanized 9C5 CDR-L2 (nucleic acid)
SEQ ID NO: 50	Humanized 9C5 CDR-L3 (nucleic acid)

DETAILED DESCRIPTION

[0040] The articles "a" and "an" are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an antibody" means one antibody or more than one antibody.

[0041] In the context of this specification, the term "about" is understood to refer to a range of numbers that a person of skill in the art would consider equivalent to the recited value in the context of achieving the same function or result.

[0042] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

[0043] As used herein, "antibody" refers to immunoglobulins and immunoglobulin fragments, whether natural or partially or wholly synthetically, such as recombinantly, produced, including any fragment thereof containing at least a portion of the variable region of the immunoglobulin molecule that retains the binding specificity ability of the

full-length immunoglobulin. Hence, an antibody includes any protein having a binding domain that is homologous or substantially homologous to an immunoglobulin antigen-binding domain (antibody combining site). Antibodies include antibody fragments capable of binding antigen. As used herein, the term antibody includes synthetic antibodies, recombinantly produced antibodies, multivalent antibodies (*e.g.*, bivalent antibodies), human antibodies, non-human antibodies, humanized antibodies, chimeric antibodies, intrabodies, domain antibodies, and antibody fragments, such as, but not limited to, Fab fragments, Fab^1 fragments, $F(ab')_2$ fragments, Fv fragments, disulfide-linked Fvs (dsFv), Fd fragments, Fd' fragments, single-chain Fvs (scFv), single-chain Fabs (scFab), diabodies, triabodies, tetrabodies or antigen-binding fragments of any of the above. Antibodies provided herein include members of any immunoglobulin type (e.g., IgG, IgM, IgD, IgE, IgA and IgY), any class (e.g. IgGl, IgG2, IgG3, IgG4, IgAl and IgA2) or subclass (e.g., IgG2a and IgG2b).

[0044] As used herein, an "antigen-binding fragment" of an antibody includes any fragment of an antibody that retains the ability to bind to the same antigen as the antibody from which it is derived. Typically, the antigen-binding fragment binds to the antigen with an affinity that is at least or about 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, or more of the binding affinity exhibited by the antibody. Exemplary antigen-binding fragments include, but are not limited to, Fab fragments, Fab^1 fragments, $F(ab')_2$ fragments, Fv fragments, disulfide-linked Fvs (dsFv), Fd fragments, Fd' fragments, single-chain Fvs (scFv), single-chain Fabs (scFab), domain antibodies, diabodies, triabodies and tetrabodies.

[0045] As used herein, "binds" with respect to an antibody or antigen-binding fragment thereof refers to the ability of the antibody or antigen-binding fragment to form one or more noncovalent bonds with a cognate antigen, by noncovalent interactions between the antibody combining site(s) of the antibody and the antigen. The antigen can be an isolated antigen or can be presented in association with another entity, such as in the context of a polypeptide on the surface of a cell.

[0046] As used herein, an antibody or antigen-binding fragment thereof that "specifically binds" to an antigen, such as CXCR3, is one that binds with an affinity constant (Ka) of about or at least 10^6-10^9 M^{-1} (or a dissociation constant (Kd) of or about 10^{-6} M (1000 nM), 10^{-7} M (100 nM), 10^{-8} M (10 nM), 10^{-9} M (1 nM) or less), and possesses no discernible binding to any other antigen. Affinity constants can be

determined by standard kinetic methodology for antibody reactions, for example, immunoassays (*e.g.* ELISA), or surface plasmon resonance (SPR). Instrumentation and methods for real time detection and monitoring of binding rates are known and are commercially available (*e.g.*, Biacore 2000, Biacore AB, Upsala, Sweden and GE Healthcare Life Sciences).

[0047] As used herein, an antibody or antigen-binding fragment thereof that "selectively binds" to a target antigen has a Kd that is greater for the target antigen relative to a non-target antigen by, *e.g.*, at least two-fold, at least three-fold, at least four fold, at least five-fold, at least 10 fold, at least 50 fold, at least 100 fold, at least 1000, at least 10,000, or at least 100,000 fold. Thus, for example, an antibody or antigen-binding fragment thereof that selectively binds to CXCR3 may have a Kd for CXCR3 that is at least one-fold, at least two-fold, at least three-fold, at least four fold, at least five-fold, at least 10 fold, at least 50 fold, at least 100 fold, at least 1000, at least 10,000, or at least 100,000 fold greater than the Kd for, *e.g.* CXCR5.

[0048] As used herein, a "complementarity-determining region" or "CDR" refers to one of a plurality of portions within each variable region of an antibody or antigen-binding fragment that together form an antigen-binding site of an antibody. Each variable region domain contains three CDRs, named CDR1, CDR2 and CDR3. Accordingly, the variable heavy chain comprises CDR-H1, CDR-H2 and CDR-H3, and the variable light chain comprises CDR-L1, CDR-L2 and CDR-L3. The three CDRs are non-contiguous along the linear amino acid sequence, but are proximate in the folded polypeptide. The CDRs are located within the loops that join the parallel strands of the beta sheets of the variable domain. One of skill in the art knows and can identify the CDRs based on Kabat or Chothia numbering (see *e.g.*, Kabat, E.A. *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, and Chothia, C. *et al.* (1987) J Mol. Biol. 196:901-917).

[0049] By "isolated" is meant material that is substantially or essentially free from components that normally accompany it in its native state. For example, an "isolated polynucleotide", as used herein, refers to a polynucleotide, which has been purified from the sequences which flank it in a naturally occurring state, e.g., a DNA fragment which has been removed from the sequences which are normally adjacent to the fragment.

[0050] As used herein, CXCR3 (or chemokine (C-X-C motif) receptor 3) refers to human and non-human CXCR3 polypeptides. Exemplary human CXCR3 polypeptides include, but are not limited to, the CXCR3 polypeptide set forth in SEQ ID NO:4, and variants thereof having at least or about 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity, including the natural variants set forth in SEQ ID NOS:5 and 6, comprising the mutations R292Q and A363T, respectively. Exemplary non-human CXCR3 polypeptides include, but are not limited to, mouse (*e.g.* SEQ ID NO:2), rat (*e.g.* SEQ ID NO:7), pig (*e.g.* SEQ ID NO:8), cow (*e.g.* SEQ ID NO:9), rhesus macaque (*e.g.* SEQ ID NO:10), and dog (*e.g.* SEQ ID NO:711) CXCR3, and variants thereof having at least or about 85%, 90%, 91%, 92%, 93%, 94%, 95%,, 96%, 97%, 98% or 99% sequence identity thereto.

[0051] The terms "nucleic acid molecule" or "polynucleotide" as used herein designates mRNA, RNA, cRNA, cDNA or DNA. The term "polynucleotide" typically refers to oligonucleotides greater than 30 nucleotides in length. A polynucleotide "variant" refers to polynucleotides displaying substantial sequence identity with a reference polynucleotide sequence or polynucleotides that hybridise with a reference sequence under stringent conditions that are defined hereinafter. These terms also encompass polynucleotides that are distinguished from a reference polynucleotide by the addition, deletion or substitution of at least one nucleotide. Accordingly, the term "variant" include polynucleotides in which one or more nucleotides have been added or deleted, or replaced with different nucleotides. In this regard, it is well understood in the art that certain alterations inclusive of mutations, additions, deletions and substitutions can be made to a reference polynucleotide whereby the altered polynucleotide retains the biological function or activity of the reference polynucleotide. The terms "polynucleotide variant" and "variant" also include naturally occurring allelic variants.

[0052] "Polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues and to variants and synthetic analogues of the same. Thus, these terms apply to amino acid polymers in which one or more amino acid residues is a synthetic non-naturally occurring amino acid, such as a chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally-occurring amino acid polymers. A polypeptide "variant" refers to a polypeptide that is distinguished from a reference polypeptide by the addition, deletion or substitution of at least one amino acid.

In certain embodiments, a polypeptide variant is distinguished from a reference polypeptide by one or more substitutions, which may be conservative or non-conservative. In certain embodiments, the polypeptide variant comprises conservative substitutions and, in this regard, it is well understood in the art that some amino acids may be changed to others with broadly similar properties without changing the nature of the activity of the polypeptide. Polypeptide variants also encompass polypeptides in which one or more amino acids have been added or deleted, or replaced with different amino acid residues.

[0053] As used herein, the term "% identical" means that in a comparison of two sequences over a specified region the two sequences have the specified number of identical residues in the same position. The term "% similar" has a similar meaning but in addition to the number of identical amino acids between the two sequences regard is also had to where the amino acids are not identical but are conservative substitutions. Percentage identity can be determined using known computer algorithms such as the "FAST A" program, using for example, the default parameters as in Pearson *et al.* Proc. Natl. Acad. Sci. USA 85: 2444 (1988) (other programs include the GCG program package (Devereux, J., et al., Nucleic Acids Research 12(1): 387 (1984)), BLASTP, BLASTN, FASTA (Atschul, S.F., et al., J. Molec. Biol. 215:403 (1990); Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego (1994), and Carillo et al. SIAM J Applied Math 48: 1073 (1988)). For example, the BLAST function of the National Center for Biotechnology Information database can be used to determine identity. Other commercially or publicly available programs include DNAStar "MegAlign" program (Madison, WI) and the University of Wisconsin Genetics Computer Group (UWG) "Gap" program (Madison WI)). Percent identity of proteins and/or nucleic acid molecules can be determined, for example, by comparing sequence information using a GAP computer program {e.g., Needleman et al. J. Mol. Biol. 48: 443 (1970), as revised by Smith and Waterman {Adv. Appl. Math. 2: 482 (1981)). Briefly, a GAP program defines similarity as the number of aligned symbols {i.e., nucleotides or amino acids) that are similar, divided by the total number of symbols in the shorter of the two sequences. Default parameters for the GAP program can include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non identities) and the weighted comparison matrix of Gribskov et al. Nucl. Acids Res. 14: 6745 (1986), as described by Schwartz and Dayhoff, eds., Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, pp. 353-358

(1979); (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps.

[0054] The terms "graft" and "transplant" can be used interchangeably herein, and include reference to cell, tissue, organ and limb grafts, as well as reference to allografts and xenografts.

[0055] The term "inhibiting" and variations thereof such as "inhibition" and "inhibits" as used herein means complete or partial inhibition, such as complete or partial inhibition of graft rejection, or complete or partial inhibition of cell migration, recruitment or accumulation. Further, inhibition may be permanent or temporary. The inhibition may be to an extent (in magnitude and/or spatially), and/or for a time, sufficient to produce the desired effect. For example, inhibition may be prevention, retardation, reduction or otherwise hindrance of graft rejection or cell migration, recruitment or accumulation. Such inhibition may be in magnitude and/or be temporal or spatial in nature.

[0056] Inhibition of graft rejection by an antibody or antigen-binding fragment can be assessed by any appropriate means as known to a skilled person. As understood by those in that art, the parameters used to measure graft rejection may vary depending on the type of graft. For example, rejection of pancreatic islet grafts can be assessed by measuring blood glucose levels. Rejection of heart grafts can be assessed by endomyocardial biopsy or intramyocardial electrocardiography (IMEG). In other examples, graft rejection can be assessed by measuring cell-free donor DNA in the blood (Snyder *et al.* (2011) PNAS 108:6229-6234). Graft rejection can be inhibited by at least or about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more compared to the rejection observed in the absence of an antibody or antigen-binding fragment of the present invention.

[0057] Inhibition of cell migration, recruitment or accumulation by an antibody or antigen-binding fragment of the present invention can be assessed by any method known to those skilled in art. Such methods can include, for example, analysis of biopsies by immunohistochemistry, flow cytometry, RT-PCR *etc*, to assess the number of cells, such as $CXCR3^+$ cells, in one or more population of cells or one or more locations with the body or within an organ. Cell migration, recruitment or accumulation can be inhibited by at least or about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%,

75%, 80%, 85%, 90%, 95% or more compared to the migration, recruitment or accumulation in the absence of an antibody or antigen-binding fragment of the present invention.

[0058] As used herein, "depletion" with respect to $CXCR3^+$ cells (*i.e.* cells expressing CXCR3) refers to the removal of these cells from a population of cells. Reference to depletion includes complete or partial depletion. Further, depletion may be permanent or temporary, and may be to varying extents in magnitude and/or spatially. Depletion may be the result of cell death, such as by apoptosis or necrosis. Depletion can be assessed by measuring the number of $CXCR3^+$ cells in a population using any method known in the art (*e.g.* flow cytometry immunohistochemistry, *etc.*), before and after exposure to an antibody or antigen-binding fragment of the present invention, or in the absence and presence of an antibody or antigen-binding fragment of the present invention. Following exposure to an antibody or antigen-binding fragment of the present invention, $CXCR3^+$ cells can be depleted by at least or about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more.

[0059] As used herein the term "effective amount" includes within its meaning a non-toxic but sufficient amount of an agent to provide the desired effect. The exact amount required will vary from subject to subject or situation to situation depending on factors such as the activity of the agent (*e.g.* the antibody or antigen-binding fragment), the severity of the condition, disease or disorder, the mode of administration of the agent, the general state of the subject, the use of other agents, and so forth. Thus, it is not possible to specify an exact "effective amount". However, for any given case, an appropriate "effective amount" may be determined by one of ordinary skill in the art using only routine experimentation.

[0060] As used herein the terms "treating", "treatment", "preventing" and "prevention" refer to any and all uses which remedy a condition or symptoms, prevent the establishment of disorder, condition or disease, or otherwise prevent, hinder, retard, or reverse the progression of an disorder, condition or disease (such as graft rejection or inflammation) or other undesirable symptoms in any way whatsoever. Thus the terms "treating" and "preventing" and the like are to be considered in their broadest context. For example, treatment does not necessarily imply that a patient is treated until total recovery.

[0061] As used herein, a "subject" includes human and non-human animals, including, for example, livestock (such as cows, sheep, goats, pigs horses and goats), companion animals (such as cats and dogs) and show or performance animals (such as horses).

[0062] Those skilled in the art will appreciate that the aspects and embodiments described herein are susceptible to variations and modifications other than those specifically described. It is to be understood that the disclosure includes all such variations and modifications. The disclosure also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

Antibodies that specifically bind CXCR3

[0063] The present invention is directed in part to isolated antibodies and antigen-binding fragments thereof that specifically bind to CXCR3. In particular, the antibodies and antigen-binding fragments specifically bind to an N-terminal epitope within amino acid residues 23 to 44 of the human CXCR3 set forth in SEQ ID NO:4 or within corresponding amino acid residues in another CXCR3 polypeptide, such as a CXCR3 polypeptide from another species or a variant of the CXCR3 polypeptide set forth in SEQ ID NO:4. Accordingly, provided are polypeptides that specifically bind to a polypeptide consisting of amino acid residues 23 to 44 of the human CXCR3 set forth in SEQ ID NO:4

[0064] In some embodiments, the antibodies and antigen-binding fragments of the present invention specifically bind to an epitope within amino acid residues 23 to 43; 23 to 42; 23 to 41; 23 to 40; 23 to 39; 23 to 38; 23 to 37; 23 to 36; 23 to 35; 23 to 34; 23 to 33; 23 to 32; 23 to 31; 23 to 30; 23 to 29; 24 to 44; 24 to 43; 24 to 42; 24 to 41; 24 to 40; 24 to 39; 24 to 38; 24 to 37; 24 to 36; 24 to 35; 24 to 34; 24 to 33; 24 to 32; 24 to 31; 24 to 30; 24 to 29; 25 to 44; 25 to 43; 25 to 42; 25 to 41; 25 to 40; 25 to 39; 25 to 38; 25 to 37; 25 to 36; 25 to 35; 25 to 34; 25 to 33; 25 to 32; 25 to 31; 25 to 30; 26 to 44; 26 to 43; 26 to 42; 26 to 41; 26 to 40; 26 to 39; 26 to 38; 26 to 37; 26 to 36; 26 to 35; 26 to 34; 26 to 33; 26 to 32; 26 to 31; 27 to 44; 27 to 43; 27 to 42; 27 to 41; 27 to 40; 27 to 39; 27 to 38; 27 to 37; 27 to 36; 27 to 35; 27 to 34; 27 to 33; 27 to 32; 28 to 44; 28 to 43; 28 to 42; 28 to 41; 28 to 40; 28 to 39; 28 to 38; 28 to 37; 28 to 36; 28 to 35; 28 to 34; 28 to 33; 28 to 32; 29 to 44; 29 to 43; 29 to 42; 29 to 41; 29 to 40; 29 to 39; 29 to 38; 29 to 37; 29 to 36; 29 to 35; 29 to

34; 30 to 44; 30 to 43; 30 to 42; 30 to 41; 30 to 40; 30 to 39; 30 to 38; 30 to 37; 30 to 36; and/or 30 to 35 of the human CXCR3 set forth in SEQ ID NO:4, or corresponding residues of another CXCR3 polypeptide.

[0065] In further embodiments, the antibodies and antigen-binding fragments of the present invention specifically bind to an epitope or polypeptide consisting of amino acid residues 23 to 43; 23 to 42; 23 to 41; 23 to 40; 23 to 39; 23 to 38; 23 to 37; 23 to 36; 23 to 35; 23 to 34; 23 to 33; 23 to 32; 23 to 31; 23 to 30; 23 to 29; 24 to 44; 24 to 43; 24 to 42; 24 to 41; 24 to 40; 24 to 39; 24 to 38; 24 to 37; 24 to 36; 24 to 35; 24 to 34; 24 to 33; 24 to 32; 24 to 31; 24 to 30; 24 to 29; 25 to 44; 25 to 43; 25 to 42; 25 to 41; 25 to 40; 25 to 39; 25 to 38; 25 to 37; 25 to 36; 25 to 35; 25 to 34; 25 to 33; 25 to 32; 25 to 31; 25 to 30; 26 to 44; 26 to 43; 26 to 42; 26 to 41; 26 to 40; 26 to 39; 26 to 38; 26 to 37; 26 to 36; 26 to 35; 26 to 34; 26 to 33; 26 to 32; 26 to 31; 27 to 44; 27 to 43; 27 to 42; 27 to 41; 27 to 40; 27 to 39; 27 to 38; 27 to 37; 27 to 36; 27 to 35; 27 to 34; 27 to 33; 27 to 32; 28 to 44; 28 to 43; 28 to 42; 28 to 41; 28 to 40; 28 to 39; 28 to 38; 28 to 37; 28 to 36; 28 to 35; 28 to 34; 28 to 33; 28 to 32; 29 to 44; 29 to 43; 29 to 42; 29 to 41; 29 to 40; 29 to 39; 29 to 38; 29 to 37; 29 to 36; 29 to 35; 29 to 34; 30 to 44; 30 to 43; 30 to 42; 30 to 41; 30 to 40; 30 to 39; 30 to 38; 30 to 37; 30 to 36; and/or 30 to 35 of the human CXCR3 set forth in SEQ ID NO:4, or corresponding residues of another CXCR3 polypeptide.

[0066] In particular embodiments, the antibodies and antigen-binding fragments of the present invention specifically bind to an epitope within amino acid residues 25 to 35; 25 to 34; 25 to 33; 25 to 32; 25 to 31; 25 to 30; 26 to 35; 26 to 34; 26 to 33; 26 to 32; 26 to 31; 27 to 35; 27 to 34; 27 to 33; 27 to 32; 28 to 35; 28 to 34; 28 to 33; 29 to 35; 29 to 34 and/or 30 to 35 of the human CXCR3 set forth in SEQ ID NO: 4 or corresponding residues of another CXCR3 polypeptide. In further embodiments the antibodies and antigen-binding fragments of the present invention specifically bind to an epitope or polypeptide consisting of amino acid residues 25 to 35; 25 to 34; 25 to 33; 25 to 32; 25 to 31; 25 to 30; 26 to 35; 26 to 34; 26 to 33; 26 to 32; 26 to 31; 27 to 35; 27 to 34; 27 to 33; 27 to 32; 28 to 35; 28 to 34; 28 to 33; 29 to 35; 29 to 34 and/or 30 to 35 of the human CXCR3 set forth in SEQ ID NO:4 or corresponding residues of another CXCR3 polypeptide.

[0067] The epitope recognized by the antibodies and antigen binding fragments of the present invention is highly conserved among species, such that the antibodies and antigen binding fragments recognize CXCR3 polypeptides across a range of species,

including, but not limited to, human CXCR3, mouse CXCR3, and marmoset CXCR3. This species cross-reactivity provides significant advantages, as preclinical safety and efficacy studies can be performed in small animal models using antibodies with the same specificity as those intended for use in humans.

[0068] The antibodies and antigen binding fragments of the present invention that specifically bind to CXCR3 can have a variety of activities. In some aspects, the antibodies and fragments thereof inhibit CXCR3 interaction with one or more ligands, including, but not limited to, CXCL9, CXCL10 and/or CXCL11. As a result, the antibodies and antigen-binding fragments of the present invention can inhibit migration of cells expressing CXCR3 (*i.e.* $CXCR3^+$ cells). In further aspects, the antibodies and antigen-binding fragments can facilitate depletion of $CXCR3^+$ cells. For example, the antibodies and antigen-binding fragments can facilitate depletion of $CD4^+/CXCR3^+$ T cells, including activated and effector $CD4^+$ T cells ($CD4^+/CXCR3^+/CD44^{high}/CD62l^{low}$) and memory $CD4^+$ T cells ($CD4^+/CXCR3^+/CD44^{high}/CD62l^{low}$). The antibodies and antigen-binding fragments can also facilitate depletion of $CD8^+/CXCR3^+$ T cells and/or $B220^+/CXCR3^+$ B cells. Depletion can be effected by any means, including but not limited to, antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC) and/or cytotoxicity mediated by a toxic moiety linked to the antibody or antigen-binding fragment thereof.

[0069] The antibodies of the present invention can be monoclonal or polyclonal antibodies. In particular aspects, the antibodies are monoclonal. Further, the antibodies and antigen-binding fragments that specifically bind to CXCR3 can be of any immunoglobulin type (*e.g.*, IgG, IgM, IgD, IgE, IgA and IgY), any class (*e.g.* IgGl, IgG2, IgG3, IgG4, IgAl and IgA2) or any subclass (*e.g.*, IgG2a and IgG2b). In embodiments of the invention that are directed to antigen-binding fragments that specifically bind to CXCR3, the antigen-binding fragments can be, for example, Fab fragments, Fab^1 fragments, $F(ab')_2$ fragments, Fv fragments, disulfide-linked Fvs (dsFv), Fd fragments, Fd' fragments, single-chain Fvs (scFv), single-chain Fabs (scFab), domain antibodies, diabodies, triabodies or tetrabodies. In some aspects, the antibodies and antigen-binding fragments of the present invention are mouse antibodies and antigen-binding fragments. In other aspects, the antibodies and antigen-binding fragments are human antibodies and

antigen-binding fragments. Still further, the antibodies and antigen-binding fragments of the present invention can be chimeric, or can be humanized.

[0070] In some aspects, the antibodies or antigen-binding fragments thereof are multivalent antibodies. Such multivalent antibodies can comprise a first binding domain comprising an anti-CXCR3 antibody or antigen-binding fragment described herein, and a second binding domain comprising the same or another anti-CXCR3 antibody or antigen-binding fragment, or comprising another antibody or antigen-binding fragment that is specific for a different epitope or antigen. Accordingly, the multivalent antibodies can be bivalent, trivalent, tetravalent, pentavalent, hexavalent, heptavalent, or of greater valency (i.e., containing 2, 3, 4, 5, 6, 7 or more antigen-binding sites). Such multivalent derivative antibodies can be monospecific, bispecific, trispecific or of greater multispecificity. In some examples, the multivalent antibodies are monospecific, containing two or more antigen-binding domains that specifically bind to the same CXCR3 epitope described herein. In other examples, the multivalent antibodies are multi specific, containing two or more antigen-binding domains that specifically bind to two or more different epitopes. The two or more epitopes may both be within CXCR3. Alternatively, at least one epitope can be the CXCR3 epitope described herein and at least one epitope can be another epitope within CXCR3 or an epitope within another polypeptide.

[0071] In particular embodiments, the antibodies and antigen-binding fragments thereof of the present invention that specifically bind to CXCR3 comprise a CDR-H1 comprising the amino acid sequence GFTFSYFA (SEQ ID NO:20) or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:20.

[0072] In some embodiments, the antibodies and antigen-binding fragments of the present invention comprise a CDR-H2 comprising the amino acid ISGGGGNT (SEQ ID NO:21) or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:21.

[0073] In further embodiments, the antibodies and antigen-binding fragments of the present invention comprise a CDR-H3 comprising the amino acid sequence VRQTYGGFGY (SEQ ID NO:22) or a sequence of amino acids having at least or at least

about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:22.

[0074] In other embodiments, the antibodies and antigen-binding fragments of the present invention comprise a CDR-L1 comprising the amino acid sequence KSLLHSNGITY (SEQ ID NO:23) or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:23.

[0075] In some embodiments, the antibodies and antigen-binding fragments of the present invention comprise a CDR-L2 comprising the amino acid sequence QMS (SEQ ID NO:24) or a sequence of amino acids having at least or at least about 66%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:24.

[0076] In further embodiments, the antibodies and antigen-binding fragments of the present invention comprise a CDR-L3 comprising the amino acid sequence AQNLELPRT (SEQ ID NO:25) or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:25.

[0077] In particular embodiments, the antibodies and antigen-binding fragments thereof of the present invention that specifically bind to CXCR3 comprise a CDR-H1 comprising the amino acid sequence set forth in SEQ ID NO:20 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:20; a CDR-H2 comprising the amino acid sequence set forth in SEQ ID NO:21 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:21; a CDR-H3 comprising the amino acid sequence set forth in SEQ ID NO:22 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:22; a CDR-L1: comprising the amino acid sequence set forth in SEQ ID NO:23 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:23; a CDR-L2 comprising the amino acid sequence set forth in SEQ ID

NO:24 or a sequence of amino acids having at least or at least about 66%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:24; and a CDR-L3 comprising the amino acid sequence set forth in SEQ ID NO:25 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:25.

[0078] In one embodiment, provided are antibodies and antigen-binding fragments that specifically bind to CXCR3 and that comprise a CDR-H1 with a sequence of amino acids set forth in SEQ ID NO:20; a CDR-H2 with a sequence of amino acids set forth in SEQ ID NO:21; a CDR-H3 with a sequence of amino acids set forth in SEQ ID NO:22; a CDR-L1 with a sequence of amino acids set forth in SEQ ID NO:23; a CDR-L2 with a sequence of amino acids set forth in SEQ ID NO:24; and a CDR-L3 with a sequence of amino acids set forth in SEQ ID NO:25.

[0079] In particular aspects of the present invention, the antibodies and antigen-binding fragments comprise a variable heavy chain comprising the sequence of amino acids set forth in SEQ ID NO:13 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:13.

[0080] In further aspects, the antibodies and antigen-binding fragments comprise a variable light chain comprising the sequence of amino acids set forth in SEQ ID NO:15 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:15.

[0081] In particular embodiments, the antibodies and antigen-binding fragments comprise a variable heavy chain comprising sequence of amino acids set forth in SEQ ID NO:13 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:13, and a variable light chain comprising a sequence of amino acids set forth in SEQ ID NO:15 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:15.

[0082] The antibodies and antigen-binding fragments of the present invention also include those that comprise a variable heavy chain comprising the sequence of amino acids set forth in SEQ ID NO:41 or a sequence of amino acids having at least or about 80%,

85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:41, and/or a variable light chain comprising a sequence of amino acids set forth in SEQ ID NO:42 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:42.

[0083] In some aspects, the antibodies and antigen binding fragments are mouse antibodies and comprise a mouse λ or κ light chain constant region, and/or a mouse α, δ, ε, γ or μ (*i.e.* IgA, IgD, IgE, IgG or IgM) heavy chain constant regions.

[0084] In other aspects, the antibodies and antigen-binding fragments of the present invention are chimeric antibodies and antigen-binding fragments. For example, provided are chimeric antibodies and antigen-binding fragments comprising mouse variable regions and human constant regions. Non-limiting examples include chimeric antibodies and antigen-binding fragments that comprise a variable heavy chain comprising a sequence of amino acids set forth in SEQ ID NO:13 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:13. In other examples, the chimeric antibodies and antigen-binding fragments comprise a variable light chain comprising a sequence of amino acids set forth in SEQ ID NO:15 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:15. In particular examples, the chimeric antibodies and antigen-binding fragments comprise a variable heavy chain comprising a sequence of amino acids set forth in SEQ ID NO:13 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:13, and a variable light chain comprising a sequence of amino acids set forth in SEQ ID NO:15 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:15. These chimeric antibodies and antigen-binding fragments can further comprise a human constant region, such as a human λ or κ light chain constant region, and/or a human α, δ, ε, γ or μ (*i.e.* IgA, IgD, IgE, IgG or IgM) heavy chain constant region.

[0085] The present invention also provides humanized antibodies and antigen-binding fragments that specifically bind to CXCR3. In particular examples, the humanized antibodies and antigen-binding fragments comprise a CDR-H1 comprising the amino acid

sequence set forth in SEQ ID NO:20 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:20; a CDR-H2 comprising the amino acid sequence set forth in SEQ ID NO:21 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:21; a CDR-H3 comprising the amino acid sequence set forth in SEQ ID NO:22 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:22; a CDR-L1: comprising the amino acid sequence set forth in SEQ ID NO:23 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:23; a CDR-L2 comprising the amino acid sequence set forth in SEQ ID NO:24 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:24; a CDR-L3 comprising the amino acid sequence set forth in SEQ ID NO:25 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:25; and human framework regions (FRs). In particular embodiments, the humanized antibodies and antigen binding fragments further comprise a human constant region, such as a human λ or κ light chain constant region, and/or a human α, δ, ε, γ or μ (*i.e.* IgA, IgD, IgE, IgG or IgM) heavy chain constant region. Non-limiting example, of humanized antibodies of the present invention include those that comprise a variable heavy chain comprising the sequence of amino acids set forth in SEQ ID NO:41 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:41, and/or a variable light chain comprising a sequence of amino acids set forth in SEQ ID NO:42 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:42.

[0086] In particular embodiments, the antibodies and antigen binding fragments provided herein comprise modifications in the Fc region to increase ADCC activity. For example, the chimeric, humanized or human antibodies and antigen binding fragments can comprise a constant region from human IgG1 that contains a triple mutation, S239D/A330L/I332E in the CH2 portion (SEQ ID NO:32). In other examples, the

antibodies and antigen binding fragments provided herein comprise modifications in the Fc region to decrease ADCC activity. For example, in some embodiments, the chimeric, humanized or human antibodies and antigen binding fragments comprise a constant region from human IgG1 that contains a triple mutation, L234F/L235E/P331S in the lower hinge and CH2 portion (SEQ ID NO:34).

Methods of producing and assessing the antibodies and antigen-binding fragments

[0087] The antibodies and antigen-binding fragments provided herein that specifically bind to CXCR3 can be produced by any suitable method known in the art for the generation and preparation of antibodies, including immunization techniques, antibody library screening, chemical synthesis, recombinant techniques and expression, and any combination thereof.

Generation and identification of antibodies

[0088] In some aspects, antibodies and antigen-binding fragments that specifically bind to an N-terminal epitope within amino acid residues 23 to 44 of the human CXCR3 set forth in SEQ ID NO:4 are generated using methods that utilize a polypeptide comprising amino acid residues 23 to 44 of the human CXCR3 set forth in SEQ ID NO:4, or comprising amino acid residues within this region, such as residues 25 to 35; 25 to 34; 25 to 33; 25 to 32; 25 to 31; 25 to 30; 26 to 35; 26 to 34; 26 to 33; 26 to 32; 26 to 31; 27 to 35; 27 to 34; 27 to 33; 27 to 32; 28 to 35; 28 to 34; 28 to 33; 29 to 35; 29 to 34 or 30 to 35 of the human CXCR3 set forth in SEQ ID NO: 4.

[0089] Any method known in the art to elicit antibodies to an antigen can be used to elicit antibodies to this region of CXCR3. Techniques for preparing monoclonal antibodies against antigens are well known in the art. For example, monoclonal antibodies specific for an antigen can be obtained by injecting a non-human subject, such as a mouse, with the antigen, then obtaining antibody-producing cells from the animal. For example, the spleen can be removed to obtain B-lymphocytes or peripheral blood lymphocytes (PBL) can be isolated. Alternatively, B lymphocytes can be isolated from peripheral blood lymphocytes (PBL) of human subjects that have been administered the antigen. The B-lymphocytes from immunised animals are then fused with myeloma cells to produce hybridomas, which are cloned. Positive clones that produce antibodies specific for the antigen are selected using standard techniques (e.g. ELISpot). The clones that produce

antibodies to the antigen are then cultured and the secreted antibodies are isolated from the hybridoma cultures. Alternatively, nucleic acid encoding antibody light and heavy chains can be amplified from the B lymphocytes to produce a recombinant antibody library, which can then be screened for antibodies that are specific for the antigen using methods well known in the art (see *e.g.* Hoogenboom (2005) Nat Biotech 23:1105-1117).

[0090] Monoclonal antibodies can be isolated and purified from hybridoma cultures or other compositions by a variety of well-established techniques including, but not limited to, affinity chromatography with Protein-A SEPHAROSE®, size-exclusion chromatography, and/or ion-exchange chromatography. After the initial raising of antibodies to the immunogen, the antibodies can be sequenced and subsequently prepared by recombinant techniques.

[0091] In instances where mouse monoclonal antibodies are produced, such antibodies can be humanised or chimerised using methods well known to those skilled in the art. Chimeric antibodies are recombinant proteins in which the variable regions of a human antibody have been replaced by the variable regions of, for example, a mouse antibody. Murine immunoglobulin variable domains can be cloned from nucleic acid encoding the antibody and chimeric antibodies produced using techniques well known in the art.

[0092] Techniques for producing humanized monoclonal antibodies by transferring the CDRs from the heavy and light variable chains of a mouse or chimeric antibody into the corresponding variable domains of a human antibody also are well known in the art. In some instances, further modifications are made in the human framework regions of the humanised antibody to increase affinity (see e.g. Tempest *et al.* (1991) Biotechnology 9:266-271). In other instances, humanized antibodies may be generated using the "Superhumanization" method described in international patent publication No. WO 2004/006955.

[0093] Alternatively, animals that have been genetically engineered to produce human antibodies can be used to generate antibodies against a polypeptide comprising amino acid residues 23 to 44 of the human CXCR3 set forth in SEQ ID NO:4, or comprising amino acid residues within this region, such as residues 25 to 35; 25 to 34; 25 to 33; 25 to 32; 25 to 31; 25 to 30; 26 to 35; 26 to 34; 26 to 33; 26 to 32; 26 to 31; 27 to 35;

27 to 34; 27 to 33; 27 to 32; 28 to 35; 28 to 34; 28 to 33; 29 to 35; 29 to 34 or 30 to 35 of the human CXCR3 set forth in SEQ ID NO: 4, using standard immunization protocols. Such mice are available commercially, for example, the XenoMouse® from Amgen (Thousand Oaks, CA) (described by Green et al., (1999) J. Immunol. Methods 231:11-23).

[0094] In further aspects, antibodies and antigen-binding fragments that specifically bind to an N-terminal epitope within amino acid residues 23 to 44 of the human CXCR3 set forth in SEQ ID NO:4 are selected by screening antibody libraries. Methods for generating and screening antibody libraries are well known in the art and can be used herein. For example, total RNA can be extracted from peripheral blood B lymphocytes and a cDNA library constructed by amplifying μ, γ and κ chain antibody repertoires. The cDNA library can then be used to make a display library. Exemplary of the display libraries for use in the described methods is a phage display library, in which antigen-binding fragments of antibodies, such as single chain Fv (scFv) fragments, are expressed on the surfaces of bacteriophages as fusion proteins with the bacteriophage coat protein. For display of antigen-binding fragments on phage, host cells capable of phage infection and packaging are transformed with phage vectors, typically phagemid vectors, containing polynucleotides encoding the antigen-binding fragments.

[0095] Peptides are then used to select ligands by panning, a process well known to those of skill in the art. For example, a polypeptide comprising amino acid residues 23 to 44 of the human CXCR3 set forth in SEQ ID NO:4, or comprising amino acid residues within this region, such as residues 25 to 35; 25 to 34; 25 to 33; 25 to 32; 25 to 31; 25 to 30; 26 to 35; 26 to 34; 26 to 33; 26 to 32; 26 to 31; 27 to 35; 27 to 34; 27 to 33; 27 to 32; 28 to 35; 28 to 34; 28 to 33; 29 to 35; 29 to 34 or 30 to 35 of the human CXCR3 set forth in SEQ ID NO: 4, can be incubated with the phage library for a sufficient time to permit binding. Following incubation, the peptide-library mixture is washed and non-binding library members are washed away. The remaining bound phage can be eluted using one of several well known elution methods. The selection methods are typically an iterative process with multiple rounds of panning. Such iteration permits identification of antibodies or antigen-binding fragments thereof with improved binding affinity. Generally, selected phage are used in multiple additional rounds of screening, by pooling the eluted phage, propagation of nucleic acids encoding the antigen-binding fragments in host cells, expression (e.g. phage display) of the selected antigen-binding fragments, and a

subsequent round of panning. Multiple rounds, e.g. 2, 3, 4, 5, 6, 7, 8, or more rounds, of screening can be performed. Negative panning steps may also be used, for example to remove phage which bind to non-relevant molecules, such as the carrier protein components of a glycan/carrier protein conjugate.

[0096] Host cells can then be infected with selected phage, and individual clones can be picked and grown up for plasmid purification using any method known to one of skill in the art. The purified plasmid can be used for nucleic acid sequencing to identify the sequence of the nucleic acid encoding the antigen-binding fragment and, by extrapolation, the sequence of antigen-binding fragment, or can be used to transfect into any cell for expression, such as by not limited to, a mammalian expression system. Purified proteins also can be tested in subsequent *in vitro* or *in vivo* assays for binding activity. If desired, the antigen-binding fragments, such as the scFv fragments, can be converted to Fab or full length IgG molecules using standard methods well known in the art.

Nucleic acids and expression

[0097] In part, the present invention is also directed to nucleic acid molecules, including isolated nucleic acid molecules, encoding the antibodies and antigen-binding fragments of the present invention. In particular aspects, therefore, provided are nucleic acid molecules encoding antibodies or antigen binding fragments comprising a CDR with a sequence of amino acids set forth in any one of SEQ ID NOS: 20-25, or a CDR with a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any one of SEQ ID NOS: 20-25. In a particular embodiment, the nucleic acid molecules comprise a sequence of nucleotides set forth in any one of SEQ ID NOS: 26-31, or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any one of SEQ ID NOS:26-31. In other embodiments, the nucleic acid molecules comprise a sequence of nucleotides set forth in any one of SEQ ID NOS: 45-50, or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to any one of SEQ ID NOS:45-50.

[0098] In one example, the present invention provides nucleic acid molecules encoding an antibody or antigen binding fragment comprising a CDR-H1 comprising the

amino acid sequence set forth in SEQ ID NO:20 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:20; a CDR-H2 comprising the amino acid sequence set forth in SEQ ID NO:21 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:21; and a CDR-H3 comprising the amino acid sequence set forth in SEQ ID NO:22 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:22. In particular examples, the nucleic acid molecules comprise a sequence of nucleotides set forth in SEQ ID NO: 26 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:26; a sequence of nucleotides set forth in SEQ ID NO: 27 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:27; and a sequence of nucleotides set forth in SEQ ID NO: 28 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:28. In other examples, the nucleic acid molecules comprise a sequence of nucleotides set forth in SEQ ID NO: 45 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:45; a sequence of nucleotides set forth in SEQ ID NO:46 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:46; and a sequence of nucleotides set forth in SEQ ID NO:47 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:47.

[0099] In another example, the present invention provides nucleic acid molecules encoding an antibody or antigen binding fragment comprising a CDR-L1: comprising the amino acid sequence set forth in SEQ ID NO:23 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:23; a CDR-L2 comprising the amino acid sequence set forth in SEQ ID NO:24 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more

sequence identity to SEQ ID NO:24; and a CDR-L3 comprising the amino acid sequence set forth in SEQ ID NO:25 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:25. In particular embodiments, the nucleic acid molecules comprise a sequence of nucleotides set forth in SEQ ID NO: 29 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:29; a sequence of nucleotides set forth in SEQ ID NO: 30 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:30; and a sequence of nucleotides set forth in SEQ ID NO: 31 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:31. In other embodiments, the nucleic acid molecules comprise a sequence of nucleotides set forth in SEQ ID NO: 48 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:48; a sequence of nucleotides set forth in SEQ ID NO: 30 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:48; and a sequence of nucleotides set forth in SEQ ID NO:48 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:48.

[00100] In further embodiments, the nucleic acid molecules of the present invention encode a variable heavy chain region comprising sequence of amino acids set forth in SEQ ID NO:13 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:13. In one example, the nucleic acid molecules comprise a sequence of nucleotides set forth in SEQ ID NO:12 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:12.

[00101] In further aspects, the nucleic acid molecules of the present invention encode a variable light chain comprising a sequence of amino acids set forth in SEQ ID NO:15 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%,

95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:15. In one example, the nucleic acid molecules comprise a sequence of nucleotides set forth in SEQ ID NO:14 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:14.

[00102] The nucleic acid molecules of the present invention may also encode a variable heavy chain region comprising sequence of amino acids set forth in SEQ ID NO:41 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:41. In one example, the nucleic acid molecules comprise a sequence of nucleotides set forth in SEQ ID NO:43 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:43.

[00103] In other aspects, the nucleic acid molecules of the present invention encode a variable light chain comprising a sequence of amino acids set forth in SEQ ID NO:42 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:42. In one example, the nucleic acid molecules comprise a sequence of nucleotides set forth in SEQ ID NO:44 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:44.

[00104] Nucleic acid molecules encoding the antibodies or antigen-binding fragments thereof provided herein can be prepared using well-known recombinant techniques for manipulation of nucleic acid molecules. In some examples, methods, such as, but not limited to, recombinant DNA techniques, site directed mutagenesis, and polymerase chain reaction (PCR) can be used to generate nucleic acid molecules encoding modified antibodies or antigen-binding fragments thereof having a different amino acid sequence, for example, to create amino acid substitutions, deletions, and/or insertions.

[00105] In particular examples, one or more of the CDRs of an antibody or antigen-binding fragment thereof provided herein is inserted within framework regions using routine recombinant DNA techniques. The framework regions can be selected from naturally occurring or consensus framework regions, including human framework regions. Generally, the polynucleotide generated by the combination of the framework regions and

CDRs encodes an antibody or antigen-binding fragment thereof that maintains the antigen-binding specificity of the parent anti-CXCR3 antibody or antigen-binding fragment thereof. Alterations to the polynucleotide can be made to improve one or more properties of the encoded antibody or antigen-binding fragment thereof and within the skill of the art. In some examples, one or more modifications of the polynucleotide can be made to produce amino acid substitutions within the framework regions, which, for example, improve binding of the antibody or antigen-binding fragment thereof to its antigen.

[00106] The nucleic acid molecules of the present invention can be incorporated into suitable vectors and constructs for expression of the encoded antibody or antigen-binding fragment. Generally, nucleic acid encoding the heavy chain of an antibody is cloned into a vector and the nucleic acid encoding the light chain of an antibody is cloned into the vector. The genes can be cloned into a single vector for dual expression thereof, or into separate vectors. If desired, the vectors also can contain further sequences encoding additional constant region(s) or hinge regions to generate other antibody forms.

[00107] Many expression vectors are available and known to those of skill in the art and can be used for expression of polypeptides. The choice of expression vector will be influenced by the choice of host expression system. Such selection is well within the level of skill of the skilled artisan. Exemplary expression systems include prokaryotic expression systems, and insect, yeast and mammalian cell expression systems. In general, expression vectors can include transcriptional promoters and optionally enhancers, translational signals, and transcriptional and translational termination signals. Expression vectors that are used for stable transformation typically have a selectable marker which allows selection and maintenance of the transformed cells. In some cases, an origin of replication can be used to amplify the copy number of the vector in the cells. Antibodies and antigen-binding fragments produced from recombinant expression of the nucleic acids molecules, or produced by any other method described herein or known in the art, can be purified or isolated by any means known to a skilled artisan.

Modification of antibodies

[00108] Any of the antibodies and antigen-binding fragments provided herein can be further modified using methods well known in the art. For example, modifications can be made to increase binding, by for example, affinity maturation, or to decrease

immunogenicity by removing predicted MHC class II-binding motifs. The therapeutic utility of the antibodies and antigen-binding fragments thereof described herein can be further enhanced by modulating their functional characteristics, such as ADCC, CDC, serum half-life, biodistribution and binding to Fc receptors or the combination of any of these. This modulation can be achieved by protein-engineering, glyco-engineering or chemical methods.

[00109] Numerous methods for affinity maturation of antibodies are known in the art. Many of these are based on the general strategy of generating panels or libraries of variant proteins by mutagenesis followed by selection and/or screening for improved affinity, such as by selection by panning methods described above. Mutagenesis is often performed at the DNA level, for example by error prone PCR, by gene shuffling, by use of mutagenic chemicals or irradiation, by use of 'mutator' strains with error prone replication machinery or by somatic hypermutation approaches that harness natural affinity maturation machinery. Mutagenesis can also be performed at the RNA level, for example by use of Qβ replicase. Library-based methods allowing screening for improved variant proteins can be based on various display technologies such as phage, yeast, ribosome, bacterial or mammalian cells, and are well known in the art. Affinity maturation can be achieved by more directed/predictive methods for example by site-directed mutagenesis or gene synthesis guided by findings from 3D protein modelling.

[00110] Methods of increasing ADCC and/or CDC have been described and are well known in the art. For example, the elimination of fucose from sugar chains on an antibody enhances ADCC, thereby enhancing anti-tumour activity of the antibody of antigen-binding fragment thereof. This can be achieved by producing the antibodies from host cells that lack the FUT8 gene, the product of which is responsible for the fucose addition to sugar chains (*i.e.* Potelligent® Cells). In other examples, mutations can be incorporated into the Fc portion of the antibody, as described above and in Example 5.

[00111] In further examples, the antibodies and antigen binding fragments may be linked to one or more moieties, such as by recombinant means to generate fusion polypeptides, or by conjugation or by other linking means to generate immuno-conjugates. Exemplary moieties include, but are not limited to, PEG moieties (*i.e.* to produce peglyated antibodies and antigen binding fragments that exhibit increased half-life and/or improved pharmacokinetic profiles), cytotoxic or cytostatic moieties (*e.g.* cytotoxic or cytostatic

small molecules, peptides or polypeptides), detectable moieties (*e.g.* fluorescent dyes, luminescent or chemiluminescent labels, radiolabels, radioisotopes, metallic ions, enzymes, colorimetric labels and magnetic beads), immunomodulating agents, including immunosuppressive agents (*e.g.* cytokines, chemokines, glucocorticoids, alkylating agents, antimetabolites, cyclosporine, rapamycin and tacrolimus), and other polypeptide moieties, such as albumin to improve half-life. The antibodies and antigen-binding fragments may also be glycoengineered to alter the glycan structures on the antibodies and fragments.

Methods of assessing the antibodies

[00112] The antibodies and antigen-binding fragments thereof provided herein can be characterized in a variety of ways well-known to one of skill in the art. For example, the anti-CXCR3 antibodies or antigen-binding fragments thereof provided herein can be assayed for the ability to specifically bind to CXCR3, or the N-terminal epitope within amino acid residues 23 to 44 of human CXCR3. Binding assays can be performed in solution, suspension or on a solid support. For example, target antigens can be immobilized to a solid support (e.g. a carbon or plastic surface, a tissue culture dish or chip) and contacted with antibody or antigen-binding fragment thereof. Unbound antibody or target protein can be washed away and bound complexes can then be detected. Binding assays can be performed under conditions to reduce nonspecific binding, such as by using a high ionic strength buffer (e.g., 0.3-0.4 M NaCl) with nonionic detergent (e.g. 0.1% Triton X-100 or Tween 20) and/or blocking proteins (e.g. bovine serum albumin or gelatin). Negative controls also can be included in such assays as a measure of background binding. Binding affinities can be determined using Scatchard analysis, surface plasmon resonance, isothermal calorimetry, or other methods known to one of skill in the art. Exemplary immunoassays which can be used to analyze specific binding and cross-reactivity include, but are not limited to, competitive and non-competitive assay systems using techniques such as, but not limited to, western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), Meso Scale Discovery (MSD, Gaithersburg, Maryland), "sandwich" immunoassays, immunoprecipitation assays, ELISPOT, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, and protein A immunoassays. In further examples, flow cytometry can be used to assess the ability of antibodies and antigen-binding fragments to recognize cells expressing CXCR3.

[00113] Binding specificity of the antibodies and fragments can be assessed using methods well known in the art, including those methods described below in Example 3. In particular examples, competition assays, such as competition ELISAs, can be used to confirm that the antibodies and fragments thereof specifically bind to the same N-terminal epitope within amino acid residues 23 to 44 of human CXCR3 as the 9C5 antibody described herein. In such assays, for example, the antigen is incubated in the presence of a predetermined limiting dilution of a labelled antibody (e.g., 50-70 % saturation concentration), and serial dilutions of an unlabeled competing antibody. Competition is determined by measuring the binding of the labelled antibody to the antigen for any decreases in binding in the presence of the competing antibody. Variations of such assays, including various labelling techniques and detection methods including, for example, radiometric, fluorescent, enzymatic and colorimetric detection, are known in the art.

[00114] The activity of the antibodies and antigen-binding fragments can be assessed using a variety of *in vitro* and *in vivo* methods well known in the art, including those exemplified in the Examples below. The ability of the antibodies and antigen-binding fragments to facilitate depletion of $CXCR3^+$ cells *in vivo* can be assessed, for example, by administering the antibodies or antigen-binding fragments to a subject, such as a mouse. The depletion of $CXCR3^+$ cells can then be determined by removing the spleen and isolating splenocytes, then staining the lymphocytes with appropriate antibodies to cell surface molecules, including $CXCR3^-$, and visualizing the cell populations by flow cytometry. In further examples, *in vitro* chemotaxis assays can be used to assess the ability of the antibodies and antigen-binding fragments to inhibit CXCR3-mediated chemotaxis. Briefly, CXCR3 transfected cells (*e.g.* L1.2 cells) can be added to the top chamber of a transwell plate with or without the antibodies or antigen-binding fragments, and CXCL9, CXCL10 and/or CXCL11 added to the bottom chamber. The number of cells that migrate to the bottom chamber can then be assessed by, for example, flow cytometry.

[00115] Small animal models also can be used to assess *in vivo* activities of the antibodies and antigen-binding fragments. For example, the ability of the antibodies and antigen-binding fragments to delay or prevent graft rejection can be assessed using the pancreatic islet allograft transplant model described in Example 7, below. Antibodies or antigen binding fragments can be administered to diabetic recipient mice receiving the graft before, during and/or after transplantation, and the graft survival monitored by

assessing blood glucose levels. In other example, a murine cardiac allograft model can be used, in which donor aorta and pulmonary artery are anastomosed to the recipient abdominal aorta and inferior vena cava, respectively. Graft function is assessed by palpation in the recipient mice with or without administration of the antibodies or antigen-binding fragments.

Uses of the antibodies and antigen-binding fragments

[00116] The antibodies and antigen-binding fragments of the present invention can be used in a variety of *in vitro* and *in vivo* methods. In particular examples, the antibodies and antigen-binding fragments of the present invention, including antibodies and antigen-binding fragments that are fusion proteins or immunoconjugates, can bind a CXCR3 polypeptide and can be used to detect, measure, select, isolate and/or purify a CXCR3 polypeptide or variants thereof or cells expressing CXCR3, and to study CXCR3 protein structure and function. The antibodies and antigen-binding fragments of the present invention also can be used in diagnostic applications (e.g., *in vitro* or *ex vivo*) and/or in therapeutic applications.

[00117] The antibodies and antigen-binding fragments can be used to detect and/or measure the level of a CXCR3 polypeptide or $CXCR3^+$ cells in a sample (*e.g.*, tissue or body fluid). In one example, a sample (e.g., tissue, cells and/or body fluid) is obtained from an individual and a suitable immunological method used to detect and/or measure CXCR3 expression and/or $CXCR3^+$ cell number and location. Suitable immunological methods for detecting or measuring CXCR3 expression include, but are not limited, to enzyme-linked immunosorbent assays (ELISA), radioimmunoassay, immunohistology and flow cytometry.

[00118] Accordingly, in particular embodiments, the present invention is directed to methods of detecting or measuring CXCR3 in a sample, comprising contacting the sample with an antibody or antigen-binding fragment of the present invention under conditions suitable for binding of the antibody or antigen-binding fragment to the CXCR3 polypeptide, and detecting and/or measuring binding of the antibody or antigen-binding fragment to the CXCR3 polypeptide. Binding of the antibody or antigen-binding fragment thereof to the CXCR3 polypeptide indicates the presence of the CXCR3 polypeptide in the sample. In some examples, binding of the antibody or antigen-binding fragment thereof to

CXCR3 is assessed by detecting the antibody or antigen-binding fragment. For example, the antibody or antigen-binding fragment can have a detectable label, such as a fluorescent or chemiluminescent label that allows direct detection of the antibody or antigen-binding fragment. In other examples, the antibody or antigen-binding fragment is detected indirectly, such as by using antibodies specific for the Fc region of the antibody or antigen-binding fragment. Samples can include, but are not limited to, blood, cells or tissue. Typically, the CXCR3 is expressed on a cell. Accordingly, the antibodies and antigen-binding fragments of the present invention can be used to detect or measure $CXCR3^+$ cells in a sample, comprising contacting the sample with an antibody or antigen-binding fragment thereof of the present invention under conditions suitable for binding of the antibody or antigen-binding fragment to a $CXCR3^+$ cell, and detecting and/or measuring binding of the antibody or antigen-binding fragment to the $CXCR3^+$ cell. In particular applications, an antibody or antigen-binding fragment of the invention can be used to analyze normal versus inflamed tissues (*e.g.*, from a human) for $CXCR3^+$ cells to detect associations between disease (*e.g.*, graft rejection) and increased expression of a CXCR3 polypeptide in affected tissues.

Therapeutic uses

[00119] In particular embodiments, the antibodies and antigen-binding fragments of the present invention are used in therapeutic methods. In some embodiments, the antibodies and antigen-binding fragments are used in methods to inhibit CXCR3 binding to one or more ligands, such as CXCL9, CXCL10 and/or CXCL11; inhibit migration, accumulation, recruitment or infiltration of $CXCR3^+$ cells, such as to a site of inflammation; and/or deplete $CXCR3^+$ cells. The $CXCR3^+$ cells include, but are not limited to, $CXCR3^+/CD4^+$ T cells, $CXCR3^+/CD8^+$ T cells and $CXCR3^+/B220^+$ B cells.

[00120] Accordingly, provided are methods for inhibiting the recruitment or migration of $CXCR3^+$ cells to an inflammation site in a subject, comprising administering to the subject an effective amount of an antibody or antigen-binding fragment of the present invention. Inhibition can include inhibition of at least or about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% compared to the recruitment or migration of $CXCR3^+$ cells to an inflammation site in the absence of the antibody or antigen-binding fragment.

[00121] Also provided are methods of depleting $CXCR3^+$ cells in a subject, comprising administering to the subject an effective amount of an antibody or antigen-binding fragment of the present invention. Depletion of $CXCR3^+$ cells can include depletion of at least or about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or more.

[00122] In particular embodiments, the antibodies and antigen-binding fragments of the present invention can be used in methods for treating or preventing a disease, disorder or condition that is associated with CXCR3-mediated recruitment or migration of cells to an inflammation site, and/or with the activity of $CXCR3^+$ cells. Exemplary diseases, disorders and conditions include, but are not limited to, inflammatory or allergic diseases and conditions, including systemic anaphylaxis or hypersensitivity responses, drug allergies, insect sting allergies; inflammatory bowel diseases, such as Crohn's disease, ulcerative colitis, celiac disease, ileitis and enteritis; sarcoidosis; vaginitis; psoriasis and inflammatory dermatoses such as dermatitis, eczema, atopic dermatitis, allergic contact dermatitis, urticaria; vasculitis (*e.g.*, necrotizing, cutaneous, and hypersensitivity vasculitis); spondyloarthropathies; scleroderma; respiratory allergic diseases such as asthma, allergic rhinitis, hypersensitivity lung diseases, hypersensitivity pneumonitis, interstitial lung diseases (ILD) (e.g., idiopathic pulmonary fibrosis, or ILD associated with rheumatoid arthritis, or other autoimmune conditions); autoimmune diseases, such as arthritis (e.g., rheumatoid arthritis, psoriatic arthritis), multiple sclerosis, systemic lupus erythematosus, myasthenia gravis, diabetes, including diabetes mellitus and juvenile onset diabetes, glomerulonephritis and other nephritides, autoimmune thyroiditis, Behcet's syndrome, Sydenham's chorea, autoimmune autonomic neuropathy; and graft or transplant rejection, including allograft or allotransplant rejection, xenograft or xenotransplant rejection or graft-versus-host disease.

[00123] In particular embodiments, provided are methods for promoting graft or transplant survival in a subject, comprising administering to the subject an antibody or antigen-binding fragment of the present invention. Accordingly, also provided are methods for inhibiting or preventing graft or transplant rejection in a subject, comprising administering to the subject an antibody or antigen-binding fragment of the present invention. The subject is one who has already received, is currently receiving or will receive, the graft or transplant. Accordingly, the antibody or antigen-binding fragment can

be administered before, at the same time, or after, the subject receives the graft or transplant, or at more than one of these times.

[00124] The methods of the present invention that are directed to promoting graft or transplant survival and/or inhibiting or preventing graft or transplant rejection in a subject are applicable to any type of graft or transplant, including but not limited to, cell, tissue, organ and limb grafts or transplants. Exemplary grafts and transplants include, but are not limited to, heart, kidney, lung, liver, pancreas, pancreatic islets, brain tissue, stomach, large intestine, small intestine, cornea, skin, trachea, bone, bone marrow, muscle, bladder or parts and/or combinations thereof. In particular embodiments the methods are for promoting the survival, or inhibiting the rejection, of solid organ transplants, such as kidney, heart, lung, liver, intestine and pancreas transplants.

Formulations, dosages and modes of administration

[00125] The antibodies and antigen-binding fragments of the present invention that specifically bind to CXCR3 can be formulated for *in vitro* or *in vivo* use. For example, in some aspects, the antibodies and antigen-binding fragments are formulated for *in vitro* use, such as for use in *in vitro* assays to detect CXCR3. In other examples, the antibodies and antigen-binding fragments are formulated for *in vivo* use, such as for administration to a subject.

[00126] In particular embodiments of the present invention, the antibodies and antigen-binding fragments are formulated as pharmaceutical compositions and administered to a subject for diagnostic or therapeutic purposes.

[00127] For *in vivo* administration to a subject, compositions containing the antibodies and antigen-binding fragments are prepared in view of approval from a regulatory agency or otherwise prepared in accordance with generally recognized pharmacopeia for use in animals and in humans. Compositions can contain, in addition to the antibody or antigen-binding fragment, a diluent such as lactose, sucrose, dicalcium phosphate, or carboxymethylcellulose; a lubricant, such as magnesium stearate, calcium stearate and talc; and a binder such as starch, natural gums, such as gum acaciagelatin, glucose, molasses, polvinylpyrrolidine, celluloses and derivatives thereof, povidone, crospovidones and other such binders known to those of skill in the art. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice,

flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, and ethanol. A pharmaceutical composition, if desired, also can contain minor amounts of wetting or emulsifying agents, or pH buffering agents, for example, acetate, sodium citrate, cyclodextrine derivatives, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, and other such agents.

[00128] The compositions can be formulated for administration by any route. The most appropriate route of administration can be determined by a person of skill in the art, taking into account the particular use, such as the particular disease, disorder or condition being treated. For example, the compositions can be formulated for parenteral, intravenous, intraarterial, subcutaneous, intramuscular, intracranial, intraorbital, ophthalmic, intraventricular, intranasal, or oral administration. In particular embodiments, pharmaceutical compositions comprising the anti-CXCR3 antibodies or antigen-binding fragments are in the form of liquid solutions or suspensions for intravenous administration. Also encompassed by the present invention are formulations for controlled release of anti-CXCR3 antibodies or antigen-binding fragments of the present invention.

[00129] The compositions comprising the antibodies and antigen-binding fragments of the present invention are formulated with an amount or concentration of antibody or antigen-binding fragment that is suitable for the embodiments of the present invention, *e.g.* at therapeutically effective concentrations, such as to promote graft survival or inhibit graft rejection when administered to a subject. The compositions can be formulated for direct administration to a subject, or can be formulated as a concentrated composition that is subsequently diluted prior to use. In particular embodiments, the compositions are in liquid form and are formulated with between about 1 ng/mL to about 100 mg/mL of an antibody or antigen-binding fragment, between about 10 ng/mL and about 10 mg/mL, between about 100 ng/mL and about 10 mg/mL, between about 1 µg/mL and about 10 mg/mL, between about 10 µg/mL and about 1 mg/mL, or between about 100 µg/mL and about 1 mg/mL of and antibody and antigen-binding fragment. The most suitable concentration to achieve the desired effect will depend on a number of factors and may be determined by those skilled in the art using routine experimentation.

[00130] The compositions comprising the anti-CXCR3 antibodies and antigen-binding fragments can also contain one or more additional active agents. For example, the

compositions provided herein can include one or more other active agents useful in the treatment of a disease, condition or disorder that is to be treated by administration of the anti-CXCR3 antibodies and antigen-binding fragments of the present invention. In particular embodiments, the compositions further comprise one or more other immunosuppressive or immunomodulatory agents, such as, for example, another antibody or antigen-binding fragments thereof, cytokine, chemokine, glucocorticoid, alkylating agent, antimetabolite, or calcineurin inhibitor. Non-limiting examples of immunosuppressive agents that can be included in the compositions of the present invention include, but are not limited to, glucocorticoids, cyclosporine, rapamycin, voclosporin, sirolimus, everolimus, tacrolimus, mycophenolate mofetil, mycophenolic acid, mizoribine, S1P-R agonists such as FTY720, malononitrilamides such as FK778, anti-CD3 monoclonal antibodies such as muromonab-CD3, anti -CD25 antibodies such as daclizumab and basiliximab, anti-CD52 antibodies such as alemtuzumab; anti-CD20 antibodies such as rituximab, and anti-tumor necrosis factor (TNF) antibodies such as infliximab, etanercept and adalimumab.

[00131] The compositions can be administered to a subject in the desired amount, such as an amount sufficient for diagnostic purposes or a therapeutically effective amount. For example, the compositions can be administered in an amount to promote graft or transplant survival, or inhibit graft or transplant rejection. The precise amount or dose of the antibody or antigen-binding fragment that is administered to the subject depends on several factors, including, but not limited to, the activity of the antibody or antigen-binding fragment, the use of other therapeutic agents, the route of administration, the number of dosages administered, and other considerations, such as the weight, age and general state of the subject. Particular dosages can be empirically determined or extrapolated from, for example, studies in animal models or previous studies in humans.

[00132] The pharmaceutical compositions containing the anti-CXCR3 antibodies and antigen-binding fragments of the present invention can be administered by any method and route understood to be suitable by a skilled artisan. Administration may be systemic, regional or local as determined by a skilled person on the basis of a variety of factors, including the condition, disease or disorder to be treated, activity of the therapeutic agent and state of the subject. For example, in some instances, regional administration may be most appropriate, as this provides the capability of delivering very high local

concentrations of the desired agent to the required site and thus is suitable for achieving the desired therapeutic or preventative effect whilst avoiding exposure of other organs of the body to the compound and thereby potentially reducing side effects.

[00133] Exemplary routes of administration include, but are not limited to, intravenous (including by discrete injection, intravenous bolus or continuous infusion), intramuscular, intradermal, transdermal, parenteral, intracranial, intraarterial, intraorbital, subcutaneous, intranasal, oral, intraperitoneal or topical administration, as well as by any combination of any two or more thereof, formulated in a manner suitable for each route of administration. The anti-CXCR3 antibody or antigen-binding fragment can be administered once or more than once, such 2, 3, 4, 5, 6 or more times, daily, weekly, bi-weekly, monthly or any combination thereof.

[00134] In the particular methods provided herein, a composition comprising an anti-CXCR3 antibody or antigen-binding fragment of the present invention is administered to a subject before, during and/or after the subject has received a graft or transplant, such as an allograft or xenograft, or allotransplant or xenotransplant. For example, a composition comprising the antibody or antigen-binding fragment can be administered to a subject at any time before, during and/or after the subject received a graft or transplant, such as 1 day before, 12 hours before, 2 hours before, at the same time, and/or 1 hour, 2 hours, 6 hours, 12 hours , 18 hours, 24 hours, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 2 weeks, 3 weeks, 4 weeks or more after the subject has received a graft or transplant, or any combination thereof.

[00135] In some embodiments of the present invention, the anti-CXCR3 antibodies or antigen-binding fragments are administered to a subject in combination with one or more other therapeutic agents. In embodiments where a subject is administered an anti-CXCR3 antibody or antigen-binding fragment and one or more other therapeutic agents, the antibody or antigen-binding fragment and the one or more other therapeutic agents can be in the same or different compositions, and can be administered by the same or different routes, at the same time or at different times.

[00136] In one embodiment, a subject about to receive, receiving or having received a graft or transplant can be administered an anti-CXCR3 antibody or antigen-binding fragment of the present invention and another therapeutic agent. For example, the anti-

CXCR3 antibody or antigen-binding fragment of the present invention may be administered in combination with an agent that induces or expands regulatory T cells (Treg cells), such as $CD4^+/CD25^+/Fox3p^+$ T cells. Exemplary agents include, but are not limited to, interleukin 2 (IL-2). In further examples, the anti-CXCR3 antibody or antigen-binding fragment may be administered in combination with an immunosuppressive agent, including but are not limited to, a glucocorticoid, cyclosporine, rapamycin, voclosporin, sirolimus, everolimus, tacrolimus, mycophenolate mofetil, mycophenolic acid, mizoribine, S1P-R agonist such as FTY720, malononitrilamide such as FK778, anti-CD3 antibody such as muromonab-CD3, anti -CD25 antibody such as daclizumab and basiliximab, anti-CD52 antibody such as alemtuzumab, anti-CD20 antibody such as rituximab, and anti-tumor necrosis factor (TNF) antibody such as infliximab, etanercept and adalimumab. In such instances, the anti-CXCR3 antibody or antigen-binding fragment can be administered simultaneously with and/or sequentially to the other therapeutic agent.

Epitope peptides and uses thereof

[00137] One aspect of the present invention is directed to isolated peptides comprising or consisting of the epitope to which the antibodies and antigen binding fragments described herein bind. Accordingly, provided herein are peptides comprising or consisting of amino acid residues 23 to 44 of SEQ ID NO:4 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 9%, 95%, 96%, 97%, 98% or 99% sequence identity to amino acid residues 23 to 44 of SEQ ID NO:4. Also provided are peptides comprising or consisting of amino acid residues 23 to 43; 23 to 42; 23 to 41; 23 to 40; 23 to 39; 23 to 38; 23 to 37; 23 to 36; 23 to 35; 23 to 34; 23 to 33; 23 to 32; 23 to 31; 23 to 30; 23 to 29; 24 to 44; 24 to 43; 24 to 42; 24 to 41; 24 to 40; 24 to 39; 24 to 38; 24 to 37; 24 to 36; 24 to 35; 24 to 34; 24 to 33; 24 to 32; 24 to 31; 24 to 30; 24 to 29; 25 to 44; 25 to 43; 25 to 42; 25 to 41; 25 to 40; 25 to 39; 25 to 38; 25 to 37; 25 to 36; 25 to 35; 25 to 34; 25 to 33; 25 to 32; 25 to 31; 25 to 30; 26 to 44; 26 to 43; 26 to 42; 26 to 41; 26 to 40; 26 to 39; 26 to 38; 26 to 37; 26 to 36; 26 to 35; 26 to 34; 26 to 33; 26 to 32; 26 to 31; 27 to 44; 27 to 43; 27 to 42; 27 to 41; 27 to 40; 27 to 39; 27 to 38; 27 to 37; 27 to 36; 27 to 35; 27 to 34; 27 to 33; 27 to 32; 28 to 44; 28 to 43; 28 to 42; 28 to 41; 28 to 40; 28 to 39; 28 to 38; 28 to 37; 28 to 36; 28 to 35; 28 to 34; 28 to 33; 28 to 32; 29 to 44; 29 to 43; 29 to 42; 29 to 41; 29 to 40; 29 to 39; 29 to 38; 29 to 37; 29 to 36; 29 to 35; 29 to 34; 30 to 44; 30 to 43; 30 to 42; 30 to 41; 30 to 40; 30 to 39; 30 to 38; 30 to 37; 30 to 36; and/or 30 to 35

of SEQ ID NO:4, or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 9%, 95%, 96%, 97%, 98% or 99% sequence identity.

[00138] In particular embodiments, the peptides consist of amino acid residues 25 to 35; 25 to 34; 25 to 33; 25 to 32; 25 to 31; 25 to 30; 26 to 35; 26 to 34; 26 to 33; 26 to 32; 26 to 31; 27 to 35; 27 to 34; 27 to 33; 27 to 32; 28 to 35; 28 to 34; 28 to 33; 29 to 35; 29 to 34 and/or 30 to 35 of SEQ ID NO: 4.

[00139] The peptides comprising or consisting of the epitope to which the antibodies and antigen binding peptides described herein bind can be fused, conjugated or otherwise linked to one or more other entities or molecules, including but not limited to, proteins, peptides, synthetic polymers, adjuvants, small molecule, drugs and/or chemical entities. For example, the peptides can be associated with an affinity tag, such as a His tag, to facilitate purification. In other examples, the peptides can be associated with a detectable moiety, such as a fluorescent, bioluminescent or chemiluminescent molecule.

[00140] In particular examples, the peptides are associated with one or more carriers, such as to facilitate the generation of an immune response following administration of the peptide to a subject. For example, the peptides can be associated with a carrier protein, including, but not limited to, KLH, OVA, BSA, thyroglobulin, tetanus toxoid, and CCH. In other examples, the peptides are associated with an adjuvant. Any adjuvant known in the art can be associated with the peptides described herein. Non-limiting examples of adjuvants include aluminium salts (*e.g.* aluminium hydroxide, aluminium phosphate and potassium aluminium sulfate (also referred to as Alum)), liposomes, virosomes, Freund's adjuvant and saponin-based adjuvants. Saponin-based adjuvants include saponins or saponin derivatives from, for example, *Quillaja saponaria, Panax ginseng Panax notoginseng, Panax quinquefolium, Platycodon grandiflorum, Polygala senega, Polygala temifolia, Quillaja brasiliensis, Astragalus membranaceus and Achyranthes bidentata.* Exemplary saponin-based adjuvants include iscoms, iscom matrix, ISCOMATRIX™ adjuvant, Matrix M™ adjuvant, Matrix C™ adjuvant, Matrix Q™ adjuvant, AbISCO®-100 adjuvant, AbISCO®-300 adjuvant, ISCOPREP™, an ISCOPREP™ derivative, adjuvant containing ISCOPREP™ or an ISCOPREP™ derivative, QS-21, a QS-21 derivative, and an adjuvant containing QS-21 or a QS21 derivative. The peptides also can be associated with immumodulatory agents, including for example, cytokines, chemokines and growth factors.

[00141] Association of the peptides with other entities, such as carriers, can be achieved using any method known to those skilled in the art. Association can be by covalent bonding or non-covalent bonding, such as with hydrogen bonds, ionic bonds, van der Waals forces, and hydrophobic interactions. In some examples, association is achieved by merely mixing the peptide with the other entity, such as an adjuvant. In further examples, the association is achieved by conjugation using methods well known to those skilled in the art.

[00142] The provided peptides can be used in *in vitro* or *in vivo* methods. For example, the peptides can be used in methods to detect, identify, select or analyze antibodies specific for CXCR3. In particular examples, the peptides are used in selection methods to select antibodies specific for CXCR3. Such methods include panning techniques useful for screening antibody libraries to select antibodies specific for CXCR3. The peptides also can be used in affinity maturation techniques. Assays and techniques for detecting, identifying, selecting and analyzing antibodies are well know to those skilled in the art.

[00143] In further examples, the peptides are used to elicit antibodies specific for CXCR3. For example, the peptides can be administered to a subject, such as a small animal, thereby eliciting an antibody response to the peptide. The anti-CXCR3 antibodies elicited in the response can be isolated or purified using well known methods. In particular examples, antibody producing cells are isolated from the subject to generate hybridomas, from which monoclonal antibodies specific for CXCR3 are produced. In particular embodiments, the peptide is linked to a carrier or adjuvant, such as described above, to enhance immunogenicity. Such methods of eliciting antibodies to an antigen are well known in the art, and any such method can be used with the peptides of the present invention.

[00144] Also provided are compositions comprising the peptides comprising or consisting of the epitope to which the antibodies and antigen binding fragments described herein bind.

[00145] The citation of any reference herein should not be construed as an admission that such reference is available as "Prior Art" to the present application. Further, the reference in this specification to any prior publication (or information derived from it), or

to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

[00146] The present disclosure is further described by reference to the following non-limiting examples.

EXAMPLES

Example 1. Generation of anti-CXCR3 antibodies

[00147] Antibodies specific for CXCR3 were generated in $CXCR3^{-/-}$ knockout mice. Briefly, L1.2 cells (a murine pre-B cell line) were transfected with the mouse *CXCR3* gene (SEQ ID NO:1) and $CXCR3^{-/-}$ knockout mice were immunized with the transfected L1.2 cells, by intraperitoneal injection, five times fortnightly, with one final intraveous boost (2×10^7 cells per injection). B cells were isolated from the spleens of immunized mice and fused with myeloma cells to produce hybridomas. Hybridoma clones were then screened for reactivity with mouse and human CXCR3 using L1.2 cells transfected with mouse CXCR3 (SEQ ID NO:1) and human CXCR3 (SEQ ID NO:40.

[00148] Clone 9C5 was identified as being strongly reactive for both mouse and human CXCR3. Total RNA from the 9C5 hybridoma was used for synthesize cDNA for sequencing analysis. The variable region genes were amplified by RT-PCR using primers annealing to the mouse light (mIgCk) and heavy (mIgG2a) constant regions, and the variable heavy chain (V_H) and variable light chain (V_L) genes were sequenced It was determined that the 9C5 mAb comprises a V_H region with a nucleic acid sequence set forth in SEQ ID NO:12 and an amino acid sequence set forth in SEQ ID NO:13, and a V_L region with a nucleic acid sequence set forth in SEQ ID NO:14 and an amino acid sequence set forth in SEQ ID NO:15.

Example 2. Characterization of the binding properties of 9C5 mAb

[00149] The ability of the 9C5 mAb to specifically bind to CXCR3 from various species was assessed by flow cytometry. Briefly, isolated mouse and marmoset

splenocytes, human peripheral blood lymphocytes and transfected and untransfected L1.2 cells were incubated with 9C5 mAb, and then with a goat anti-mouse antibody conjugated to phycoerythrin (PE). Following washing, the cells were analysed by flow cytometry.

[00150] The 9C5 mAb was able to recognize L1.2 cells transfected with mouse CXCR3 (mCXCR3) or human CXCR3 (hCXCR3), but not untransfected L1.2 cells or cells transfected with other chemokine receptors (*i.e.* mCXCR2) (Figure 1A). The 9C5 mAb was also able to stain both mouse and marmoset splenocytes, as well as human peripheral blood mononuclear cells (PBMC) derived from healthy donors (Figure 1B and 1C).

Example 3. Epitope mapping of 9C5 mAb

[00151] Epitope mapping studies were performed to determine the region within CXCR3 that is recognized by 9C5 mAb. Initially, biotinylated peptides corresponding to the N-terminal region and the first, second and third extracellular loops of CXCR3 were used in an ELISA. The results of this preliminary mapping study indicated that the 9C5 mAb recognized the N-terminal region of CXCR3.

[00152] Four overlapping biotinylated peptides spanning the entire N-terminal region of human CXCR3 were then synthesized and used in more defined 9C5 mAb epitope mapping studies. Peptide 1 (MVLEVSDHQVLNDAEVAALLENF: SEQ ID NO: 16) corresponds to amino acid position 1-23 of the human CXCR3 set forth in SEQ ID NO:4; Peptide 2 (NDAEVAALLENFSSSYDYGENE: SEQ ID NO: 17) corresponds to amino acid position 12-33 of the human CXCR3 set forth in SEQ ID NO:4; Peptide 3 (FSSSYDYGENESDSCCTSPPCP: SEQ ID NO: 18) corresponds to amino acid position 23-44 of the human CXCR3 set forth in SEQ ID NO:4; and Peptide 4 (ENESDSCCTSPPCPQDFSLN: SEQ ID NO: 19) corresponds to amino acid position 31-50 of the human CXCR3 set forth in SEQ ID NO:4. Briefly, multiwell plates were coated with streptavidin and washed before the biotinylated peptides were added to separate wells and incubated to facilitate binding of the peptides to the plate. Different anti-mCXCR3 antibodies (4H8; clone CXCR3-173, BioLegend; and 6C5, clone 220803, R&D Systems, as well as anti-hCXCR3 antibodies (1C6, Pharmingen; humanized 6C5, R&D Systems; 5H6; and humanized 5H6 (both produced in this laboratory) were then tested in parallel

with 9C5 mAb by adding the respective antibodies to the wells of the plate and incubating the plate. An isotype control and buffer only were included as negative controls. Following washing, appropriate conjugated antibodies were added and the plates were incubated. The plates were washed again and binding of the antibodies to the immobilised peptides was visualised.

[00153] As shown in Figure 2, the 9C5 mAb specifically recognized Peptide 3, while the other anti-CXCR3 antibodies specifically recognized Peptide 1. Peptide 3 encompasses a highly conserved region between species (Figure 3), demonstrating why this antibody recognizes at least mouse, human and marmoset CXCR3.

Example 4. *In vivo* effect of 9C5 mAb in mice

Depletion of CXCR3$^+$ cells

[00154] To assess the effect of 9C5 mAb *in vivo* in C57/Bl6 mice, various concentrations (0.5; 2; 5 and 10 mg/kg) of the 9C5 mAb were injected into the mice intravenously. Saline solution alone was injected into one group of mice as a negative control. The mice were sacrificed after 4 days and their spleens removed. Splenocytes were isolated and lymphocytes were stained using anti-CD4, anti-CD8 and anti-B220 antibodies conjugated with APC. Cells were also stained with the PE-conjugated anti-mCXCR3 mAb from clone 4H8 (BioLegend) that recognizes a distinct epitope to 9C5. Flow cytometry was then performed to visualize and quantify the number of CXCR3$^+$ cells in the spleens of injected mice.

[00155] As shown in the FACS plots (Figure 4), the 9C5 mAb almost completely depleted CD4$^+$/CXCR3$^+$; CD8+/CXCR3$^+$ and B220$^+$/CXCR3$^+$ cells at a concentration as low as 2 mg/kg. Table 2 sets forth the percentage of various CXCR3$^+$ cell subsets following the various treatments.

Table 2.

	% cells following treatment				
	Saline	**0.5 mg/ml 9C5 mAb**	**2 mg/ml 9C5 mAb**	**5 mg/ml 9C5 mAb**	**10 mg/ml 9C5 mAb**
$CD4^+/CXCR3^+$	2.71	2.82	0.22	0.24	0.22
$CD4^-/CXCR3^+$	6.51	7.29	0.86	0.87	0.86
CD8+/$CXCR3^+$	3.31	4.33	0.17	0.19	0.11
CD8-/$CXCR3^+$	5.49	5.75	1.04	1.03	1.03
$B220^+/CXCR3^+$	1.97	1.90	1.16	1.37	1.64
$B220^+/CXCR3^+$	8.09	10.0	0.55	0.63	0.62

Duration of depletion of $CXCR3^+$ cells

[00156] To determine the duration of depletion of $CXCR3^+$ cells, C57/Bl6 mice were injected intravenously with 5 mg/kg and the mice were sacrificed after 24 or 36 days and their spleens removed. The isolated splenocytes were stained with anti-CD4, anti-CD8, anti-B220 and anti-mCXCR3 antibodies as described above and sorted by flow cytometry. As shown in Figure 5, $CXCR3^+$ cell depletion was maintained for more than 3 weeks.

Depletion of effector cells

[00157] CXCR3 on $CD4^+$ T-cells is mainly expressed by activated and effector $CD4^+$ T-cells ($CD44^{high}/CD62^{low}$) and memory $CD4^+$ T-cells ($CD44^{high}/CD62L^{high}$), while naive CD4+ T-cells ($CD44^{low}/CD62L^{high}$) do not express this receptor. To ascertain whether the decrease in $CXCR3^+$ cells after administration of 9C5 was due to the depletion of the population and not to the internalization of the receptor, the number of effector and memory $CD4^+$ T-cells was monitored by flow cytometry both before, and 4 days after, injection with 5 mg/kg 9C5 mAb or 5 mg/kg of an isotype control mAb.

[00158] As shown in Figure 6, a significant decrease in effector $CD4^+$ T-cells ($CD44^{high}/CD62^{low}$) and memory $CD4^+$ T-cells ($CD44^{high}/CD62L^{high}$) was observed, reduced from 27.1% in the isotype control sample to 13.5% in the sample obtained from 9C5-treated mice. This confirmed that the observed reduction in $CXCR3^+$ cells shown in

Figures 4 and 5 was due to the depletion of the target cells rather than the internalization of the receptor.

Example 5. Generation of chimeric 9C5 mAb

[00159] Chimeric 9C5 mAbs comprising the mouse V_H region set forth in SEQ ID NO: 13 (nucleic acid sequence set forth in SEQ ID NO:12) fused to the human IgG1 constant region set forth in SEQ ID NO:36 (nucleic acid sequence set forth in SEQ ID NO:37), and the mouse V_L region set forth in SEQ ID NO: 15 (nucleic acid sequence set forth in SEQ ID NO:14) fused to the human kappa constant region set forth in SEQ ID NO:38 (nucleic acid sequence set forth in SEQ ID NO:39) were generated using a human IgG1/Kappa mammalian expression system.

[00160] Fc variants of the chimeric 9C5 antibody were generated by standard site directed mutagenesis techniques in order to enhance or decrease antibody-dependent cell-mediated cytotoxicity (ADCC). The triple mutation S239D/A330L/I332E (known as "3M") was introduced into the Fc region to enhance ADCC, resulting in the chimeric 9C5-3MFc antibody having a C_H region set forth in SEQ ID NO:32 (nucleic acid sequence set forth in SEQ ID NO:33). The triple mutation L234F/L235E/P331S was also introduced into the Fc region to reduce ADCC, resulting in the chimeric 9C5-Fc-KO antibody having a C_H region set forth in SEQ ID NO:34 (nucleic acid sequence set forth in SEQ ID NO:35).

Example 6. Characterization of chimeric 9C5-3MFc and 9C5-Fc-KO mAb

[00161] The activities of 9C5-3MFc and 9C5-Fc-KO chimeric antibodies were analyzed as described above. Briefly, the antibodies were injected intravenously into C57/Bl6 mice at 2 mg/kg or 5 mg/kg. After 4 days, the animals were sacrificed and the $CD4^+/CXCR3^+$ T-cell population was monitored by flow cytometry (Figure 7). The 9C5-3MFc chimeric antibody was able to deplete the Th1 cell population as efficiently as the parental mouse 9C5 IgG2a mAb. A significant decrease in $CD4^+/CXCR3^+$ T-cell depletion occurred after injection of 9C5 Fc-KO chimeric antibody (see also Table 3, below).

Table 3

	% cells as a percentage of total cells following treatment				
	Saline	2 mg/ml 9C5-Fc-KO	5 mg/ml 9C5-Fc-KO	**2 mg/ml 9C5-3MFe-**	**5 mg/ml 9C5-3MFc**
$CD4^+/CXCR3^+$	3.77	1.51	1.61	0.79	0.41
$CD4^-/CXCR3^+$	6.70	5.77	6.08	2.67	1.63
$CD4^+/CXCR3^-$	17.0	21.7	22.8	20.5	23.1
$CD4^-/CXCR3^-$	72.5	71.1	69.5	76.0	74.9

Example 7. *In vivo* effect of 9C5 mAb on pancreatic islet allografts

[00162] Several studies have demonstrated that CXCR3 and CCR5, two receptors preferentially expressed by $CD4^+$ Th1 cells, as well as their corresponding ligands, are present in various murine and human allografts. Accordingly, experiments were performed to determine whether depletion of $CXCR3^+$ cells using the 9C5 mAb, or would in a pancreatic islet allograft transplant model would result in immunosuppression and prolonged graft survival.

[00163] To induce diabetes, recipient mice (C57BL/6, $H2^b$) were injected with 200 mg/kg streptozotocin in 10 mM citrate buffer, pH 4.2, and blood glucose levels (BGLs) were determined using ACCU-CHECK Advantage. Mice with a blood glucose value >16 mmol/liter were selected as transplant recipients. Islets were prepared from the pancreata of donor (BALB/c, $H2^d$) mice at a ratio of three pancreata per recipient. For the transplant, the kidney was accessed by a left-flank incision and brought into the wound by gentle blunt dissection. A small nick was made in the kidney capsule at the inferior renal pole, and the islets were deposited through the nick toward the superior pole of the kidney. BGLs were analyzed on postoperative days (POD) 1, 2, and 5, and then three times per week until rejection. Graft rejection was defined as an increase of blood glucose to >16 mmol/liter after a period of euglycemia. Nephrectomy was performed at POD ≥100 to determine if the euglycemia was graft dependent.

[00164] In a first set of experiments, the allograft recipients were intravenously administered 5mg/kg of 9C5 mAb (one dose) or saline solution 4-5 hours prior to

transplantation. It was observed that the single dose of 9C5mAb increased the mean survival time (MST) of islet grafts from 17 to 33 days (Figure 8A).

[00165] In a second set of experiments, 2 mg/kg 9C5 mAb or saline injections were administered weekly on day 0, 7, 14, and 21 (multi-dose) after islet transplantation (5 mice per group). This weekly administration of 9C5 mAb increased MST to 35 days. Two out of seven recipients showed graft survival for >100 days without further treatment. (Figure 8B).

[00166] In a third set of experiments, the allograft recipients were treated with rapamycin (1, 0.35, 0.1 or 0 mg/kg) on the day of transplantation and every day thereafter for 7 days, or with a combination protocol of 0.1 mg/kg rapamycin and 2 mg/kg isotype control or 9C5 mAb on day 0, 7, 14, and 21. Recipient mice given 1 mg/kg rapamycin on the day of transplantation and every day thereafter for 7 days, permanently accepted their grafts (>100 days survival). Gradual reduction of this dose from 0.35, 0.1 to 0 gave reduced MSTs of 43, 22 and 19 days, respectively (Figure 8B). Combining the homeopathic 0.1 mg/kg dose of rapamycin with 9C5 mAb at weekly intervals on days 0, 7, 14 and 21, a 50 % graft survival at 60 days was achieved.

Example 8. Generation of humanized 9C5 mAb

[00167] Humanized 9C5 mAbs were generated by transferring the CDRs of the 9C5 mAb (CDR-H1: SEQ ID NO:20; CDR-H2: SEQ ID NO:21; CDR-H3: SEQ ID NO:22; CDR-L1: SEQ ID NO:23; CDR-L2: SEQ ID NO:24; and CDR-L3: SEQ ID NO:25) onto human framework regions using standard molecular techniques. IMGT/V-QUEST and IMGT/Junctions analysis tools were used to identify human germline genes in which sequences from the variable regions of both the heavy and light chains were closely aligned with those of murine antibody. Framework sequences of these selected human germline genes were used as acceptor sequences for the mouse 9C5 CDRs (IGHV3-23*04 and IGKV2-28*01 human genes according to IMGT database). However, murine residues were retained in the critical "Vernier" zone. The humanized V_H and V_L genes, which were also codon optimized for expressed in CHO cells, were synthesized by Genescript.

[00168] The nucleic acid sequence of the humanized 9C5 V_H gene is set forth in SEQ ID NO:43 and the nucleic acid sequence of the humanized 9C5 V_L gene is set forth in SEQ

ID NO:44. The amino acid sequence of the V_H region of the humanized 9C5 mAb is set forth in SEQ ID NO:41 and the amino acid sequence of the V_L region is set forth in SEQ ID NO:42.

[00169] The binding specificity of the humanized 9C5 antibody was assessed using flow cytometry. Briefly, human CXCR3 transfected cells (L1.2 cells) were stained with the humanized 9C5 antibody (0.25 μg/ml), the mouse 9C5 antibody (0.25 μg/ml) or an isotype control (1 μg/ml). The antibody binding was revealed with Fc specific antibodies conjugated to PE. As shown in Figure 9, the humanized 9C5 antibody recognized human CXCR3.

[00170] BALB/c $H2^d$ islets were transplanted under the kidney capsule of streptozotocin-induced diabetic C57BL/6 $H2^b$ mice. Mice were administered with a single dose (5 mg/kg (i.v.)) of humanized 9C5 3MFc mAb, humanized 9C5 Fc-KO mAb or isotype control antibody 4-5 h prior to transplantation. As shown in Figure 10, humanized 9C5 3MFc mAb (depleting antibody) increased the mean survival time of the islet grafts.

CLAIMS:

1. An isolated antibody or antigen-binding fragment thereof that specifically binds to an epitope within amino acid residues 23 to 44 of the human CXCR3 set forth in SEQ ID NO:4.

2. The antibody or antigen-binding fragment of claim 1, wherein the epitope is within amino acid residues 23 to 43; 23 to 42; 23 to 41; 23 to 40; 23 to 39; 23 to 38; 23 to 37; 23 to 36; 23 to 35; 23 to 34; 23 to 33; 23 to 32; 23 to 31; 23 to 30; 23 to 29; 24 to 44; 24 to 43; 24 to 42; 24 to 41; 24 to 40; 24 to 39; 24 to 38; 24 to 37; 24 to 36; 24 to 35; 24 to 34; 24 to 33; 24 to 32; 24 to 31; 24 to 30; 24 to 29; 25 to 44; 25 to 43; 25 to 42; 25 to 41; 25 to 40; 25 to 39; 25 to 38; 25 to 37; 25 to 36; 25 to 35; 25 to 34; 25 to 33; 25 to 32; 25 to 31; 25 to 30; 26 to 44; 26 to 43; 26 to 42; 26 to 41; 26 to 40; 26 to 39; 26 to 38; 26 to 37; 26 to 36; 26 to 35; 26 to 34; 26 to 33; 26 to 32; 26 to 31; 27 to 44; 27 to 43; 27 to 42; 27 to 41; 27 to 40; 27 to 39; 27 to 38; 27 to 37; 27 to 36; 27 to 35; 27 to 34; 27 to 33; 27 to 32; 28 to 44; 28 to 43; 28 to 42; 28 to 41; 28 to 40; 28 to 39; 28 to 38; 28 to 37; 28 to 36; 28 to 35; 28 to 34; 28 to 33; 28 to 32; 29 to 44; 29 to 43; 29 to 42; 29 to 41; 29 to 40; 29 to 39; 29 to 38; 29 to 37; 29 to 36; 29 to 35; 29 to 34; 30 to 44; 30 to 43; 30 to 42; 30 to 41; 30 to 40; 30 to 39; 30 to 38; 30 to 37; 30 to 36; and/or 30 to 35 of the human CXCR3 set forth in SEQ ID NO:4.

3. The antibody or antigen-binding fragment of claim 1 or claim 2, wherein the epitope consists of amino acid residues 25 to 35; 25 to 34; 25 to 33; 25 to 32; 25 to 31; 25 to 30; 26 to 35; 26 to 34; 26 to 33; 26 to 32; 26 to 31; 27 to 35; 27 to 34; 27 to 33; 27 to 32; 28 to 35; 28 to 34; 28 to 33; 29 to 35; 29 to 34 or 30 to 35 of the human CXCR3 set forth in SEQ ID NO:4

4. An isolated antibody or antigen-binding fragment thereof that specifically binds to a polypeptide consisting of amino acid residues 23 to 44 of SEQ ID NO:4.

5. The antibody or antigen-binding fragment of claim 4, wherein the polypeptide consists of amino acid residues 23 to 43; 23 to 42; 23 to 41; 23 to 40; 23 to 39; 23 to 38; 23 to 37; 23 to 36; 23 to 35; 23 to 34; 23 to 33; 23 to 32; 23 to 31; 23 to 30; 23 to 29; 24 to 44; 24 to 43; 24 to 42; 24 to 41; 24 to 40; 24 to 39; 24 to 38; 24 to 37; 24 to 36; 24 to 35; 24 to 34; 24 to 33; 24 to 32; 24 to 31; 24 to 30; 24 to 29; 25 to 44; 25 to 43; 25 to 42; 25 to 41; 25 to 40; 25 to 39; 25 to 38; 25 to 37; 25 to 36; 25 to 35; 25 to 34; 25 to 33; 25 to 32; 25 to 31; 25 to 30; 26 to 44; 26 to 43; 26 to 42; 26 to 41; 26 to 40; 26 to 39; 26 to 38; 26 to 37;

26 to 36; 26 to 35; 26 to 34; 26 to 33; 26 to 32; 26 to 31; 27 to 44; 27 to 43; 27 to 42; 27 to 41; 27 to 40; 27 to 39; 27 to 38; 27 to 37; 27 to 36; 27 to 35; 27 to 34; 27 to 33; 27 to 32; 28 to 44; 28 to 43; 28 to 42; 28 to 41; 28 to 40; 28 to 39; 28 to 38; 28 to 37; 28 to 36; 28 to 35; 28 to 34; 28 to 33; 28 to 32; 29 to 44; 29 to 43; 29 to 42; 29 to 41; 29 to 40; 29 to 39; 29 to 38; 29 to 37; 29 to 36; 29 to 35; 29 to 34; 30 to 44; 30 to 43; 30 to 42; 30 to 41; 30 to 40; 30 to 39; 30 to 38; 30 to 37; 30 to 36; and/or 30 to 35 of the human CXCR3 set forth in SEQ ID NO:4.

6. The antibody or antigen-binding fragment of claim 4 or claim 5, wherein the polypeptide consists of amino acid residues 25 to 35; 25 to 34; 25 to 33; 25 to 32; 25 to 31; 25 to 30; 26 to 35; 26 to 34; 26 to 33; 26 to 32; 26 to 31; 27 to 35; 27 to 34; 27 to 33; 27 to 32; 28 to 35; 28 to 34; 28 to 33; 29 to 35; 29 to 34 or 30 to 35 of the human CXCR3 set forth in SEQ ID NO:4

7. The antibody or antigen-binding fragment of any one of claims 1 to 6, comprising:

a CDR-H1 comprising the amino acid sequence set forth in SEQ ID NO:20 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:20;

a CDR-H2 comprising the amino acid sequence set forth in SEQ ID NO:21 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:21;

a CDR-H3 comprising the amino acid sequence set forth in SEQ ID NO:22 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:22;

a CDR-L1: comprising the amino acid sequence set forth in SEQ ID NO:23 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:23;

a CDR-L2 comprising the amino acid sequence set forth in SEQ ID NO:24 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:24; and

a CDR-L3 comprising the amino acid sequence set forth in SEQ ID NO:25 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:25.

8. The antibody or antigen-binding fragment of any one of claims 1 to 7, comprising:

a CDR-H1 with a sequence of amino acids set forth in SEQ ID NO:20;

a CDR-H2 with a sequence of amino acids set forth in SEQ ID NO:21;

a CDR-H3 with a sequence of amino acids set forth in SEQ ID NO:22;

a CDR-L1 with a sequence of amino acids set forth in SEQ ID NO:23;

a CDR-L2 with a sequence of amino acids set forth in SEQ ID NO:24; and

a CDR-L3 with a sequence of amino acids set forth in SEQ ID NO:25.

9. The antibody or antigen-binding fragment of any one of claims 1 to 8, comprising:

a variable heavy chain comprising sequence of amino acids set forth in SEQ ID NO:13 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:13, and

a variable light chain comprising a sequence of amino acids set forth in SEQ ID NO:15 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:15.

10. The antibody or antigen-binding fragment of any one of claims 1 to 8, comprising:

a variable heavy chain comprising sequence of amino acids set forth in SEQ ID NO:41 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:41, and

a variable light chain comprising a sequence of amino acids set forth in SEQ ID NO:42 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:42.

11. An isolated antibody or antigen binding fragment thereof that binds to CXCR3, comprising:

a CDR-H1 comprising the amino acid sequence set forth in SEQ ID NO:20 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:20;

a CDR-H2 comprising the amino acid sequence set forth in SEQ ID NO:21 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:21;

a CDR-H3 comprising the amino acid sequence set forth in SEQ ID NO:22 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:22;

a CDR-L1: comprising the amino acid sequence set forth in SEQ ID NO:23 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:23;

a CDR-L2 comprising the amino acid sequence set forth in SEQ ID NO:24 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:24; and

a CDR-L3 comprising the amino acid sequence set forth in SEQ ID NO:25 or a sequence of amino acids having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:25.

12. The antibody or antigen-binding fragment of claim 11, comprising:

a CDR-H1 with a sequence of amino acids set forth in SEQ ID NO:20;

a CDR-H2 with a sequence of amino acids set forth in SEQ ID NO:21;

a CDR-H3 with a sequence of amino acids set forth in SEQ ID NO:22;

a CDR-L1 with a sequence of amino acids set forth in SEQ ID NO:23;

a CDR-L2 with a sequence of amino acids set forth in SEQ ID NO:24; and

a CDR-L3 with a sequence of amino acids set forth in SEQ ID NO:25.

13. The antibody or antigen-binding fragment of claim 11 or claim 12, comprising:

a variable heavy chain comprising sequence of amino acids set forth in SEQ ID NO:13 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:13, and

a variable light chain comprising a sequence of amino acids set forth in SEQ ID NO:15 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:15.

14. The antibody or antigen-binding fragment of claim 11 or claim 12, comprising:

a variable heavy chain comprising sequence of amino acids set forth in SEQ ID NO:41 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:41, and

a variable light chain comprising a sequence of amino acids set forth in SEQ ID NO:42 or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:42.

15. The antibody or antigen-binding fragment of any one of claims 1 to 14, wherein the antibody is a monoclonal antibody.

16. The antibody or antigen-binding fragment of any one of claims 1 to 14, wherein the antibody or antigen-binding fragment is multivalent.

17. The antibody or antigen-binding fragment of any one of claims 1 to 16, wherein the antibody or antigen-binding fragment is a chimeric or humanized antibody or antigen-binding fragment.

18. The antibody or antigen-binding fragment of any one of claims 1 to 17, wherein the wherein the fragment is selected from among a Fab fragment, Fab^3 fragment, $F(ab')_2$ fragment, Fv fragment, disulfide-linked Fv (dsFv), Fd fragment, Fd' fragment, single-chain Fv (scFv), single-chain Fab (scFab), domain antibody, diabody, triabody and tetrabody .

19. The antibody or antigen-binding fragment of any one of claims 1 to 18, that exhibits antibody-dependent cellular cytotoxicity (ADCC) activity.

20. The antibody or antigen-binding fragment of any one of claims 1 to 19, that exhibits complement dependent cytotoxicity (CDC) activity.

21. The antibody or antigen-binding fragment of any one of claims 1 to 19, that is linked to one or more other moieties.

22. The antibody or antigen-binding fragment of claim 21, wherein the one or more other moieties is elected from among a polymer, small molecule, peptide or polypeptide.

23. The antibody or antigen-binding fragment of claim 21, wherein the one or more other moieties is a cytotoxic agent.

24. An isolated nucleic acid molecule, encoding the antibody or antigen-binding fragment of any one of claims 1 to 23.

25. An isolated nucleic acid molecule, comprising:

a sequence of nucleotides set forth in SEQ ID NO: 26 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:26;

a sequence of nucleotides set forth in SEQ ID NO: 27 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:27; and

a sequence of nucleotides set forth in SEQ ID NO: 28 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:28.

26. The nucleic acid molecule of claim 25, comprising a sequence of nucleotides set forth in SEQ ID NO:12 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:12.

27. An isolated nucleic acid molecule, comprising:

a sequence of nucleotides set forth in SEQ ID NO: 29 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:29;

a sequence of nucleotides set forth in SEQ ID NO: 30 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:30; and

a sequence of nucleotides set forth in SEQ ID NO: 31 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:31.

28. The isolated nucleic acid molecule of claim 27, comprising a sequence of nucleotides set forth in SEQ ID NO:14 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:14.

29. An isolated nucleic acid molecule, comprising:

a sequence of nucleotides set forth in SEQ ID NO:45 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:45;

a sequence of nucleotides set forth in SEQ ID NO:46 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:46; and

a sequence of nucleotides set forth in SEQ ID NO:47 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:47.

30. The nucleic acid molecule of claim 29, comprising a sequence of nucleotides set forth in SEQ ID NO:43 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:43.

31. An isolated nucleic acid molecule, comprising:

a sequence of nucleotides set forth in SEQ ID NO:48 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:48;

a sequence of nucleotides set forth in SEQ ID NO:49or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:49; and

a sequence of nucleotides set forth in SEQ ID NO:50 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:50.

32. The isolated nucleic acid molecule of claim 31, comprising a sequence of nucleotides set forth in SEQ ID NO:44 or a sequence of nucleotides having at least or at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity to SEQ ID NO:44.

33. A vector comprising the nucleic acid molecule of any one of claims 24 to 32.

34. A cell comprising the nucleic acid molecule of any one of claims 24 to 32 or the vector of claim 33.

35. A recombinant cell that produces the antibody or antigen-binding fragment of any one of claims 1 to 23.

36. A composition comprising the antibody or antigen-binding fragment of any one of claims 1 to 23 or the nucleic acid molecule of any one of claims 22 to 32.

37. A method for depleting $CXCR3^+$ cells in a subject, comprising administering to the subject an effective amount of the antibody or antigen-binding fragment of any one of claims 1 to 23.

38. A method for inhibiting the recruitment or migration of $CXCR3^+$ cells to an inflammation site in a subject, comprising administering to the subject an effective amount of the antibody or antigen-binding fragment of any one of claims 1 to 23.

39. The method of claim 30 or claim 31, wherein the cells are $CXCR3^+/CD4^+$ T cells, $CXCR3^+/CD8^+$ T cells and/or $CXCR3^+/B220^+$ B cells.

40. The method of any one of claims 37 to 39, wherein the subject has received, is receiving or is to receive a graft or transplant.

41. A method for inhibiting graft or transplant rejection in a subject that has received, is receiving or is to receive a graft or transplant, comprising administering to the subject an effective amount of the antibody or antigen-binding fragment of any one of claims 1 to 21.

42. The method of claim 40 or claim 41, wherein the graft is an allograft or xenograft, or the transplant is an allotransplant or xenotransplant.

43. The method of any one of claims 40 to 42, wherein the graft or transplant is selected from among a heart, kidney, lung, liver, pancreas, pancreatic islets, brain tissue, stomach, large intestine, small intestine, cornea, skin, trachea, bone, bone marrow, muscle and bladder graft or transplant.

44. The method of any one of claims 40 to 43, wherein the antibody or antigen-binding fragment is administered to the subject before, at the same time and/or after the subject has received the graft or transplant.

45. The method of any one of claims 37 to 44, wherein the antibody or antigen-binding fragment is administered to the subject two or more times.

46. The method of any one of claims 37 to 45, further comprising administering to the subject one or more other therapeutic agents.

47. The method of claim 46, wherein the therapeutic agent is a cytokine, chemokine or antibody.

48. The method of claim 47, wherein the cytokine is interleukin 2.

49. The method of claim 46, wherein the therapeutic agent is an immunosuppressive agent.

50. The method of claim 49, wherein the immunosuppressive agent is selected from among a glucocorticoid, cyclosporine, rapamycin, voclosporin, sirolimus, everolimus, tacrolimus, mycophenolate mofetil, mycophenolic acid, mizoribine, an S1P-R agonists, a malononitrilamide, an anti-CD3 antibody, an anti -CD25 antibody, an anti-CD52 antibody, an anti-CD20 antibody and an anti-tumor necrosis factor antibody.

51. A method for measuring or detecting CXCR3 in a sample, comprising contacting the sample with an antibody or antigen-binding fragment of any one of claims 1 to 23 and measuring or detecting binding of the antibody or antigen-binding fragment to the CXCR3.

52. The method of claim 51, wherein the sample comprises one or more cells expressing CXCR3.

53. The method of claim 51 or 52, wherein the sample comprises blood, cells or tissue.

54. Use of an antibody or antigen-binding fragment of any one of claims 1 to 23, for the preparation of a medicament for depleting $CXCR3^+$ cells.

55. Use of an antibody or antigen-binding fragment of any one of claims 1 to 23, for the preparation of a medicament for inhibiting the recruitment or migration of $CXCR3^+$ cells to an inflammation site.

56. Use of an antibody or antigen-binding fragment of any one of claims 1 to 23, for the preparation of a medicament for inhibiting graft rejection in a subject that has received, is receiving or is to receive a graft.

57. An isolated peptide, consisting of amino acid residues 23 to 44; 23 to 43; 23 to 42; 23 to 41; 23 to 40; 23 to 39; 23 to 38; 23 to 37; 23 to 36; 23 to 35; 23 to 34; 23 to 33; 23 to 32; 23 to 31; 23 to 30; 23 to 29; 24 to 44; 24 to 43; 24 to 42; 24 to 41; 24 to 40; 24 to 39; 24 to 38; 24 to 37; 24 to 36; 24 to 35; 24 to 34; 24 to 33; 24 to 32; 24 to 31; 24 to 30; 24 to 29; 25 to 44; 25 to 43; 25 to 42; 25 to 41; 25 to 40; 25 to 39; 25 to 38; 25 to 37; 25 to 36; 25 to 35; 25 to 34; 25 to 33; 25 to 32; 25 to 31; 25 to 30; 26 to 44; 26 to 43; 26 to 42; 26 to 41; 26 to 40; 26 to 39; 26 to 38; 26 to 37; 26 to 36; 26 to 35; 26 to 34; 26 to 33; 26 to 32; 26 to 31; 27 to 44; 27 to 43; 27 to 42; 27 to 41; 27 to 40; 27 to 39; 27 to 38; 27 to 37; 27 to 36; 27 to 35; 27 to 34; 27 to 33; 27 to 32; 28 to 44; 28 to 43; 28 to 42; 28 to 41; 28 to 40; 28 to 39; 28 to 38; 28 to 37; 28 to 36; 28 to 35; 28 to 34; 28 to 33; 28 to 32; 29 to 44; 29 to 43; 29 to 42; 29 to 41; 29 to 40; 29 to 39; 29 to 38; 29 to 37; 29 to 36; 29 to 35; 29 to 34; 30 to 44; 30 to 43; 30 to 42; 30 to 41; 30 to 40; 30 to 39; 30 to 38; 30 to 37; 30 to 36; or 30 to 35 of SEQ ID NO:4, or a sequence of amino acids having at least or about 80%, 85%, 90%, 91%, 92%, 93%, 9%, 95%, 96%, 97%, 98% or 99% sequence identity thereto.

58. The peptide of claim 57, that is linked to a protein, peptide, synthetic polymer, adjuvant, or small molecule.

59. A method for inducing antibodies that specifically and/or selectively bind to CXCR3, comprising administering to a subject the peptide of claim 57 or claim 58.

60. A method for selecting antibodies or antigen-binding fragments thereof that specifically and/or selectively bind to CXCR3, comprising contacting a sample comprising antibodies or antigen-binding fragments with the peptide of claim 57 or claim 58, and selecting those antibodies and antigen-binding fragments that specifically bind to the peptide.

Figure 1

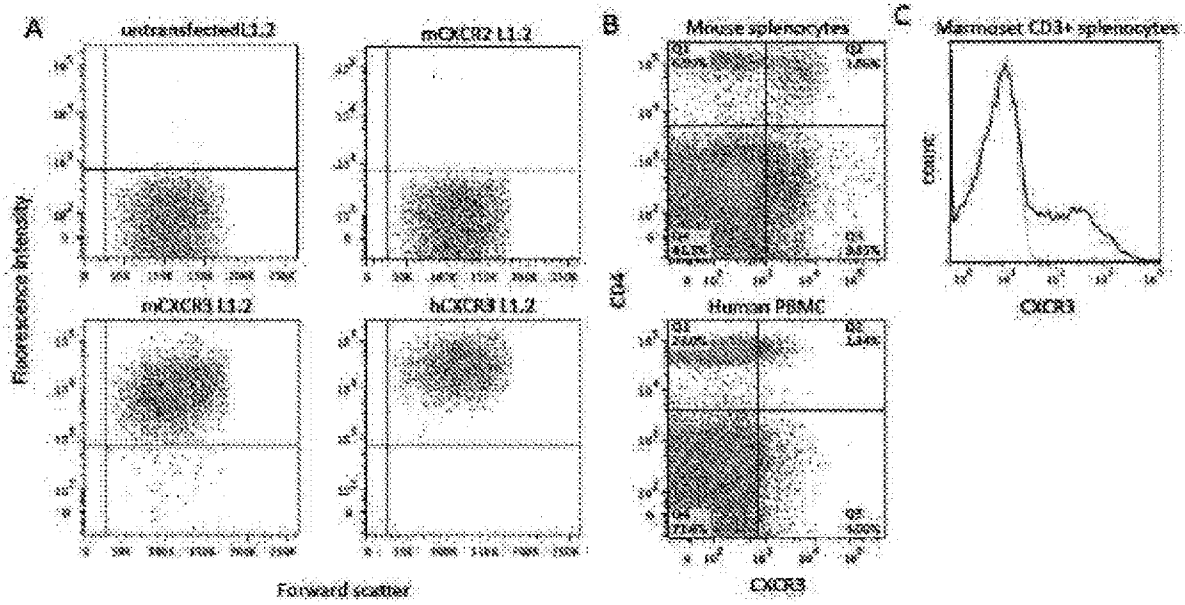

A
untransfected L1.2
mCXCR2 L1.2
mCXCR3 L1.2
hCXCR3 L1.2
Fluorescence intensity
Forward scatter
B
Mouse splenocytes
Human PBMC
CD4
CXCR3
C
Marmoset CD3+ splenocytes
COUNT
CXCR3

Figure 2

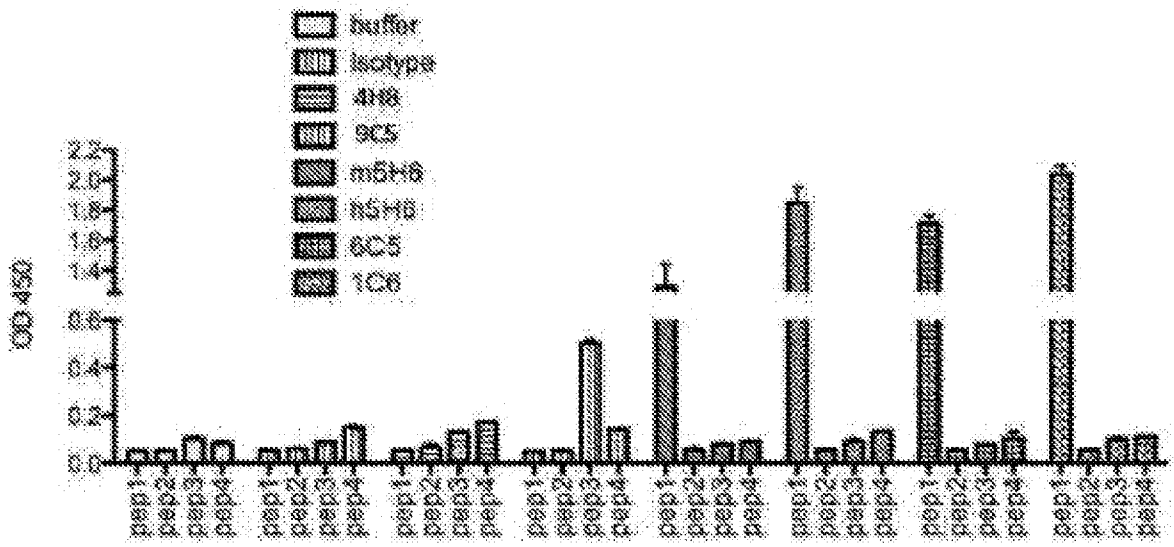

buffer
isotype
4H8
9C5
m5H8
h5H8
6C5
1C6
OD 450
0.0
0.2
0.4
0.6
1.4
1.6
1.8
2.0
2.2
pep1
pep2
pep3
pep4

Figure 3

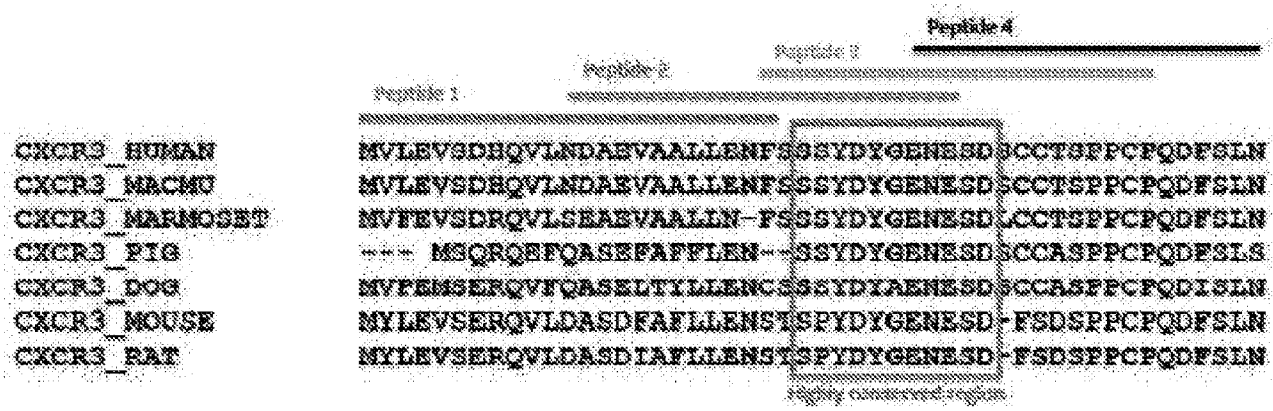

Peptide 4
Peptide 3
Peptide 2
Peptide 1
CXCR3_HUMAN MVLEVSDHQVLNDAEVAALLENFSSSYDYGENESDSCCTSPPCPQDFSLN
CXCR3_MACMU MVLEVSDRQVLNDAEVAALLENFSSSYDYGENESDSCCTSPPCPQDFSLN
CXCR3_MARMOSET MVFEVSDRQVLSEAEVAALLN-FSSSYDYGENESDLCCTSPPCPQDFSLN
CXCR3_PIG --- MSQRQEFQASEFAFFLEN--SSYDYGENESDSCCASPPCPQDFSLS
CXCR3_DOG MVFEMSERQVFQASELTILLENCSSSYDYAENESDSCCASPPCPQDISLN
CXCR3_MOUSE MYLEVSERQVLDASDFAFLLENSISPYDYGENESD-FSDSPPCPQDFSLN
CXCR3_RAT MYLEVSERQVLDASDIAFLLENSISPYDYGENESD-FSDSPPCPQDFSLN

Figure 4

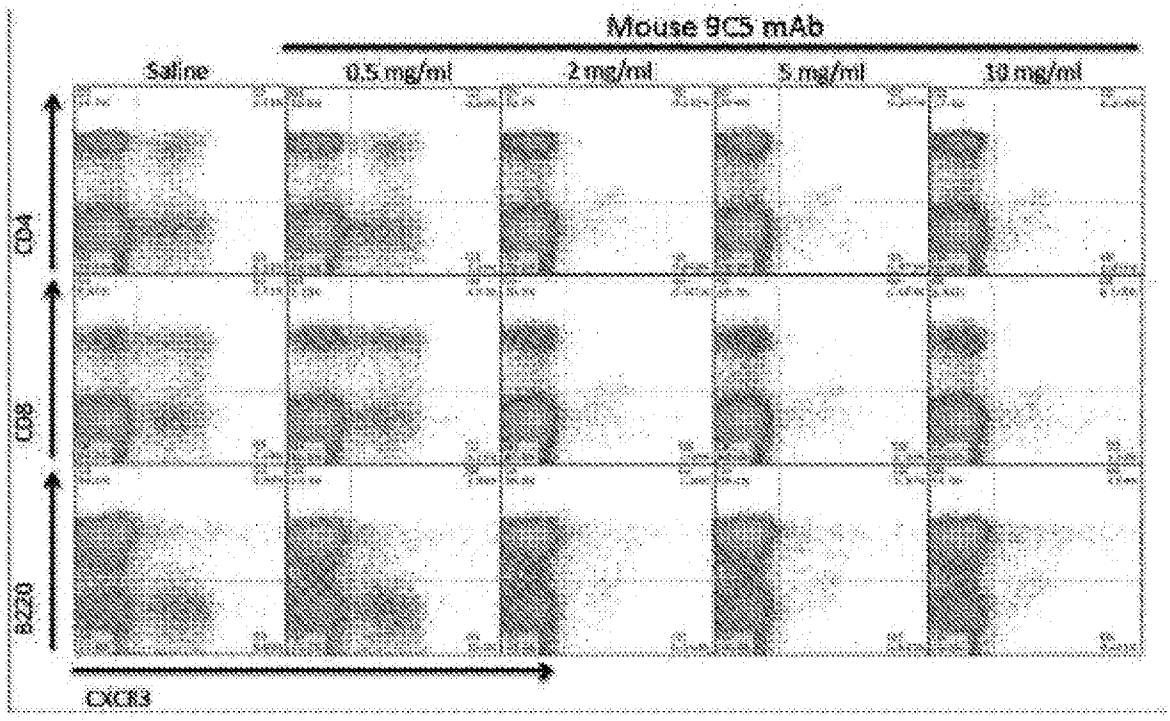

Mouse 9C5 mAb
Saline
0.5 mg/ml
2 mg/ml
5 mg/ml
10 mg/ml
CD4
CD8
B220
CXCR3

Figure 5

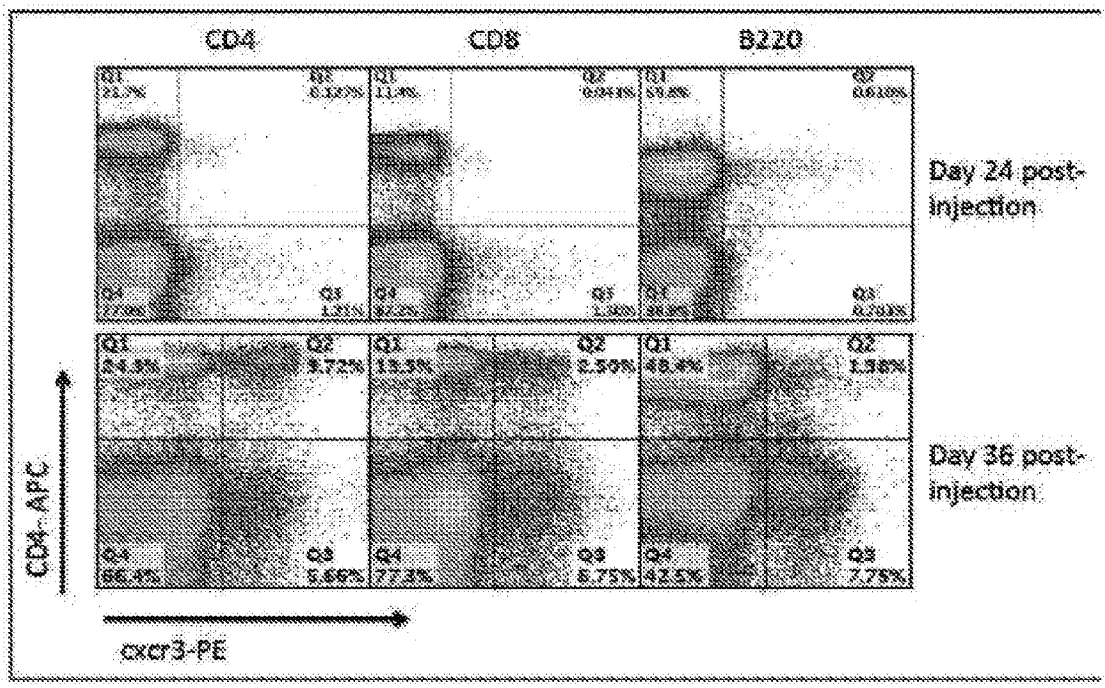

CD4
CD8
B220
Day 24 post-injection
Day 36 post-injection
CD4-APC
cxcr3-PE
Q1
Q2
Q3
Q4
24.5%
9.72%
66.4%
5.66%
35.5%
2.59%
77.3%
5.75%
48.4%
3.36%
42.5%
7.75%

Figure 6

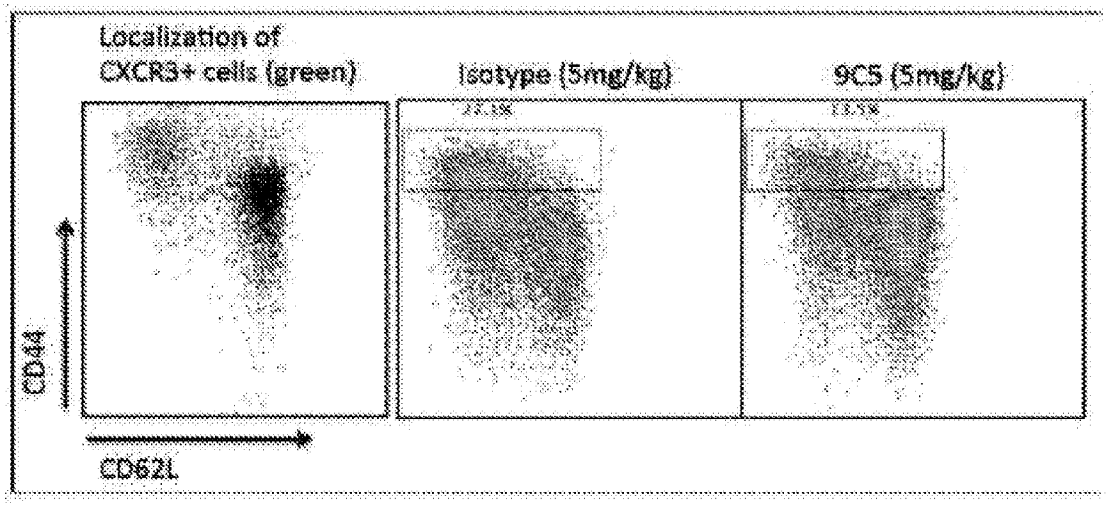

Localization of
CXCR3+ cells (green)
Isotype (5mg/kg)
9C5 (5mg/kg)
CD44
CD62L

Figure 7

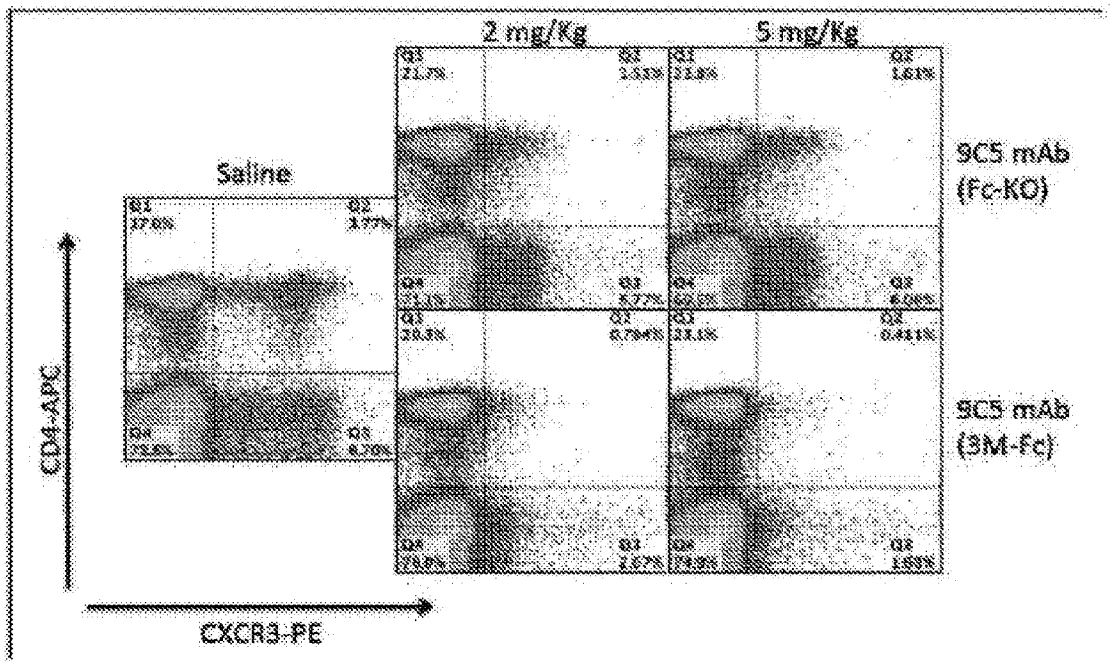


Figure 8

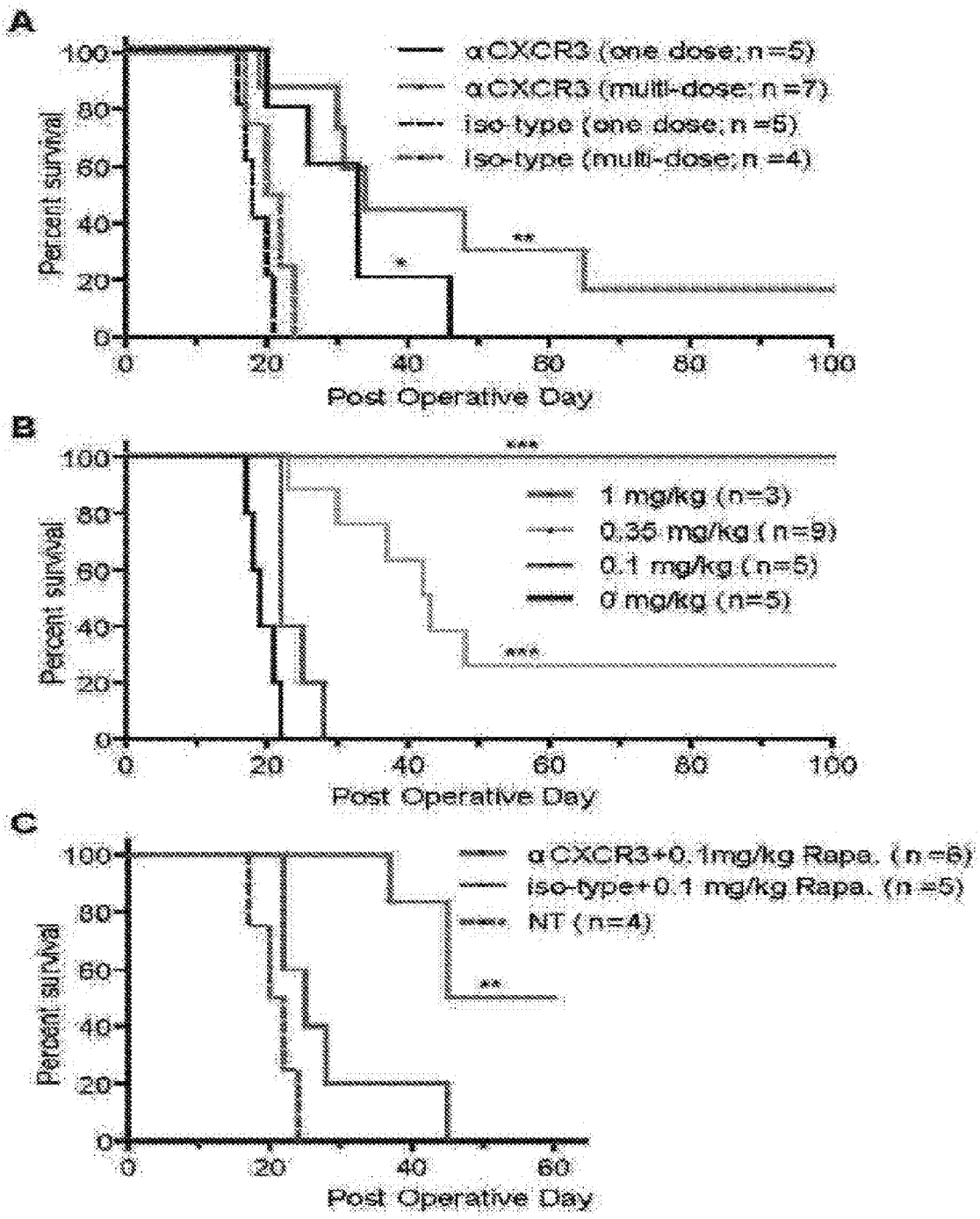

A
Percent survival
100
80
60
40
20
0
0
20
40
60
80
100
Post Operative Day
αCXCR3 (one dose; n=5)
αCXCR3 (multi-dose; n=7)
Iso-type (one dose; n=5)
Iso-type (multi-dose; n=4)
**
*
B
Percent survival
Post Operative Day
***
***
1 mg/kg (n=3)
0.35 mg/kg (n=9)
0.1 mg/kg (n=5)
0 mg/kg (n=5)
C
Percent survival
Post Operative Day
αCXCR3+0.1mg/kg Rapa. (n=6)
Iso-type+0.1 mg/kg Rapa. (n=5)
NT (n=4)
**

Figure 9

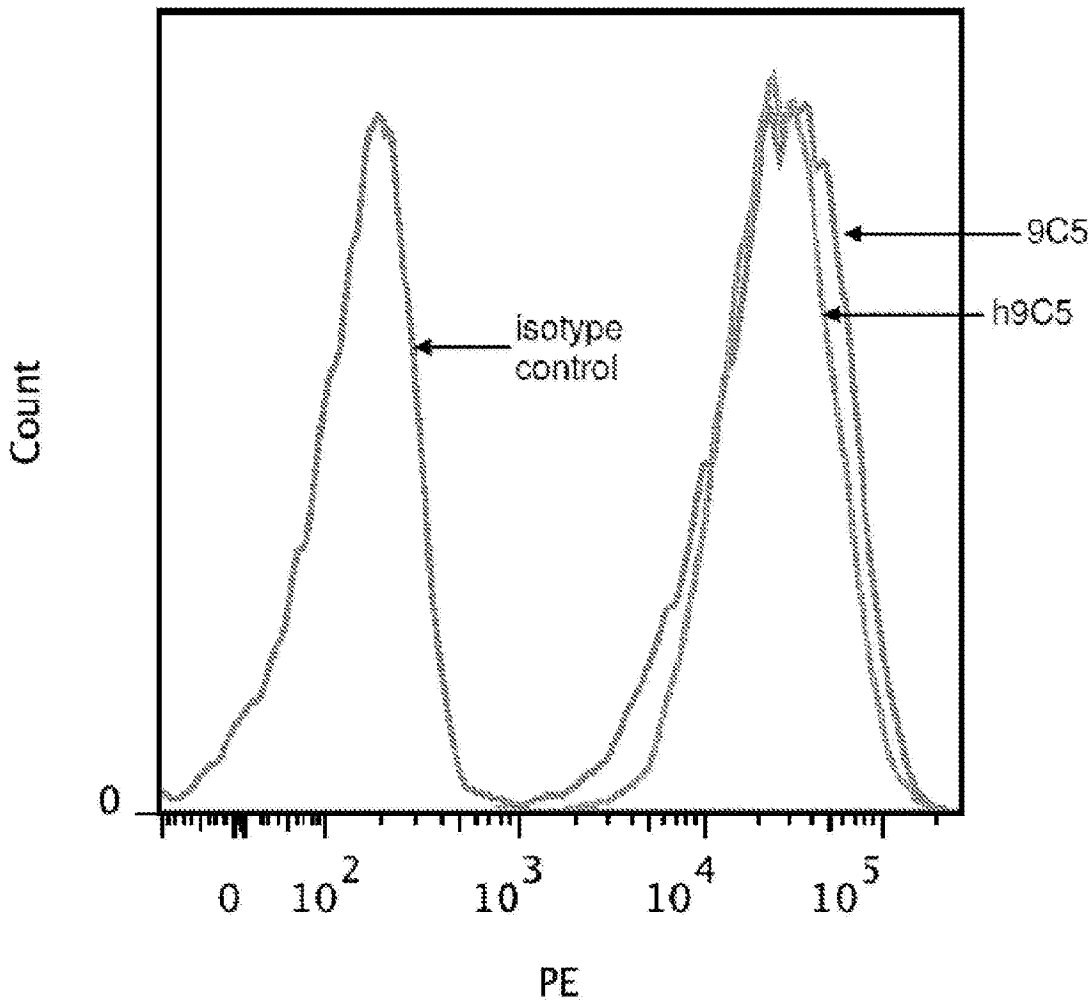
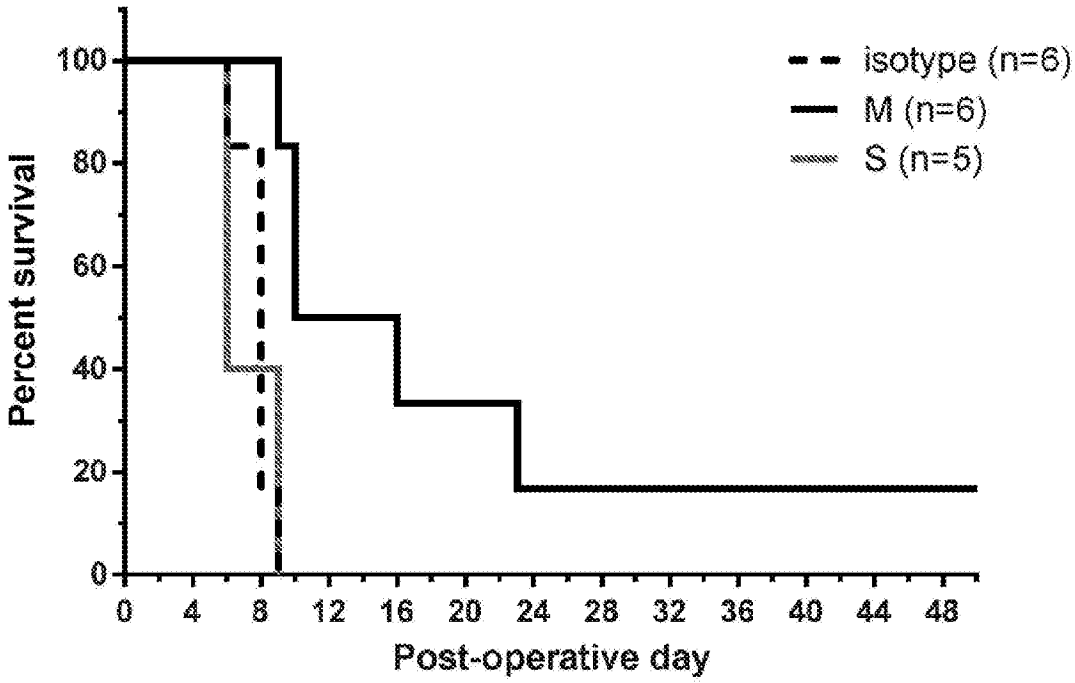


Figure 10


isotype (n=6)
M (n=6)
S (n=5)
Percent survival
100
80
60
40
20
0
0
4
8
12
16
20
24
28
32
36
40
44
48
Post-operative day

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2014/050048

A. CLASSIFICATION OF SUBJECT MATTER

C07K 16/28 (2006.01) ***A61K 39/395 (2006.01)*** ***C07K 7/04 (2006.01)*** ***C07K 14/705 (2006.01)*** ***A61K 31/436 (2006.01)*** ***A61K 38/20 (2006.01)*** ***A61P 37/06 (2006.01)***

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, EPODOC, MEDLINE, Patentscope, PubMed. Keywords: CXCR3, GPR9, CD183, IP10-R, Mig-R, antibody, depletion, rejection, transplant, and related terms.

GenomeQuest: Sequence search on SEQ ID NOs: 12-15, 18, 20-31, and 41-50.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 July 2014	28 July 2014
Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au	Michael Amon AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. 0262832083

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	UPPALURI, R., *et al.*, "Prolongation of Cardiac and Islet Allograft Survival by a Blocking Hamster Anti-Mouse CXCR3 Monoclonal Antibody," Transplantation, 2008, Vol. 86, No. 1, pages 137-147. See, in particular: Abstract; 'Hamster mAb Production', 'Allografts', and 'Antibodies', section of the 'Materials and Methods' section; 'CXCR3-173 does not deplete the critical CD4+ T cells required for allograft rejection' section; 'CXCR3 expression on mouse lymphocyte subsets' section; 'CXCR3-173 prolongs allograft survival' section, paragraph 1; 'CXCR3-173 plus rapamycin therapy induces long-term allograft survival' section, paragraph 1; 'Discussion' section, paragraphs 1 , 5, and the penultimate paragraph; and Figure 2.	1-6, 15, 16, 19-23, 35, 36, 38-53, 55, and 56
X	HANCOCK, W.W., *et al.*, "Requirement of the Chemokine Receptor CXCR3 for Acute Allograft Rejection," The Journal of Experimental Medicine, 2000, Vol. 192, No. 10, pages 1515-1519. See, in particular: Abstract; page 1516, 'Cellular and Molecular Studies' and 'Transplantation' sections; and page 1518-1519, bridging paragraph.	1-6, 15, 16, 19-23 and 36-56
X	WO 2001/078708 A1 (MILLENNIUM PHARMACEUTICALS, INC.) 25 October 2001 See, in particular: page 9, lines 1-13; page 13, line 16 to page 15, line 1; page 23, line 9 to page 24, line 10; page 28, lines 22-30; page 31-32 bridging paragraph; and Examples 3 and 5.	1-6, 15-24, and 33-56
X	WO 1998/011218 A1 (THEODOR-KOCHER INSTITUTE, ET AL.) 19 March 1998 See, in particular: P2: VAALLENFSSSYDYG; page 10, lines 20-24; page 11-12, bridging paragraph; page 28, lines 22-30; page 34, line 11 to page 36 line 14; page 37, lines 13-26; page 38, lines 3-10; page 61, line 25 to page 62, line 27; page 65, line 11 to page 66, line 30; Examples 5, 8, and 9; Figure 15; and Table 2 on page 94.	1-6, 15-24, 33-36, 38-42, 44, 46-53, 55, and 56
X	SONG, C.-H., *et al.*, "Identification of Chemoattractive Factors Involved in the Migration of Bone Marrow-Derived Mesenchymal Stem Cells to Brain Lesions Caused by Prions," Journal of Virology, 2011, Vol. 85, No. 21, pages 11069-11078. See, in particular: Abstract; page 11070, 'In vitro migration assay' section; page 11071-11072, bridging paragraph and 'IFA' section; and Figures 2 and 4.	1-6, 15, 16, 19-23, 36, and 51-53
X	XANTHOU, G., *et al.*, "Molecular characterization of the chemokine receptor CXCR3: evidence for the involvement of distinct extracellular domains in a multi-step model of ligand binding and receptor activation," European Journal of Immunology, 2003, Vol. 33, pages 2927-2936. See, in particular: page 2929, '2.3 Expression profiles of chimeric receptors' section; page 2934, '4.1 Materials' and '4.4 Flow cytometric analysis of chemokine receptor expression' sections; and Figures 3 and 4.	1-6, 15, 16, 19-23, 36, and 51-53
X	US 6,171,590 B1 (HOWARD, M., ET AL.) 09 January 2001 See, in particular: SEQ ID NOs:2 and 11; column 2, lines 11-40; column 8, line 22 to column 9, line 35; column 9, line 50 to column 10, line 15; column 12, lines 17-35; and Example 2.	57-60
X	WO 2001/042277 A2 (PROTEOM LIMITED) 14 June 2001 See, in particular: SEQ ID NOs: 1669, 1707, 1709, 1721, 1723, and 1731; page 5, paragraph 4; and page 6, paragraph 4.	57
X	WO 2002/061087 A2 (LIFESPAN BIOSCIENCES, INC.) 08 August 2002 See, in particular: SEQ ID NO:860; and paragraph [14].	57

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/AU2014/050048**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2001/078708 A1	25 October 2001	AU 5349601 A	30 Oct 2001
		US 2002018776 A1	14 Feb 2002
WO 1998/011218 A1	19 March 1998	AU 4260897 A	02 Apr 1998
		AU 734090 B2	07 Jun 2001
		CA 2265915 A1	19 Mar 1998
		EP 0925358 A1	30 Jun 1999
		EP 0925358 B1	25 Apr 2007
		JP 2002513388 A	08 May 2002
		JP 4502408 B2	14 Jul 2010
		JP 2009278990 A	03 Dec 2009
		JP 4955040 B2	20 Jun 2012
		US 6140064 A	31 Oct 2000
		US 6184358 B1	06 Feb 2001
		US 6686175 B1	03 Feb 2004
		US 6833439 B1	21 Dec 2004
		US 7029862 B1	18 Apr 2006
		US 2003158392 A1	21 Aug 2003
		US 7407655 B2	05 Aug 2008
		US 2010061983 A1	11 Mar 2010
US 6,171,590 B1	09 January 2001	AU 6285399 A	17 Apr 2000
		CA 2345978 A1	06 Apr 2000
		CN 1328470 A	26 Dec 2001
		EP 1117429 A1	25 Jul 2001
		JP 2002525338 A	13 Aug 2002
		NO 20011537 A	28 May 2001
		US 2001006942 A1	05 Jul 2001
		US 2002039578 A1	04 Apr 2002
		WO 0018431 A1	06 Apr 2000

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/AU2014/050048**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2001/042277 A2	14 June 2001	AU 2196101 A	18 Jun 2001
		EP 1237907 A2	11 Sep 2002
		US 2003078374 A1	24 Apr 2003
WO 2002/061087 A2	08 August 2002	US 2003113798 A1	19 Jun 2003

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2014/050048

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☒ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments: