

ANTI-CXCR3 ANTIBODIES

[001] This application claims the benefit of priority under 35 U.S.C. § 119 of United States Provisional Application No. 61/588,936, filed Jan. 20, 2012, which is incorporated herein by reference in its entirety.

[002] The invention relates to antibodies and methods of using antibodies to treat disorders associated with CXCR3 signaling such as diabetes mellitus type 1 (type I diabetes; T1D).

[003] Diabetes is characterized by chronic hyperglycemia resulting from a lack of insulin action, along with various characteristic metabolic abnormalities. Diabetes can be broadly divided into type I and type II. T1D is characterized by the loss of pancreatic β -cells of the Langerhans' islets, while type II diabetes is characterized by reductions in both insulin secretion and insulin sensitivity (insulin resistance). In the United States, the prevalence of diabetes is about 2 to 4 percent of the population, with type I diabetes (also known as insulin-dependent or IDDM) making up about 7 to 10 percent of all cases.

[004] Type I diabetes is characterized by the failure to produce sufficient insulin to maintain glucose homeostasis. This disorder is believed to be caused by autoimmune-mediated destruction of the pancreatic β -cells. Autoimmunity associated with type I diabetes involves the participation of both B and T autoreactive lymphocytes. Indeed, up to 98% of type I diabetes patients have

antibodies against one or more of their own β -cell antigens, including insulin, glutamic acid decarboxylase (GAD), insulinoma antigen-2 and insulinoma antigen-2b (IA-2 and IA-2 β), and heterogeneous islet cell cytoplasmic antigens (ICAs). Although it may not always be determinative, the level of one or more autoantibodies generally correlates with the state of β -cell destruction. Irvine, *et al.*, *Diabetes*, 26:138-47 (1997); Riley, *et al.*, *N. Engl. J. Med.*, 323:1167-72 (1990). Accordingly, autoantibodies can serve as indicators of the development of autoimmune diabetes and together with metabolic changes can predict the risk of developing diabetes in relatives of T1D patients

[005] The development of type I diabetes may be mediated by autoreactive T cells, as evidenced by tissue biopsies obtained near the time of T1D diagnosis that show the islets infiltrated with activated T cells. Bottazzo *et al.*, *N. Engl. J. Med.*, 313:353-60 (1985); Hanninen *et al.*, *J. Clin. Invest.*, 90:1901-10 (1992); Itoh *et al.*, *J. Clin. Invest.*, 92:2313-22 (1993); Imagawa, *et al.*, *Diabetes*, 50:1269-73 (2001).

[006] Chemokine (C-X-C motif) receptor 3 (CXCR3), also known as G protein-coupled receptor 9 (GPR9), CD183, IP-10 receptor, and Mig receptor, is a chemokine receptor expressed on autoreactive T cells that have been implicated in a range of physiological processes and related disorders, such as T1D. CXCR3 is largely absent from naïve T cells but is upregulated upon activation with antigen and recruits activated cells to sites of tissue inflammation in response to its primary ligands: CXCL9, CXCL10, and CXCL11. β cells have been shown to predominately express CXCL10, with lower levels of CXCL9, in

mouse models of T1D (Christen et al *The Journal of Immunology*, 2003, 171: 6838–6845; Morimoto et al. *J Immunol* 2004;173;7017-7024; Sarkar et al. *Diabetes*. 2012 Feb;61(2):436-46); and in islets from T1D patients having insulinitis (Uno et al 2010; Roep et al *Clinical and Experimental Immunology*, 2003, 159: 338–343; Sarkar et al. *Diabetes*. 2012 Feb;61(2):436-46). In addition, T cells that have infiltrated the pancreas have been shown to express CXCR3 in T1D mice models and type 1 diabetes patient pancreas samples (Christen et al, *The Journal of Immunology*, 2003, 171: 6838–6845; Van Halteren et al., *Diabetologia* 48:75-82 (2005); Uno et al 2010; Roep et al., *Clinical and Experimental Immunology*, 2003, 159: 338–343; Sarkar et al., *Diabetes*. 2012 Feb;61(2):436-46). Furthermore, knockout mice deficient in CXCR3 demonstrate a significant delay in onset and a reduction in incidence of T1D (Frigerio et al., *Nature Medicine* 8:1414-1420 (2002)), while overexpression of CXCL10 in the islets of transgenic mice promotes T cell infiltration and accelerates the onset of T1D (Rhode et al., *J. Immunol.* 175(6): 3516-24 (2005)). Neutralization of CXCL10 by antibody treatment has been shown to be protective (Christen et al., *The Journal of Immunology*, 2003, 171: 6838–6845).

[007] There are three isoforms of CXCR3, denoted A, B, and Alt., that have been identified in humans (Lasagni et al. *J. Exp. Med.* 2003 197:1537; Ehlert et al *J. Immunol.* 2004;173;6234-6240). CXCR3-A binds to the CXC chemokines CXCL9 (MIG), CXCL10 (IP-10), and CXCL11 (I-TAC); CXCR3-B also binds to these targets but also binds CXCL4; CXCR3-Alt appears to

interact with CXCL11. Although alternative splicing leads to the generation of several protein isoforms of CXCR3, CXCR3-A is the predominant form in vivo as the CXCR3-B and CXCR3-Alt are expressed at much lower levels at the protein levels. Lasagni et al. *J. Exp. Med.* 2003 197:1537; Ehlert et al *J. Immunol.* 2004;173:6234-6240.

[008] Efforts to disrupt the CXCR3 pathway using small molecule inhibitors of CXCR3 have not proved fully effective. Christen et al., *Clin Exp. Immunol.* 165: 318-328 (2011). Accordingly, research has focused on antibodies and other methods of disrupting CXCL10, primarily before the onset of diabetes. Morimoto et al., *J. Immun.* 173: 7017-7024 (2004); Oikawa et al., *Rev. Diabet. Stud.* 7: 209-224 (2010).

[009] In view of the prevalence of T1D and other disorders in which CXCR3 has been implicated, a need exists for additional methods that target CXCR3 signaling, e.g., to treat or reduce the progression of a disorder such as T1D in a patient.

[010] Disclosed herein are antibodies and methods of using antibodies that are capable of binding to CXCR3. In some embodiments, the antibodies can be used to prevent, treat or reduce the early progression of T1D in a subject by targeting the CXCR3 pathway. The antibodies and methods rely, at least in part, on the surprising result that neutralizing antibodies directed to CXCR3 can prevent onset of T1D in NOD mice when administered prior to disease onset, or can reverse the course of disease when administered in the new-onset stage of T1D in NOD mice. Furthermore, neutralization of CXCR3

activity is not associated with a significant impairment of the normal operation of the patient's immune system, thereby reducing the undesirable side effects of antibody therapy.

[011] Accordingly, in one aspect, disclosed herein are antibodies and antigen binding fragments capable of neutralizing the activity of CXCR3. In certain embodiments, CXCR3 neutralizing antibodies may be characterized by the ability to bind to a peptide selected from residues 1-58, 1-16, or 1-37 of SEQ ID NO:1. In some embodiments, the antibodies comprise all or portions of antibody clones (CI) designated CI 12, CI 135, CI 82, CI 53 and/or CI 4. In certain embodiments, variants of antibodies CI 12, CI 135, CI 82, CI 53 and/or CI 4 are provided, including CDR-grafted, humanized, back mutated, and fully human variants of the disclosed antibodies. In particular embodiments, the antibody comprises one or more complementary determining regions (CDRs), e.g., one or more of heavy chain CDR1, CDR2, and CDR3, and/or one or more of light chain CDR1, CDR2, and CDR3, from clones CI 12, CI 135, CI 82, CI 53 and/or CI 4 or any of the variants of clones 4, 12, 53, 82, and 135 disclosed herein. In some embodiments, the antibodies from CI 12, CI 135, CI 82, CI 53 and/or CI 4, or the chimeric and humanized versions thereof, exhibit certain beneficial properties, as compared to anti-CXCR3 clones 5H7, 7H5, V44D7, 1C6, and/or 49801. For example, the antibodies disclosed herein can exhibit increased binding affinity as compared to the anti-hCXCR3 clones 5H7, 7H5, V44D7, 1C6, and 49801. For example, the antibody may exhibit 1, 2, 3, 4, 5, or more fold better affinity (or any value in between) over anti-CXCR3 antibodies

such as 1C6, e.g., as measured by surface Plasmon resonance (e.g., using a BIACORE™ assay). The humanized antibodies disclosed herein also have a predicted reduction in immunogenicity as compared to the mouse anti-hCXCR3 clones 5H7, 7H5, V44D7, 1C6, and 49801. In addition, heavy chain clones 4.7-4.11 disclosed herein have been optimized to remove a deamidation site at positions 58 and 59 (using IMGT numbering) and thereby enhance stability over the initial mouse anti-hCXCR3 heavy chain variable domain (VH) CDR2 sequence.

[012] In another aspect, the present disclosure provides methods of prophylaxis prior to T1D onset, as well as methods of treating or reducing the progression of new onset T1D in a subject by administering an effective amount of a CXCR3 neutralizing antibody. In particular embodiments, the subject is a mammal, such as a human.

[013] In certain embodiments, the subject having new onset T1D is treated by the methods disclosed herein within 6 months of clinical diagnosis. In other embodiments, the subject is treated more than 6 months after clinical diagnosis, wherein the subject retains residual fasting integrated serum C-peptide levels of at least about 0.2 nmol/L.

[014] In some embodiments, subjects may be characterized by elevated fasting blood glucose levels in the absence of exogenous insulin above 120 mg/dL or an abnormally low fasting integrated serum C-peptide level of about 0.033 to 1.0 nmol/L x min during C-peptide stimulation. In particular embodiments the CXCR3 neutralizing antibody is administered at a dose of

about 0.03-3.7 mg/kg/dose. In some embodiments, the subject is administered at least one dose of antibody. In certain embodiments, the subject is administered repeat doses of antibody (e.g., at least yearly, quarterly, bimonthly, monthly, biweekly, weekly, or daily). In further embodiments, the methods described above may further comprise the step of administering an immunosuppressant and/or β -cell stimulating agent concurrently or sequentially (before or after) administering the CXCR3 neutralizing antibody.

[015] In various embodiments, the anti-CXCR3 antibodies disclosed herein are administered to treat a condition characterized by abnormal CXCR3 expression. In some embodiments, the anti-CXCR3 antibodies are administered to treat any condition that can benefit from the downregulation and/or neutralization of CXCR3 activity. In some embodiments, the anti-CXCR3 antibodies disclosed herein are administered to treat T1D.

[016] Additional embodiments and advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice of the invention. The embodiments and advantages of the invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims.

[017] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

[018] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate one (several) embodiment(s) of the invention and together with the description, serve to explain the principles of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[019] Figure 1 shows expression of insulin (left panel), CXCL10 (center panel), and CD3 (right panel) in pancreas sections from 6 week old female NOD mice (first row), 10 week old female NOD mice (second row) and new onset diabetic female NOD mice (third row).

[020] Figure 2 is a flow cytometry analysis of CXCR3 expression on T cells from the pancreas of female NOD mice with new-onset diabetes. CD4⁺ and CD8⁺ T cells were identified and stained for CXCR3 expression, as shown by the solid line in the bottom two graphs. Isotype control staining is shown by the shaded curve in the same two graphs.

[021] Figure 3 shows the percentage of non-diabetic female NOD mice over time for animals treated with PBS, anti-CXCR3, and control IgG starting at 10 weeks of age, before diabetes onset. Results from two independent studies are shown in Fig. 3A and 3B.

[022] Figure 4 shows pancreas sections from 26 week old non-diabetic female NOD mice treated with anti-CXCR3 antibody prophylactically starting at 10 weeks of age and stained for insulin (left panel) or CD3/Foxp3 (center and

right panels). The right panel is an increased magnification image of the section shown in the center panel.

[023] Figure 5 shows daily morning blood glucose values for female NOD mice treated with PBS, anti-CXCR3 antibody, control IgG, and murine anti-thymocyte globulin (murine thymoglobulin, mATG) antibody starting within 3-4 days after mouse was deemed diabetic. Each line represents an individual mouse. Arrows indicate days when treatment was provided.

[024] Figure 6A is a bar graph showing the percentage of T cells from female NOD mice treated with PBS, anti-CXCR3 antibody, control IgG, and mATG antibody that were CD4+ (left graph) and CD8+ (right graph). The pancreas was harvested from mice during the treatment course after the fifth injection of test article and from age-matched mATG treated mice. Figure 6B is a plot of CD44 expression (vertical axis) against CD62L expression (horizontal axis) on CD4+ T cells from the pancreas of mice treated with PBS, control antibody, or anti-CXCR3 antibody. G1 and G2 refer to gated high CD44 / low CD62L and low CD44 / low CD62L T cells, respectively. Figure 6C shows the expression of CXCR3 on CD4+ T cells in G1 and G2, as compared to CXCR3 expression on cells stained with isotype control antibody and gated on lymphocytes.

[025] Figure 7 shows pancreas sections from female NOD mice treated with control IgG (left panels), anti-CXCR3 antibody (center panels), and mATG (right panels) and stained for insulin (top row) or CD3 / Foxp3 (bottom row).

[026] Figure 8A-D is a plot of blood glucose levels following glucose challenge in age-matched non-diabetic female NOD mice (Fig. 8A), diabetic NOD mice treated with PBS (Fig. 8B), NOD mice in disease remission following anti-CXCR3 antibody treatment (Fig. 8C), and diabetic NOD mice treated with control IgG antibody (Fig. 8D). Glucose challenge was performed on mice 100 days after initial diabetes diagnosis and study enrollment. Each line represents data from an individual animal.

[027] Figure 9A-B shows the percentage of non-diabetic mice over time for NOD.Scid recipients receiving pooled donor CD4⁺ and CD8⁺ T cells isolated from female NOD mice treated with PBS, anti-CXCR3, control IgG, or mATG antibodies. T cells were isolated from diabetic female NOD mice around 80-90 days following treatment with PBS or control IgG, or from female NOD mice in disease remission around 80-90 days following treatment with anti-CXCR3 or mATG antibodies. Results from two independent studies are shown in Fig. 9A and 9B.

[028] Figure 10A shows the percentage of CD4⁺ and CD8⁺ donor T cells isolated from female NOD mice treated with PBS, anti-CXCR3, control IgG, or mATG antibodies (left panel) as described in Figure 9. The percentage of effector and central memory cells in the CD4⁺ and CD8⁺ T cell donor pools for each treatment group are shown in the right panels of Fig. 10A. Figure 10B shows the percentage of regulatory T cells in the donor T cell pools, identified by the expression of CD4 and CD25 or by the expression of CD4, CD25, and

Foxp3. Figure 10C shows the percentage of CD8+ (left panel) and CD4+ (right panel) in the donor T cell pools that also express CXCR3.

[029] Figure 11A-B shows the percentage of non-diabetic mice over time following adoptive transfer of T cells from donor OVA-specific TCR transgenic mice into RIP-OVA recipient mice that were left untreated or treated with anti-CXCR3 antibody or control IgG. Results from two studies are shown in Fig. 11A and 11B.

[030] Figure 12A shows CXCR3 expression on donor T cells analyzed by flow cytometry before adoptive transfer into RIP-OVA recipient mice (solid curve). Staining with isotype control antibody is shown in the shaded curve. Figure 12B shows the percentage of donor cells in the blood, spleen and pancreatic lymph nodes of recipient mice treated with anti-CXCR3 or control IgG antibody on days 2, 4, 7, 9, and 15 post adoptive transfer. Figure 12C shows the percentage of proliferating donor cells in the blood, spleen and pancreatic lymph nodes of recipient mice treated with anti-CXCR3 or control IgG antibody following adoptive transfer. Figure 12D shows the percentage of CXCR3+ donor cells in the blood, spleen, and pancreatic lymph nodes of recipient mice treated with anti-CXCR3 or control IgG antibody following adoptive transfer.

[031] Figure 13 shows sections of pancreas from RIP-OVA recipient mice left untreated and stained for insulin (upper left) or CD3 (upper right), or treated with anti-CXCR3 antibody and stained for insulin (bottom left) or CD3

(bottom right). The pancreas was harvested 60 days after adoptive transfer of donor T cells.

[032] Figures 14A-C show the level of inhibition of CXCR3-mediated chemotaxis to CXCL11 mediated by clones CI 4, 12, 53, 82, and 135. The data is shown as mean relative fluorescence units (RFUs) of the cells that migrate in the chemotaxis assay. Figure 14D shows the concentration of antibody needed to inhibit calcium mobilization by 50% for antibody clones CI 4, 12, 53, and 135.

[033] Figure 15A-C show the level of inhibition of CXCR3-mediated chemotaxis to CXCL9 (Fig. 15A), CXCL10 (Fig. 15B), and CXCL11 (Fig. 15C) mediated by clones CI 4, 12, 53, 82, and 135. The data is shown as mean relative fluorescence units (RFUs) of the cells that migrate in the chemotaxis assay.

[034] Fig. 16 shows histogram plots of antibody binding to cells expressing various different chemokine receptors. The concentration of bound antibody increases along the horizontal axis for each histogram plot.

[035] Fig. 17A shows an alignment of the heavy (VH) and light (VK) chain variable domains for clone 4.0 (labeled "parent") and certain humanized variants (labeled VH1-3 and 7-11 and VK 1-3). Fig. 17B shows an alignment of the heavy (VH) and light (VK) chain variable domains for clone 12.0 (labeled "parent") and certain humanized variants (labeled VH1-3 and VK 1-3). Fig. 17C shows an alignment of the heavy (VH) and light (VK) chain variable domains for clone 53.0 (labeled "parent") and certain humanized variants (labeled VH1-6 and VK 1-9). Fig. 17D shows an alignment of the heavy (VH) and light (VK)

chain variable domains for clone 82.0 (labeled "parent") and certain humanized variants (labeled VH1-3 and VK 1-3). Fig. 17E shows an alignment of the heavy (VH) and light (VK) chain variable domains for clone 135.0 (labeled "parent") and certain humanized variants (labeled VH1-3 and VK 1-3). Fig. 17F shows an alignment of the heavy (VH) and light (VK) chain variable domains for clone 4.0 (labeled "parent") and certain 4D humanized variants (labeled VH4-6 and VK4-7). Fig. 17G shows an alignment of the heavy (VH) and light (VK) chain variable domains for clone 53.0 (labeled "parent") and certain 4D humanized variants (labeled VH7-10 and VK10-13). The bottom sequence in each alignment in Fig. 17A-G represent the closest human germline sequences. Black boxes indicate CDR domains, shaded residues vary in sequence from the corresponding germline residue (Fig. 17A-E) or the corresponding parent residue (Fig. 17F-G), IMGT numbering and CDR delimitation is used. Fig. 17H shows an alignment of the heavy (VH) and light (VK) chain variable domains for clones 4.0, 12.0, 82.0, and 135, as well as the antibody clones 5H7 and 7H5. Black boxes indicate CDR domains, shaded residues vary in sequence from the previous sequence in the alignment, IMGT numbering is used.

[036] Fig. 18 shows the boundaries of the minimum epitope residues for antibody clones 4, 12, 53, 82, and 135. Residues important for binding activity are indicated by an X.

[037] Fig. 19 is a histogram plot showing antibody binding in human CXCR3 transfected 300.19 cells for chimeric clones 4, 12, 53, 82, and 135, as well as the humanized variants Hu1, Hu2, Hu3. Antibody was administered at 5

µg/ml (black line), 0.5 µg/ml (dark gray line), or 0.1 µg/ml (black dashed line) or 5 µg/ml secondary antibody alone (filled gray histogram), and data is plotted as number of cells (horizontal axis) against percentage of maximum fluorescence.

[038] Fig. 20A-C shows percentage inhibition of migration (vertical axis) of human CXCR3-transfected cells to CXCL9 (Fig. 20A), CXCL10 (Fig. 20B), and CXCL11 (Fig. 20C) in the presence or absence of 10 µg/ml of chimeric (Chim) or humanized (Hu1, Hu2 or Hu3) antibody variants of clones 4, 12, 53, 82, and 135, or the commercial clone 1C6.

[039] Fig. 21 is a plot showing the ability of chimeric (Chim) and humanized (Hu1, Hu2 or Hu3) antibody variants of clones 4, 12, 53, 82, and 135 and the commercial clone 1C6 to inhibit calcium mobilization in human CXCR3-Gqi4qi4 transfected CHO cells. Antibody concentration (horizontal axis) is plotted against percent maximal inhibition (vertical axis).

[040] Fig. 22A-D show the effects of anti-CXCR3 antibody treatment on the percentage of CD3+/CD4+ T cells (Fig. 22A), CD3+/CD8+ T cells (Fig. 22B), CXCR3+/CD3+/CD4+ T cells (Fig. 22C), and CXCR3+/CD3+/CD8+ T cells (Fig. 22D) in NOD-*scid* *IL2γ*^{null} (NSG) mice. HuIgG1 indicates human IgG1 (Herceptin), clones 4, 12, 53, 82, and 135 refer to the chimeric antibody clones.

[041] Fig. 23A depicts the amino acid sequences for heavy and light chain clones 12.0. Fig. 23B depicts the amino acid sequences for heavy and light chain clones 12.1. Fig. 23C depicts the amino acid sequences for heavy

and light chain clones 12.2. Fig. 23D depicts the amino acid sequences for heavy and light chain clones 12.3.

[042] Fig. 24A depicts the amino acid sequences for heavy and light chain clones 135.0. Fig. 24B depicts the amino acid sequences for heavy and light chain clones 135.1. Fig. 24C depicts the amino acid sequences for heavy and light chain clones 135.2. Fig. 24D depicts the amino acid sequences for heavy and light chain clones 135.3.

[043] Fig. 25A depicts the amino acid sequences for heavy and light chain clones 4.0. Fig. 25B depicts the amino acid sequences for heavy and light chain clones 4.1. Fig. 25C depicts the amino acid sequences for heavy and light chain clones 4.2. Fig. 25D depicts the amino acid sequences for heavy and light chain clones 4.3. Fig. 25E depicts the amino acid sequences for heavy chain clone 4.4. Fig. 25F depicts the amino acid sequences for heavy chain clone 4.5. Fig. 25G depicts the amino acid sequences for heavy chain clone 4.6. Fig. 25H depicts the amino acid sequences for heavy chain clone 4.7. Fig. 25I depicts the amino acid sequences for heavy chain clone 4.8. Fig. 25J depicts the amino acid sequences for heavy chain clone 4.9. Fig. 25K depicts the amino acid sequences for heavy chain clone 4.10. Fig. 25L depicts the amino acid sequences for heavy chain clone 4.11. Fig. 25M depicts the amino acid sequences for light chain clone 4.4. Fig. 25N depicts the amino acid sequences for light chain clone 4.5. Fig. 25O depicts the amino acid sequences for light chain clone 4.6. Fig. 25P depicts the amino acid sequences for light chain clone 4.7.

[044] Fig. 26A depicts the amino acid sequences for heavy and light chain clones 53.0. Fig. 26B depicts the amino acid sequences for heavy and light chain clones 53.1. Fig. 26C depicts the amino acid sequences for heavy and light chain clones 53.2. Fig. 26D depicts the amino acid sequences for heavy and light chain clones 53.3. Fig. 26E depicts the amino acid sequences for heavy chain clone 53.4. Fig. 26F depicts the amino acid sequences for heavy chain clone 53.5. Fig. 26G depicts the amino acid sequences for heavy chain clone 53.6. Fig. 26H depicts the amino acid sequences for light chain clone 53.4. Fig. 26I depicts the amino acid sequences for light chain clone 53.5. Fig. 26J depicts the amino acid sequences for light chain clone 53.6. Fig. 26K depicts the amino acid sequences for light chain clone 53.7. Fig. 26L depicts the amino acid sequences for light chain clone 53.8. Fig. 26M depicts the amino acid sequences for light chain clone 53.9. Fig. 26N depicts the amino acid sequences for heavy chain clone 53.7. Fig. 26O depicts the amino acid sequences for heavy chain clone 53.8. Fig. 26P depicts the amino acid sequences for heavy chain clone 53.9. Fig. 26Q depicts the amino acid sequences for heavy chain clone 53.10. Fig. 26R depicts the amino acid sequences for light chain clone 53.10. Fig. 26S depicts the amino acid sequences for light chain clone 53.11. Fig. 26T depicts the amino acid sequences for light chain clone 53.12. Fig. 26U depicts the amino acid sequences for light chain clone 53.13.

[045] Fig. 27A depicts the amino acid sequences for heavy and light chain clones 82.0. Fig. 27B depicts the amino acid sequences for heavy and

light chain clones 82.1. Fig. 27C depicts the amino acid sequences for heavy and light chain clones 82.2. Fig. 27D depicts the amino acid sequences for heavy and light chain clones 82.3.

[046] Fig. 28A-P show the nucleic acid sequences for heavy chain clones 12.0-12.3 and light chain clones 12.0-12.3, heavy chain clones 135.0-135.3 and light chain clones 135.0-135.3, heavy chain clones 4.0-4.11 and light chain clones 4.0-4.7, heavy chain clones 53.0-53.6 and light chain clones 53.0-53.9, and heavy chain clones 82.0-82.3 and light chain clones 82.0-82.3.

EXEMPLARY EMBODIMENTS

[047] Reference will now be made in detail to certain exemplary embodiments according to the present disclosure, certain examples of which are illustrated in the accompanying drawings.

[048] CXCR3

[049] CXCR3 (MIM: 300574, human GeneID: 2833, chemokine (C-X-C motif) receptor 3; also known as CD182, CD183, CKR-L2, CMKAR3, GPR9, IP10-R, Mig-R, MigR, G protein-coupled receptor 9, IP-10 receptor, IP10 receptor, Mig receptor, chemokine (C-X-C) receptor 3, interferon-inducible protein 10 receptor) is a chemokine receptor that is largely absent from naïve T cells but is upregulated upon activation with antigen and recruits these cells to sites of tissue inflammation in response to its primary ligands: CXCL9 (human GeneID: 4283), CXCL10 (human GeneID: 3627), and CXCL11 (human GeneID: 6373). β cells in the islet of Langerhans express CXCL9 and CXCL10 (Frigerio

et al., *Nature Medicine* 8:1414-1420 (2002) and T cells that have infiltrated the pancreas express CXCR3 (Christen *et al.*, *The Journal of Immunology*, 2003, 171: 6838–6845; Van Halteren *et al.*, *Diabetologia* 48:75-82 (2005); Uno *et al.* 2010; Roep *et al.*, *Clinical and Experimental Immunology*, 2003, 159: 338–343; Tanaka *et al.*, *Diabetes* 58: 2285-2291 (2009); Sarkar *et al.*, *Diabetes*. 2012 Feb;61(2):436-46).

[050] CXCR3 is expressed in a variety of organisms, including, for example, human, mouse, rat, cow, chimp, macaque, dog, frog, platypus, pig, and zebrafish. Table 1 lists the U.S. National Center for Biotechnology Information (NCBI) GeneID and protein reference sequence for CXCR3 from a variety of organisms. SEQ ID NO:1 is the full length human CXCR3 sequence (splice variant A). The peptide sequence of splice variant B is provided by reference sequence NP_001136269.1. Predicted extracellular domains of human CXCR3 splice variant A are described in Colvin *et al.*, *Mol. Cell. Bio.*, 26: 5838-49 (2006) and include residues 1-58, 1-16, 111-126, 190-223, 278-301 of SEQ ID NO:1, as shown below.

SEQ ID NO:1 NP_001495 Human CXCR3, isoform A

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1 mvlevsdhqv lndaevaall enfsssydyg enesdsccs ppcpqdfsln
fdraflpaly

61 slflglglg ngavaavlls rrtalsstdt filhlavadt llvltiplwa vdaavqwvfg
121 sgckkvagal fninfyagal llacisfdry lnivhatqly rrgpparvtl tclavwglcl
181 lfalpdfilf sahhderlna thcqynfpqv gtralrvlql vagflpllv maycyahila
241 vllvsrgqrr lramrlvvvv vvafalcwtp yhlvvlvdil mdlgalarnc gresrvdvak

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301 svtslglymh cclnplyaf vgkfrermw mllrlgcpn qrglqrqps

srrdsswset

361 seasysgl

Table 1

Species	GeneID	Protein Sequence
<i>Homo sapiens</i>	2833	NP_001495.1 (A) NP_001136269.1 (B)
<i>Mus musculus</i>	12766	NP_034040.1
<i>Rattus norvegicus</i>	84475	NP_445867.1
<i>Bos taurus</i>	497018	NP_001011673.1
<i>Macaca mulatta</i>	699438	NP_001138512.1
<i>Xenopus tropicalis</i>	496477	NP_001011067.1
<i>Xenopus laevis</i>	443669	AAH73571.1
<i>Canis lupus familiaris</i>	491952	NP_001011887.1
<i>Pan troglodytes</i>	465704	XP_521125.2 XP_001137964.1 XP_001137867.1
<i>Sus scrofa</i>	492278	CAH64842.1
<i>Danio rerio</i>	791973 654692	NP_001007315.1, XP_001330996.1 NP_001082899.2, XP_001923160.1
<i>Salmo salar</i>	100195464	NP_001133965.1
<i>Ornithorhynchus anatinus</i>	100085584	XP_001515888.1

[051] CXCR3 and CXCL10 are expressed in human T1D patients.

Uno et al., *Endocrine J.* 57: 991-996 (2010); Roep et al., *Clin. and Exp. Immun.* 159: 338-343 (2009); Tanaka et al., *Diabetes* 58: 2285-2291 (2009). In these patients, CXCL10 is expressed in the remaining insulin-producing beta cells in the islets. CXCR3 is expressed in invading T cells surrounding the islets. Similar expression patterns have been reproduced in non-obese diabetic (NOD) mice, a mouse models of diabetes. Morimoto et al., *J. Immun.* 173: 7017-7024

(2004); Li et al., *World J Gastroenterol.* 11(30): 4750-4752 (2005); Sarkar et al. *Diabetes.* 2012 Feb;61(2):436-46).

[052] CXCR3 is also expressed in T cells present in certain types of inflamed tissues, while CXCL9, CXCL10 and CXCL11 are often produced by local cells in inflammatory lesions. Accordingly, in some embodiments, therapies are disclosed for disrupting CXCR3 to treat T1D.

[053] Antibodies

[054] The term "antibody," as used herein, refers to any polypeptide comprising an antigen-binding site regardless of the source, species of origin, method of production, and/or characteristics, and encompasses immunoglobulins or antigen-binding parts or fragments thereof. As a non-limiting example, the term "antibody" includes human, orangutan, mouse, rat, goat, sheep, and chicken antibodies. The term includes but is not limited to polyclonal, monoclonal, monospecific, polyspecific, non-specific, humanized, fully human, camelized, single-chain, chimeric, synthetic, recombinant, hybrid, mutated, back-mutated, and CDR-grafted antibodies. For the purposes of the present invention, it also includes, unless otherwise stated, antibody fragments such as Fab, F(ab')₂, Fv, scFv, Fd, dAb, VHH (also referred to as nanobodies), and other antibody fragments that retain antigen-binding function, including bi-specific or multi-specific antibodies. The term "antibody" also refers to antigen-binding molecules that are not based on immunoglobulins. For example, non-immunoglobulin scaffolds known in the art include small modular

immunopharmaceuticals (see, e.g., U.S. Patent Application Publication Nos. 20080181892 and 20080227958 published July 31, 2008 and September 18, 2008, respectively), tetranectins, fibronectin domains (e.g., AdNectins, see U.S. Patent Application Publication No. 2007/0082365, published April 12, 2007), protein A, lipocalins (see, e.g., U.S. Patent No. 7,118,915), ankyrin repeats, and thioredoxin.

[055] The term “antigen-binding domain” refers to the part of an antibody molecule that comprises the area specifically binding to or complementary to a part or all of an antigen. Where an antigen is large, an antibody may only bind to a particular part of the antigen. In certain embodiments, a CXCR3 antibody or antigen-binding fragment comprises at least one antigen-binding domain. In some embodiments, the antibody or fragment is multi-specific and comprises two or more (e.g., 2, 3, 4, 5, or more) antigen-binding domains, such that the antibody or fragment is capable of binding two or more CXCR3 molecules at the same or different epitopes, or capable of binding to CXCR3 and at least one other antigen with high affinity. The antigen-binding portion of an antibody can comprise one or more fragments of an antibody that retains the ability to specifically bind to an antigen. These fragments may comprise the heavy and/or light chain variable region from a parent antibody or from a variant of a parent antibody.

[056] The “epitope” or “antigenic determinant” is a portion of an antigen molecule that is responsible for specific interactions with the antigen-binding domain of an antibody. An antigen-binding domain may be provided by one or

more antibody variable domains. An antigen-binding domain can comprise at least one antibody light chain variable region (VL) and at least one antibody heavy chain variable region (VH). An antigen-binding domain can also comprise only VH or only VL regions. For example, antibodies from camels and llamas (Camelidae, camelids) include a unique kind of antibody, which is formed by heavy chains only and is devoid of light chains. The antigen-binding site of such antibodies is one single domain, referred to as VHH. These have been termed "camelized antibodies" or "nanobodies". See, *e.g.*, U.S. Patent Nos. 5,800,988 and 6,005,079 and International Application Publication Nos. WO 94/04678 and WO 94/25591, which are incorporated by reference.

[057] The anti-CXCR3 antibodies disclosed herein can be generated by any suitable method known in the art. For example, the antibodies may comprise polyclonal antibodies or monoclonal antibodies. Methods of preparing polyclonal antibodies are known to the skilled artisan (Harlow et al., *Antibodies: a Laboratory Manual*, Cold Spring Harbor Laboratory Press, 2nd ed. (1988)). Immunogens comprising polypeptides of CXCR3, fragments thereof (*e.g.*, one or more extracellular domains, or the N-terminal 58 amino acids, or the N-terminal 37 amino acids, or the N-terminal 20 amino acids, or the N-terminal 16 amino acids, etc.), fusion proteins, or variants thereof can be used in generating the anti-CXCR3 antibodies.

[058] The immunogen may be produced by a cell that produces or overproduces CXCR3, which may be a naturally occurring cell, a naturally occurring mutant cell or a genetically engineered cell. Depending on the nature

of the polypeptides (e.g., percent hydrophobicity, percent hydrophilicity, stability, net charge, isoelectric point etc.), the immunogen may be modified or conjugated to alter its immunogenicity. For example, CXCR3 or a portion thereof can be conjugated to a carrier. The conjugation can include either chemical conjugation by derivatizing with active chemical functional groups, or through fusion-protein based methodology, or other methods known to the skilled artisan. Examples of carriers and/or other immunogenicity altering proteins include, but are not limited to, KLH, ovalbumin, serum albumin, bovine thyroglobulin, soybean trypsin inhibitor, and promiscuous T helper peptides.

[059] Various adjuvants may also be used with the CXCR3 immunogen to increase the immunological response. Examples of adjuvants include, but are not limited to, Freund's adjuvant (complete and incomplete), mineral oils, gels, alum (aluminum hydroxide), surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins (KLH), dinitrophenol, and human adjuvants, such as BCG (Bacille Calmette-Guerin) and *Corynebacterium parvum*. Additional examples of adjuvants which may be employed include the MPL-TDM adjuvant (monophosphoryl lipid A, synthetic trehalose dicorynomycolate). Immunization protocols are well known in the art and may be performed by any method that elicit an immune response in the animal host chosen. Thus, various administration routes can be used over various time periods as a design choice.

[060] For example, an immunogen, as exemplified herein, can be administered to various host animals including, but not limited to, rabbits, mice,

camelids, rats etc., to induce the production of serum containing polyclonal antibodies specific for CXCR3. The administration of the immunogen may involve one or more injections of an immunizing agent and, optionally, an adjuvant. In some embodiments, the immunogen (with or without adjuvant) is injected into the mammal by multiple subcutaneous or intraperitoneal injections, or intramuscularly or intravenously. In some embodiments, once a suitable polyclonal preparation is obtained, particular antibodies can be isolated by known separation techniques, such as affinity chromatography, panning, absorption, etc., such that an individual antibody species can be obtained. In some embodiments, the individual antibody species is subjected to further study, for example, sequencing to obtain the amino acid sequences of one or more CDRs.

[061] In some embodiments, the CXCR3 antibodies are monoclonal. A monoclonal antibody includes any antibody derived from a single eukaryotic, phage or prokaryotic clone that expresses the antibody. Monoclonal antibodies can be made, for example, via traditional hybridoma techniques (Kohler and Milstein, *Nature* 256: 495-499 (1975) and U.S. Pat. No. 4,376,110, incorporated herein by reference), recombinant DNA methods (U.S. Patent No. 4,816,567, incorporated herein by reference), or phage display techniques using antibody libraries (Clackson *et al.*, *Nature* 352: 624-628 (1991); Marks *et al.*, *J. Mol. Biol.* 222: 581-597 (1991)). For various other antibody production techniques, see *Antibodies: A Laboratory Manual*, eds. Harlow *et al.*, Cold Spring Harbor Laboratory, 1988. Other examples of methods which may be employed to

produce monoclonal antibodies include, but are not limited to, the human B-cell hybridoma technique (Kosbor et al., *Immunology Today* 4:72 (1983); and Cole et al., *Proc Natl Acad Sci USA* 80:2026 (1983)), and the EBV-hybridoma technique (Cole et al., *Monoclonal Antibodies and Cancer Therapy*, pp. 77-96, Alan R. Liss (1985)). Such antibodies may be of any immunoglobulin class, including IgG, IgM, IgE, IgA and IgD, and any subclass or variant thereof. The hybridoma producing the mAb of the invention may be cultivated in vitro or in vivo.

[062] In some embodiments, an immunogen comprising polypeptides of CXCR3, fragments thereof (e.g., one or more extracellular domains, or the N-terminal 58 amino acids, or the N-terminal 37 amino acids, or the N-terminal 20 amino acids, or the N-terminal 16 amino acids, etc.), fusion proteins, or variants thereof can be used to immunize a host animal (e.g., rabbits, mice, camelids, rats etc.) to generate the hybridomas that produce the monoclonal antibodies. Lymphocytes that produce or are capable of producing antibodies that specifically bind to CXCR3 can be collected from the immunized host and fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form hybridoma cells (Goding, *Monoclonal Antibodies: Principles and Practice*, Academic Press, pp. 59-103 (1986)).

[063] Multiple hybridomas producing monoclonal antibodies can be generated and those that exhibit beneficial properties or suggest therapeutic potential, for example, by preventing binding of CXCR3 ligand to its receptor, can be selected. The selected antibodies can be further modified to obtain or

enhance beneficial properties, such as having enhanced stability in vivo. For example, after hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned using dilution procedures and grown by standard culture methods (Goding, *Monoclonal Antibodies: Principles and Practice*, Academic Press, pp. 59-103 (1986)). Suitable culture media include, for example, Dulbecco's Modified Eagle's Medium (D-MEM) or RPMI-1640 medium. In addition, the hybridoma cells may be grown in vivo as tumors in an animal. The subclones can be assayed for specificity, affinity, and/or activity, and the subclones exhibiting the most beneficial properties can be selected for further characterization.

[064] A variety of alternative methods exist in the art for the production of monoclonal antibodies, any of which may be used to produce the anti-CXCR3 antibodies disclosed herein. For example, the monoclonal antibodies may be made by recombinant DNA methods, such as those described in U.S. Pat. No. 4,816,567, which is incorporated by reference in its entirety.

[065] DNA encoding monoclonal antibodies can be isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding to genes encoding the heavy and light chains of murine antibodies, or the chains from human, humanized or other antibodies) (Innis et al. *PCR Protocols, A Guide to Methods and Applications*, Academic (1990), and Sanger et al., *Proc Natl Acad Sci USA* 74:5463 (1977)). Hybridoma cells can serve as a source of such DNA. Once isolated, the DNA can be placed into expression vectors, which can be transfected into host cells

such as *E. coli* cells, NSO cells, COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA can also be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences (U.S. Pat. No. 4,816,567; and Morrison et al., *Proc Natl Acad Sci USA* 81:6851 (1984)) or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence from a non-immunoglobulin polypeptide. In some embodiments, a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody, or can be substituted for the variable domains of one CXCR3-combining site of an antibody to create a chimeric bivalent antibody.

[066] In some embodiments, the antibodies described herein can be modified to generate CDR grafted and/or otherwise humanized antibodies. CDR grafting is a form of humanization, but other humanizing techniques known in the art can also be used. CDR grafting procedures are known to the skilled artisan and may be based on CDR numbering designations including IMGT (the international ImMunoGeneTics information system®, Montpellier, France), Kabat, Chothia and modified-Chothia numbering schemes. See, e.g., imgt.org (summarizing the use of the IMGT continuous numbering system, which takes into account and combines the definition of the framework and complementarity determining regions, structural data from X-ray diffraction studies, and the characterization of the hypervariable loops, to provide unique numbering for all

IG and TR V-regions from all species); Abhinandan and Martin, *Mol Immunol.*, 45:3832-9 (2008); see also Abhinandan and Martin, *J. Mol. Biol.*, 369(3):852-62 (2007) (describing methods to assess the "humanness" of a chimeric antibody); Retter *et al.*, *Nucleic Acids Res.* 33(Database issue):D671-4 (2005) (describing the VBASE2 database of variable domain genes); and Johnson and Wu, *Int. Immunol.* 10(12):1801-5 (1998) (describing the distribution of lengths of CDRH3s).

[067] For example, using the IMGT numbering system, conserved amino acids always have the same position. The hydrophobic amino acids of the framework regions are also numbered in conserved positions, allowing for framework amino acids (and codons) located at the same positions in different sequences to be compared without requiring sequence alignments. In another example, the Kabat numbering system is as follows, CDR-H1 begins at approximately amino acid 31 (*i.e.*, approximately 9 residues after the first cysteine residue), includes approximately 5-7 amino acids, and ends at the next tyrosine residue. CDR-H2 begins at the fifteenth residue after the end of CDR-H1, includes approximately 16-19 amino acids, and ends at the next arginine or lysine residue. CDR-H3 begins at approximately the thirty third amino acid residue after the end of CDR-H2; includes 3-25 amino acids; and ends at the sequence W-G-X-G, where X is any amino acid. CDR-L1 begins at approximately residue 24 (*i.e.*, following a cysteine residue); includes approximately 10-17 residues; and ends at the next tyrosine residue. CDR-L2 begins at approximately the sixteenth residue after the end of CDR-L1 and

includes approximately 7 residues. CDR-L3 begins at approximately the thirty third residue after the end of CDR-L2; includes approximately 7-11 residues and ends at the sequence F-G-X-G, where X is any amino acid. Antibodies containing at least one of these CDRs can be used in the methods of the present disclosure.

[068] CDR-grafted antibodies can comprise heavy and light chain variable region sequences from a human antibody, wherein one or more of the CDR regions of VH and/or VL are replaced with CDR sequences from the donor antibodies e.g., from the murine antibodies described below that bind CXCR3. A framework sequence from any human antibody may serve as the template for CDR grafting. However, straight CDR chain replacement onto such a framework may lead to some loss of binding affinity to the antigen. The more homologous a human antibody is to the original, e.g. murine antibody, the less likely the possibility that combining the donor CDRs with the human framework will introduce distortions in the CDRs that could reduce affinity. Therefore, in some embodiments, the CDR-grafted CXCR3 antibodies of the present disclosure comprise a human variable framework that has at least a 65% sequence identity with the variable region framework of the donor murine CXCR3 neutralizing antibody. Methods for producing such antibodies are known in the art (see EP 239,400; PCT Publication No. WO 91/09967; and U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), and include veneering or resurfacing (EP 592,106; EP 519,596; Padlan (1991) *Mol. Immunol.* 28(4/5): 489-498; Studnicka et al. (1994) *Prot. Engineer.* 7(6): 805-814; and Roguska et

al. (1994) Proc. Acad. Sci. USA 91: 969-973), chain shuffling (U.S. Patent No. 5,565,352), and anti-idiotypic antibodies.

[069] In some embodiments, the antibodies described herein can be humanized. "Humanized antibodies" are antibody molecules that bind the desired antigen, have one or more CDRs from a non-human species, and have framework regions and/or constant domains from a human immunoglobulin molecule. Known human Ig sequences are disclosed in, e.g., www.ncbi.nlm.nih.gov/entrez-/query.fcgi; www.atcc.org/phage/hdb.html; www.sciquest.com/; www.abcam.com/; www.antibodyresource.com/onlinecomp.html; and Kabat et al., Sequences of Proteins of Immunological Interest, U.S. Dept. Health (1983). Imported human sequences can be used to reduce immunogenicity or reduce, enhance or modify binding, affinity, on-rate, off-rate, avidity, specificity, half-life, or any other suitable characteristic, as known in the art. Antibodies can be humanized using a variety of techniques known in the art, such as, but not limited to those described in Jones et al. (1986) Nature 321: 522; Verhoeyen et al. (1988) Science 239: 1534; Sims et al. (1993) J. Immunol. 151: 2296; Chothia and Lesk (1987) J. Mol. Biol. 196: 901; Carter et al. (1992) Proc. Natl. Acad. Sci. USA 89: 4285; Presta et al. (1993) J. Immunol. 151: 2623; U.S. Patent Nos. 5,589,205; 5,665,332; 6,180,370; 6,632,927; 7,241,877; 7,244,615; 7,244,832; 7,262,0505; and U.S. Patent Publication No. 2004/0236078 (filed Apr 30, 2004), which are hereby incorporated by reference in their entirety.

[070] In certain embodiments, framework residues in humanized or CDR-grafted antibodies may be substituted with the corresponding residue from the CDR donor antibody, e.g. substituted with framework residues from an anti-mouse CXCR3 neutralizing antibody, in order to alter, e.g., improve, antigen binding. See Queen et al., *Proc. Nat'l. Acad. Sci. USA* 86:10029-33 (December 1989). These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. See, e.g., U.S. Patent No. 5,585,089; and Riechmann et al. (1988) *Nature* 332:323, which are hereby incorporated by reference in their entirety. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, framework residues can be selected and combined from the consensus and import sequences so that the desired antibody characteristic, such as increased affinity for CXCR3, is achieved.

[071] Antibodies can be humanized or CDR-grafted, and framework residues from CDR-donors that are useful for improving antigen binding can be

identified, using a variety of techniques known in the art, such as but not limited to those described in Jones et al. (1986) *Nature* 321: 522; Verhoeyen et al. (1988) *Science* 239: 1534; Sims et al. (1993) *J. Immunol.* 151: 2296; Chothia and Lesk (1987) *J. Mol. Biol.* 196: 901; Carter et al. (1992) *Proc. Natl. Acad. Sci. USA* 89: 4285; Presta et al. (1993) *J. Immunol.* 151: 2623; and U.S. Patent Nos. 5,565,332; 5,723,323; 5,976,862; 5,824,514; 5,817,483; 5,814,476; 5,763,192; 5,723,323; 5,766,886; 5,714,352; 6,204,023; 6,180,370; 5,693,762; 5,530,101; 5,585,089; 5,225,539; and 4,816,567. In some embodiments, 4D humanization is used to prepare antibody variants of the present disclosure (e.g., to prepare the 4D humanized variants of clone 4, comprising any one of heavy chains 4.4-4.6 and any one of light chains 4.4-4.7). See WO 2009/032661 (which is incorporated herein by reference in its entirety), e.g., at paragraphs [0037]-[0044] for methods used in 4D humanization. Briefly, 4D humanization can comprise: a) building a 3-D model of the variable domain that is to be humanized; b) identifying the flexible residues in the variable domain using a molecular dynamics simulation of the 3-D model of the domain; c) identifying the closest human germline by comparing the molecular dynamics trajectory of the 3-D model to the molecular dynamics trajectories of 49 human germlines; and d) mutating the flexible residues, which are not part of the CDR, into their human germline counterpart (as identified in step c).

[072] In some embodiments, the CDR grafted and/or otherwise humanized antibodies can comprise CDR grafted and/or humanized variants of clones 4, 12, 53, 82, and 135. For instance, corresponding heavy and light

chain regions from any one of clones 4, 12, 53, 82, and 135 (e.g., clone 4 heavy chain and clone 4 light chain) can be joined to human constant domains to form chimeric antibodies. Chimeric antibodies can be further humanized by changing one or more framework or CDR amino acid to the corresponding human residue. Likewise, in some embodiments the six heavy and light chain CDR regions from any one of clones 4, 12, 53, 82, and 135 (e.g., clone 4 heavy chain CDR1, CDR2, and CDR3, and clone 4 light chain CDR1, CDR2, and CDR3) or from any of the variants of clones 4, 12, 53, 82, and 135 can be subcloned into human framework and/or constant domains to form humanized antibodies. Humanization can include using human variable domains, excluding the amino acids of the CDRs and/or any Vernier position residues. The humanized antibodies can also include further backmutated changes at residues positioned within four amino acids of the CDRs and/or at positions identified as "very dissimilar" between the original antibody sequence and human sequences, e.g., using IMGT-based modeling. Further mutations in the framework or CDR regions can be introduced to enhance stability or therapeutic effectiveness of the antibody, for example by introducing mutations to remove a deamidation site at positions 58 and 59 (IMGT numbering) of clone 4 VH CDR2.

[073] For instance, the antibodies, chimeric antibodies, and humanized antibodies disclosed herein can comprise the six CDRs and/or the heavy and light chain variable domains from any of clones 4, 12, 53, 82, and 135 and their chimeric or humanized variants. For example, the antibody or fragment capable of binding CXCR3 can comprise the three CDRs from any one of heavy

chains 4.0-4.11, heavy chains 12.0-12.3, heavy chains 53.0-53.10, heavy chains 82.0-82.3, and heavy chains 135.0-135.3. Similarly, the antibody or fragment can comprise the three CDRs from any one of light chains 4.0-4.7, light chains 12.0-12.3, light chains 53.0-53.13, light chains 82.0-82.3, and light chains 135.0-135.3. In some embodiments, the heavy and light chain CDRs are from the same clone, but can be from different variants of that clone (e.g., the three CDRs from heavy chain 4.1 paired with the three CDRs from light chain 4.2). Heavy and light chains 4.0, 12.0, 82.0, and 135.0 refer to the variable domain in the mouse antibody clones and the chimeric antibodies (where the antibodies comprise mouse variable domains and human framework regions). The remaining heavy and light chains refer to the humanized chains as shown in Table 11.

[074] In some embodiments, the antibody or fragment capable of binding CXCR3 can comprise any one of heavy chains 4.0-4.11, heavy chains 12.0-12.3, heavy chains 53.0-53.10, heavy chains 82.0-82.3, and heavy chains 135.0-135.3. Similarly, the antibody or fragment can comprise any one of light chains 4.0-4.7, light chains 12.0-12.3, light chains 53.0-53.13, light chains 82.0-82.3, and light chains 135.0-135.3.

[075] In some embodiments, the heavy and light chains are selected such that the three CDRs from a heavy chain of a particular clone (e.g., the CDRs from a clone 4 heavy chain) are paired with the three CDRs from any of the light chains for that clone (e.g., the CDRs from a clone 4 light chain). In some embodiments, the heavy and light chains are selected such that a heavy

chain from a particular clone (e.g., a clone 4 heavy chain) is paired with any of the light chains for that clone (e.g., a clone 4 light chain).

[076] In some embodiments, the three CDRs from any one of heavy chain variable domains 4.0-4.11 can be paired with the three CDRs from any one of light chain variable domains 4.0-4.7; the three CDRs from any one of heavy chain variable domains 12.0-12.3 can be paired with the three CDRs from any one of light chain variable domains 12.0-12.3; the three CDRs from any one of heavy chain variable domains 53.0-53.10 can be paired with the three CDRs from any one of light chain variable domains 53.0-53.13; the three CDRs from any one of heavy chain variable domains 82.0-82.3 can be paired with the three CDRs from any one of light chain variable domains 82.0-82.3; or the three CDRs from any one of heavy chain variable domains 135.0-135.3 can be paired with the three CDRs from any one of light chain variable domains 135.0-135.3.

[077] In some embodiments, any one of heavy chain variable domains 4.0-4.11 can be paired with any one of light chain variable domains 4.0-4.7, any one of heavy chain variable domains 12.0-12.3 can be paired with any one of light chain variable domains 12.0-12.3, any one of heavy chain variable domains 53.0-53.10 can be paired with any one of light chain variable domains 53.0-53.13, any one of heavy chain variable domains 82.0-82.3 can be paired with any one of light chain variable domains 82.0-82.3, or any one of heavy chain variable domains 135.0-135.3 can be paired with any one of light chain variable domains 135.0-135.3.

[078] An alignment of certain heavy and light chain variable domains is shown in Fig. 17. In some embodiments, an antibody as disclosed herein can comprise the paired heavy and light chain variable domains as shown in Table 2 (Ch = chimeric, Hu = humanized, VH = heavy chain, VK = light chain). For example, the first entry in Table 2 indicates a clone 4 variant comprising heavy chain 4.0 and light chain 4.0. The second entry indicates a clone 4 variant comprising heavy chain 4.1 and light chain 4.1. Each antibody, comprising the indicated heavy chain and light chain sequence, was also assigned an antibody identifier in the second column of Table 2. For instance, the first entry in the table (comprising heavy chain 4.0 and light chain 4.0) was assigned the antibody identifier 4Ch, while the second antibody in the table (comprising heavy chain 4.1 and light chain 4.1) was assigned the identifier 4Hu1.

Clone	Antibody	Heavy Chain	VH SEQ ID NO	Light Chain	VK SEQ ID NO
Clone 4	4Ch	VH		VK	
Clone4	4Hu1	VH1		VK1	
Clone4	4Hu2	VH2		VK2	
Clone4	4Hu3	VH3		VK3	
Clone4	4Hu4	VH2		VK3	
Clone4	4Hu5	VH3		VK2	
Clone4	4Hu6	VH4		VK4	
Clone4	4Hu7	VH4		VK7	
Clone4	4Hu8	VH5		VK5	
Clone4	4Hu9	VH5		VK6	
Clone4	4Hu10	VH6		VK4	
Clone4	4Hu11	VH2		VK1	
Clone4	4Hu12	VH1		VK2	
Clone4	4Hu13	VH3		VK1	
Clone4	4Hu14	VH1		VK3	
Clone4	4Hu15	VH7		VK2	
Clone4	4Hu16	VH8		VK2	
Clone4	4Hu17	VH9		VK2	

Clone4	4Hu18	VH10		VK2	
Clone4	4Hu19	VH11		VK2	
Clone 12	12Ch	VH		VK	
Clone12	12Hu1	VH1		VK1	
Clone12	12Hu2	VH2		VK2	
Clone12	12Hu3	VH3		VK3	
Clone 82	82Ch	VH		VK	
Clone82	82Hu1	VH1		VK1	
Clone82	82Hu2	VH2		VK2	
Clone82	82Hu3	VH3		VK3	
Clone135	135Ch	VH		VK	
Clone135	135Hu1	VH1		VK1	
Clone135	135Hu2	VH2		VK2	
Clone135	135Hu3	VH3		VK3	
Clone 53	53Ch	VH		VK	
Clone53	53Hu1	VH1		VK1	
Clone53	53Hu2	VH2		VK2	
Clone53	53Hu3	VH3		VK3	
Clone53	53Hu4	VH1		VK2	
Clone53	53Hu5	VH2		VK1	
Clone53	53Hu6	VH2		VK4	
Clone53	53Hu7	VH2		VK5	
Clone53	53Hu8	VH2		VK6	
Clone53	53Hu9	VH2		VK7	
Clone53	53Hu10	VH2		VK8	
Clone53	53Hu11	VH2		VK9	
Clone53	53Hu12	VH4		VK2	
Clone53	53Hu13	VH5		VK2	
Clone53	53Hu14	VH6		VK2	
Clone53	53Hu15	VH1		VK4	
Clone53	53Hu16	VH1		VK6	
Clone53	53Hu17	VH6		VK4	
Clone53	53Hu18	VH6		VK6	
Clone53	53Hu19	VH7		VK10	
Clone53	53Hu20	VH7		VK11	

Table 2

[079] The term "specific interaction," or "specifically binds," or the like, means that two molecules form a complex that is relatively stable under

physiologic conditions. Specific binding is characterized by a high affinity and a low to moderate capacity. Nonspecific binding usually has a low affinity with a moderate to high capacity. Typically, the binding is considered specific when the affinity constant K_a is higher than 10^6 M^{-1} , or preferably higher than 10^8 M^{-1} . In some embodiments, antibodies, variants, and fragments thereof bind their antigen(s) with association constants of at least 10^6 , 10^7 , 10^8 , 10^9 M^{-1} , or higher. In some embodiments, the antibodies, variants, and fragments thereof bind CXCR3 with at least the binding kinetics shown in any one of Tables 7A-B, 8-10, and/or 12. If necessary, non-specific binding can be reduced without substantially affecting specific binding by varying the binding conditions. Such conditions are known in the art, and a skilled artisan using routine techniques can select appropriate conditions. The conditions are usually defined in terms of concentration of antibodies, ionic strength of the solution, temperature, time allowed for binding, concentration of blocking molecules, such as serum albumin and milk casein.

[080] Disclosed herein are anti-CXCR3 antibodies that can, in some embodiments, neutralize CXCR3. A "CXCR3 neutralizing antibody," binds to CXCR3 and blocks the activity of the receptor, such as the typical physiological and genetic responses resulting from CXCR3 ligands binding to CXCR3. Neutralizing activity may be complete (100% neutralization) or partial, *e.g.*, approximately 10, 20, 30, 40, 50, 60, 70, 80, 90, 95 (or any percentage in between) or more neutralizing and will depend on various factors known to the skilled artisan, such as antibody concentration, affinity, and epitope as well as

the particular assay used to evaluate neutralizing activity. The neutralizing activity of a CXCR3 neutralizing antibody may be shown by assays to measure inhibition of, e.g., ligand binding, GTP binding, calcium mobilization, cell chemotaxis, and/or receptor internalization. Numerous assays for determining the activity of neutralizing antibodies, and particularly CXCR3 neutralizing antibody, are known to the skilled artisan and may be readily adapted to verify that a particular antibody is neutralizing.

[081] For example, in some embodiments, the neutralizing activity of an antibody for use in the methods of the invention may be assessed by a chemotaxis assay, substantially as set forth in the package insert for the antibody produced by clone 49801 and sold by R&D Systems® (Cat. no. MAB160). The Neutralization Dose-50 (ND₅₀) is defined as the concentration of antibody required to yield one-half maximal inhibition of the cell surface CXCR3 mediated rhI-TAC response in a responsive cell line, at a specific rhI-TAC concentration. To measure the ability of the antibody to block rhI-TAC induced chemotaxis of hCXCR3 transfected BaF/3 cells, rhI-TAC at 7 ng/mL is added to the lower compartment of a 96-well chemotaxis chamber (NeuroProbe, Cabin John, MD). The chemotaxis chamber is then assembled using a PVP-free polycarbonate filter (5μ pore size). Serial dilutions of the antibody (e.g., from 0.001 to 10000 μg/mL) and 0.25 x 10⁶ cells/well are added to the top wells of the chamber. After incubation for 3 hours at 37 °C in a 5% CO₂ humidified incubator, the chamber is disassembled, and the cells that migrate through to

the lower chamber are transferred to a working plate and quantitated using, for example, Resazurin Fluorescence.

[082] Colvin *et al.*, *Mol. Cell. Bio.*, 26: 5838-49 (2006) describe additional assays that can be used, in certain embodiments, to determine the neutralizing activity of neutralizing CXCR3 antibodies for use in the invention. Briefly, 300-19 cells, a murine pre-B-cell leukemia cell line that functionally expresses CXCR4 may be used. Following transfection, this line can functionally express other chemokine receptors, e.g., human CXCR3 (*see, e.g.*, paragraphs 201-209 of U.S. Patent Application Publication No. 2010/0061983, which are incorporated by reference). 300-19 cells expressing human CXCR3 may be grown in complete RPMI medium containing 10% fetal bovine serum (FBS). To assess binding of CXCR3 ligands to CXCR3 in the presence of candidate neutralizing CXCR3 antibodies, 400,000 CXCR3/300-19 cells are placed into 96-well tissue culture plates in a total volume of 150 μ L of binding buffer (0.5% BSA, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 50 mM HEPES, pH 7.4). A total of 0.04 nM of ^{125}I -labeled CXCL10 (New England Nuclear, Boston, MA) or CXCL11 (Amersham Biosciences, Piscataway, NJ) and 5×10^6 nM to 500 nM of unlabeled CXCL10 or CXCL11 (Peprotech, Rocky Hill, NJ) may be added to the cells and incubated for 90 min at room temperature with shaking. The cells are transferred onto 96-well filter plates (Millipore, Billerica, MA) that are presoaked in 0.3% polyethyleneimine and washed three times with 200 μ l binding buffer supplemented with 0.5 M NaCl. The plates are dried, and the radioactivity is measured after the addition of scintillation fluid in a Wallac Microbeta

scintillation counter (Perkin-Elmer Life Sciences, Boston, MA). Binding of CXCL9 may be assessed analogously to CXCL10 and 11.

[083] In certain embodiments, the antibodies disclosed herein can prevent or reduce calcium flux into CXCR3-expressing cells. In some embodiments, calcium flux may be detected in cells such as CXCR3/300-19 cells. Approximately 5×10^6 cells are suspended in 2 ml of RPMI medium with 1% BSA. Fifteen micrograms of Fura-2 (Molecular Probes, Eugene, OR) are added and the cells are incubated at 37 °C for 20 min. The cells are washed twice in PBS and resuspended in 2 ml of calcium flux buffer (145 mM NaCl, 4 mM KCl, 1 mM NaHPO₄, 1.8 mM CaCl₂, 25 mM HEPES, 0.8 mM MgCl₂, and 22 mM glucose). Fluorescence readings are measured at 37 °C in a DeltaRAM fluorimeter (Photon Technology International, Lawrenceville, NJ). Before and after the addition of chemokines (*e.g.*, CXCL9, 10, or 11), intracellular calcium concentrations are recorded as the excitation fluorescence intensity emitted at 510 nm in response to sequential excitation at 340 nm and 380 nm and presented as the relative ratio of fluorescence at 340 nm to that at 380 nm.

[084] In certain embodiments, CXCR3 neutralization can be evaluated by measuring a reduction in receptor internalization. In some embodiments, receptor internalization assays may be performed by incubating about 2.5×10^5 cells, such as CXCR3/300-19 cells in RPMI medium with 1% BSA with various concentrations of CXCL10, CXCL11, or CXCL9 for 30 min at 37 °C. The cells may then be washed with ice-cold fluorescence-activated cell sorter buffer and

subsequently analyzed for surface expression of CXCR3 using a PE-conjugated CXCR3 antibody.

[085] Additional assays for assessing neutralizing activity are disclosed in, for example, Examples 2-4 of U.S. Patent No. 7,405,275, which are incorporated by reference.

[086] As assessed by any of the above assays, a neutralizing CXCR3 antibody may have, in certain embodiments, an ND_{50} of approximately 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 40, 50, or 100 $\mu\text{g/mL}$. In particular embodiments, the ND_{50} may be 0.5-12 $\mu\text{g/mL}$, and in more particular embodiments, 1-6 $\mu\text{g/mL}$.

[087] Isolated CXCR3 antibodies disclosed herein may include those that bind specific epitopes of CXCR3. For example, antibodies for use in the invention may bind a peptide comprising all or part (e.g., a fragment of at least 5, 6, 8, 10, 12, 14, 15, 16, 18, or 20 residues) of a sequence selected from residues 1-58, 1-16, or 1-37 of SEQ ID NO:1. In some embodiments, the antibodies disclosed herein include those that bind one or more of the epitopes identified in Fig. 18. In some embodiments, an anti-CXCR3 antibody can comprise an antibody that binds to a CXCR3 epitope comprising SDHQVLNDAE (SEQ ID NO:). In some embodiments, the epitope comprises SDHQVLND (SEQ ID NO:), DHQVLND (SEQ ID NO:), and/or VLNDAE (SEQ ID NO:). In certain embodiments, the epitope comprises the sequence VLND (SEQ ID NO:). In some embodiments, the epitope comprises XDXXVXNDXX (SEQ ID NO:), where X indicates any amino acid. In some embodiments, the

epitope comprises XDXXVXND (SEQ ID NO:), DXXVXND (SEQ ID NO:), and/or VXNDXX (SEQ ID NO:), where X indicates any amino acid. In certain embodiments, the epitope comprises the sequence VXND (SEQ ID NO:), where X indicates any amino acid.

[088] Anti-CXCR3 antibodies may be pan-specific for CXCR3 sequences from different species or selective for CXCR3 sequences from a particular species or a particular isotype of CXCR3. In particular embodiments, the CXCR3 antibody is specific for the subject species to which it is administered. Accordingly, in some embodiments, a CXCR3 antibody may be specific for a human CXCR3 sequence (e.g., capable of binding a peptide comprising a sequence homologous to any of the subsequences of SEQ ID NO:1 listed above). Homologous sequence will be readily identified by a person having ordinary skill in the art by means such as protein sequence alignments (e.g., BLASTp, ClustalW, *et cetera*). In particular embodiments, an antibody for use in the invention binds to a peptide comprising a sequence at least 90, 95, or 99% (or any percentage in between) similar or identical to SEQ ID NO:1 over the entire length of the sequence or a window of at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, or 70 residues (or any value in between). In some embodiments, the antibody is able to bind to an epitope at least 80, 85, 90, 95, or 99% (or any percentage in between) similar or identical to one of the epitopes described above (see also Fig 18).

[089] Particular antibodies disclosed herein include, for example, antibody clones (CI) 12, CI 135, CI 82, CI 53, and CI 4, as well as their chimeric and humanized variants.

[090] In some embodiments, the antibodies disclosed herein exhibit certain improved properties over antibodies known in the art, including antibodies 5H7 and 7H5 (disclosed in, e.g., U.S. Patent No. 7,405,275; CDRs for the antibodies are disclosed in Tables 1 and 2 and in the referenced sequence listings, which are incorporated by reference); V44D7 (described in International Publication WO 2008/094942), 1C6 (described in U.S. Patent No. 7,407,655; with epitope mapping described in Examples 8 and 9, which are incorporated by reference), and 49801, sold by R&D Systems as catalog no. MAB160.

[091] In some embodiments, the antibody clones disclosed herein (clones 4, 12, 53, 82, and 135 and their chimeric and humanized counterparts) exhibit certain surprising benefits over the known antibodies 5H7, 7H5, V44D7, 1C6, and 49801. For example, the clones disclosed herein exhibit increased binding affinity as compared to the anti-hCXCR3 clones 5H7, 7H5, V44D7, 1C6, and 49801. The humanized clones disclosed herein may also have reduced immunogenicity as compared to the mouse anti-hCXCR3 clones 5H7, 7H5, V44D7, 1C6, and 49801. In addition, the antibodies disclosed herein, such as those comprising heavy chain clones 4.7-4.11 have been optimized by modification at positions 58 and 59 (using IMGT numbering) to remove a

deamidation site to enhance stability. In some embodiments, the antibodies disclosed herein retain CXCR3 neutralizing activity.

[092] In certain embodiments, the antibodies or fragments disclosed herein can comprise VH and/or VL CDR sequences that are about 80% to about 100% (e.g., about 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the VH and/or VL CDR sequences in any one of antibodies CI 12, CI 135, CI 82, CI 53, and CI 4 or in the chimeric or humanized variants of those clones (e.g., 80-100% identical to the six CDRs in CI 12, or to the six CDRs in CI 12.1, etc). In some embodiments, the antibodies or fragments can comprise VH and VL CDR sequences that contain 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions (including additions, deletions, and substitutions, such as conservative substitutions) relative to the VH and/or VL CDR sequences in any one of antibodies CI 12, CI 135, CI 82, CI 53, and CI 4.

[093] As used herein, the terms "percent (%) sequence identity" or "homology" are defined as the percentage of amino acid residues or nucleotides in a candidate sequence that are identical with the amino acid residues or nucleotides in the reference sequences after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and excluding conservative nucleic acid substitutions. Optimal alignment of the sequences for comparison may be produced, besides manually, by means of local homology algorithms known in the art or by means of computer programs which use these algorithms (e.g., BLAST P).

[094] In some embodiments, an isolated CXCR3 antibody or antigen-binding fragment as disclosed herein comprises a VH amino acid sequence comprising at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% (or any percentage in between) identity to the amino acid sequence of any one of heavy chains 4.0-4.11, heavy chains 12.0-12.3, heavy chains 53.0-53.10, heavy chains 82.0-82.3, and heavy chains 135.0-135.3. In certain embodiments the antibody or fragment comprises a VH amino acid sequence having 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mutations (including additions, deletions, and substitutions, such as conservative substitutions) in the amino acid sequence of SEQ any one of heavy chains 4.0-4.11, heavy chains 12.0-12.3, heavy chains 53.0-53.10, heavy chains 82.0-82.3, and heavy chains 135.0-135.3. As used herein, a "conservative substitution" refers to the replacement of a first amino acid by a second amino acid that does not substantially alter the chemical, physical and/or functional properties of the antibody or fragment (e.g., the antibody or fragment retains the same charge, structure, polarity, hydrophobicity/hydrophilicity, and/or preserves functions such as the ability to recognize, bind to, and/or neutralize CXCR3 activity).

[095] In certain embodiments, an isolated CXCR3 antibody or antigen-binding fragment as disclosed herein comprises a VL amino acid sequence comprising at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% (or any percentage in between) identity to the amino acid sequence of any one of light chains 4.0-4.7, light chains 12.0-12.3, light chains 53.0-53.13, light chains 82.0-82.3, and light chains 135.0-135.3. In various embodiments the antibody

or fragment comprises a VL amino acid sequence having 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mutations (including additions, deletions, and substitutions, such as conservative substitutions) in the amino acid sequence of any one of light chains 4.0-4.7, light chains 12.0-12.3, light chains 53.0-53.13, light chains 82.0-82.3, and light chains 135.0-135.3.

[096] In certain embodiments, an isolated CXCR3 antibody or antigen-binding fragment as disclosed herein comprises a heavy chain variable domain comprising at least 80% identity to the amino acid sequence of any one of heavy chains 4.0-4.11, heavy chains 12.0-12.3, heavy chains 53.0-53.10, heavy chains 82.0-82.3, and heavy chains 135.0-135.3, and comprises a light chain variable domain comprising at least 80% identity to the amino acid sequence of any one of light chains 4.0-4.7, light chains 12.0-12.3, light chains 53.0-53.13, light chains 82.0-82.3, and light chains 135.0-135.3. In some embodiments, the heavy and light chains are selected such that a heavy chain from a particular clone (e.g., a clone 4 heavy chain) is paired with any of the light chains for that clone (e.g., a clone 4 light chain). In some embodiments, the heavy and light chains are paired as shown in Table 2. In further embodiments, the antibody or fragment comprising the disclosed VH and/or VL sequences retains the ability to neutralize CXCR3 activity.

[097] In some embodiments, the antibody disclosed herein is a humanized variant of CI 12, 135, 82, 53, and/or 4. In other embodiments, the antibody is fully human. In certain embodiments, the antibody is a humanized or fully human derivative of an antibody selected from clones 12, 135, 82, 53,

and 4. In some embodiments, the antibody has an affinity constant of at least 10^8 M^{-1} (e.g., at least 10^8 M^{-1} , at least 10^9 M^{-1} , at least 10^{10} M^{-1} , or at least 10^{11} M^{-1} , or any value in between). In some embodiments, the antibody is capable of binding to all CXCR3 isoforms. In certain embodiments, the antibody is capable of binding to both the A and B isoforms of CXCR3. In some embodiments, the antibody does not bind the B-isoform of CXCR3.

[098] In some embodiments, an isolated CXCR3 antibody or antigen-binding fragment comprises VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2 and/or VL CDR3 comprising amino acid sequences about 90% to about 100% (e.g., about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the VH and VL CDR sequences from any one of clones 12, 135, 82, 53, and 4 and their chimeric and humanized variants. In some embodiments, an isolated CXCR3 antibody or antigen-binding fragment comprises VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2 and/or VL CDR3 comprising amino acid sequences identical to, or comprising 1, 2, 3, 4, or 5 amino acid residue mutations (including additions, deletions, and substitutions, such as conservative substitutions) relative to the VH and VL CDR sequences from any one of clones 12, 135, 82, 53, and 4 and their chimeric and humanized variants.

[099] In some embodiments, the anti-CXCR3 antibody or fragment comprises a heavy chain having three CDRs (heavy chain CDR1, CDR2, and CDR3) and a light chain having three CDRs (light chain CDR1, CDR2, and CDR3). In some embodiments, the VH CDR1 has 1, 2, or 3 amino acid

mutations relative to the VH CDR1 sequence of any one of clones 12, 135, 82, 53, and 4 and their chimeric and humanized variants. In some embodiments, the VH CDR2 has 1, 2, or 3 amino acid mutations relative to the VH CDR2 sequence of any one of clones 12, 135, 82, 53, and 4 and their chimeric and humanized variants. In some embodiments, the VH CDR3 has 1, 2, or 3 amino acid mutations relative to the VH CDR3 sequence of any one of clones 12, 135, 82, 53, and 4 and their chimeric and humanized variants. In some embodiments, the VL CDR1 has 1, 2, or 3 amino acid mutations relative to the VL CDR1 sequence of any one of clones 12, 135, 82, 53, and 4 and their chimeric and humanized variants. In some embodiments, the VL CDR2 has 1 or 2 amino acid mutations relative to the VL CDR2 sequence of any one of clones 12, 135, 82, 53, and 4 and their chimeric and humanized variants. In some embodiments, the VL CDR3 has 1, 2, or 3 amino acid mutations relative to the VL CDR3 sequence of any one of clones 12, 135, 82, 53, and 4 and their chimeric and humanized variants. In certain embodiments, the heavy and light chain CDR 1, CD2, and CDR3 are the CDRs from any one of clones 12, 135, 82, 53, and 4 and their chimeric and humanized variants, or comprise 1-3 amino acid mutations relative to the CDR set in the selected from any one of the antibody clones or their chimeric/humanized variants. In some embodiments, the mutations are at the highlighted positions shown in the alignments in Fig. 17A-H. In some embodiments, the mutation is at one or more of positions 58 and 59 in VH CDR2 from any one of clones 4.0-4.11.

[0100] In some embodiments, the anti-CXCR3 antibody or fragment comprises a heavy chain and a light chain. In some embodiments, the heavy chain is at least about 90% identical (e.g., at least about 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical, or any percentage in between), or has 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid mutations relative to, any one of heavy chains 4.0-4.11, heavy chains 12.0-12.3, heavy chains 53.0-53.10, heavy chains 82.0-82.3, and heavy chains 135.0-135.3. In some embodiments, the light chain is at least about 90% identical (e.g., at least about 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical, or any percentage in between), or has 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid mutations relative to, any one of light chains 4.0-4.7, light chains 12.0-12.3, light chains 53.0-53.13, light chains 82.0-82.3, and light chains 135.0-135.3. In some embodiments, the heavy chain is at least about 90% identical (e.g., at least about 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical, or any percentage in between), or has 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid mutations relative to, any one of heavy chains 4.0-4.11, heavy chains 12.0-12.3, heavy chains 53.0-53.10, heavy chains 82.0-82.3, and heavy chains 135.0-135.3 and/or the light chain is at least about 90% identical (e.g., at least about 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% identical, or any percentage in between), or has 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid mutations relative to, any one of light chains 4.0-4.7, light chains 12.0-12.3, light chains 53.0-53.13, light chains 82.0-82.3, and light chains 135.0-135.3. In some embodiments, the mutations are at the positions shown in the alignments in Fig. 17A-H.

[0101] In certain embodiments, an isolated CXCR3 antibody or antigen-binding fragment comprising the VH and/or VL CDR sequences disclosed above retains CXCR3 neutralizing activity.

[0102] In various embodiments, the heavy and light chain variable domains of a CXCR3 antibody or fragment can comprise at least one framework region (e.g., at least one of FR1, FR2, FR3, and FR4). The framework regions of the heavy chain are designated VH FR, while the framework regions of the light chain are here designated VL FR. In certain embodiments the framework regions can contain substitutions, insertions, or other alterations. In certain embodiments, these alterations result in an improvement or optimization in the binding affinity of the antibody. Non-limiting examples of framework region residues that can be modified include those that non-covalently bind CXCR3 directly, interact with or effect the conformation of a CDR, and/or participate in the VL-VH interface.

[0103] In certain embodiments, the heavy chain (VH) of a CXCR3 antibody or fragment may comprise FR1, FR2, FR3 and/or FR4 having amino acid sequences that are about 80% to about 100% identical (e.g., 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, or any percentage in between) to the corresponding VH framework regions within any one of clones 12, 135, 82, 53, and 4 and their chimeric and humanized variants. In certain embodiments, a CXCR3 antibody or fragment may comprise at least one VH FR (FR1, FR2, FR3 and/or FR4) having an amino acid sequence identical to, or having 1, 2, 3, 4, or 5 amino acid mutations (including additions,

deletions, and substitutions, such as conservative substitutions) relative to, the corresponding VH FR regions within any one of clones 12, 135, 82, 53, and 4 and their chimeric and humanized variants.

[0104] In certain embodiments, the light chain (VL) of a CXCR3 antibody or fragment may comprise FR1, FR2, FR3 and/or FR4 having amino acid sequences that are about 80% to about 100% identical (e.g., 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%, or any percentage in between) to the corresponding VL framework regions within any one of clones 12, 135, 82, 53, and 4 and their chimeric and humanized variants. In certain embodiments, a CXCR3 antibody or fragment may comprise at least one VL FR (FR1, FR2, FR3 and/or FR4) having an amino acid sequence identical to, or having 1, 2, 3, 4, or 5 amino acid mutations (including additions, deletions, and substitutions, such as conservative substitutions) relative to, the corresponding VL FR regions within any one of clones 12, 135, 82, 53, and 4 and their chimeric and humanized variants.

[0105] In certain embodiments, a CXCR3 antibody or fragment comprises VH FR regions (FR1, FR2, FR3 and/or FR4) having amino acid sequences identical to, or comprising 1, 2, 3, 4, or 5 amino acid mutations relative to, the corresponding VH FR regions within any one of clones 12, 135, 82, 53, and 4 and their chimeric and humanized variants, and comprises VL FR regions (FR1, FR2, FR3 and/or FR4) having an amino acid sequence identical to, or comprising 1, 2, 3, 4, or 5 amino acid mutations relative to, the corresponding VL FR of within any one of clones 12, 135, 82, 53, and 4 and

their chimeric and humanized variants. In certain embodiments, a CXCR3 antibody or fragment comprises VH FR regions (FR1, FR2, FR3 and/or FR4) having amino acid sequences about 80-100% identical to the corresponding VH FR regions within any one of clones 12, 135, 82, 53, and 4 and their chimeric and humanized variants, and comprises VL FR regions (FR1, FR2, FR3 and/or FR4) having an amino acid sequence about 80-100% identical to the corresponding VL FR of within any one of clones 12, 135, 82, 53, and 4 and their chimeric and humanized variants.

[0106] The CDR and FR regions disclosed herein can be combined in a variety of combinations, as each of the CDRs and FR regions can be independently selected and combined with any other CDR or FR region for a given antibody. In certain embodiments, the VH and/or VL CDR and FR sequences can be present in any combination in an antibody or fragment that retains the ability to neutralize CXCR3 activity.

[0107] Antibodies and fragments, as disclosed herein, can comprise one or more amino acid sequences that do not substantially alter the amino acid sequences described herein. Amino acid sequences that are substantially the same include sequences comprising conservative amino acid substitutions, as well as amino acid deletions and/or insertions that do not impair the ability of the antibody or fragment to neutralize CXCR3 activity.

[0108] Antibodies and fragments disclosed herein can be further conjugated to one or more additional molecules. For example, a conjugate can comprise an antibody joined directly or through a linker to one or more

therapeutic agents, solubilizing agents, stabilizing agents, immunosuppressants, receptors and fragments thereof, antigen binding peptides and/or other ligand targeting moieties. In some embodiments, the therapeutic agent is an agent useful for treating T1D and/or other disorders associated with CXCR3. In some embodiments, the antibody or fragment is conjugated to a β -cell stimulating agent or insulin.

[0109] Nucleotide Sequences

[0110] In addition to the amino acid sequences described above, disclosed herein, in certain embodiments, are nucleotide sequences corresponding to those amino acid sequences. In some embodiments, a nucleotide sequence encodes an antibody or fragment capable of neutralizing CXCR3 activity. In certain embodiments, the nucleotide sequences can be used to prepare expression vectors for the expression of anti-CXCR3 antibodies in cells (e.g., expression in mammalian cells).

[0111] Also disclosed herein, in certain embodiments, are polynucleotides substantially identical to those coding for the amino acid sequences disclosed herein. Substantially identical sequences may be polymorphic sequences, *i.e.*, alternative sequences or alleles in a population. Substantially identical sequences may also comprise mutagenized sequences, including sequences comprising silent mutations. A mutation may comprise one or more nucleotide residue changes, a deletion of one or more nucleotide residues, or an insertion of one or more additional nucleotide residues. Substantially identical sequences may also comprise various nucleotide

sequences that encode for the same amino acid at any given amino acid position in an amino acid sequence disclosed herein, due to the degeneracy of the nucleic acid code. Also included within substantially identical sequences are sequences that encode a chain or chains of an antibody that retains the ability to neutralize CXCR3.

[0112] Also disclosed herein, in certain embodiments, are polynucleotides that hybridize under highly stringent or lower stringency hybridization conditions to polynucleotides that encode a CXCR3 neutralizing antibody or fragment. The term "stringency" as used herein refers to the experimental conditions (*e.g.*, temperature and salt concentration) of a hybridization experiment conducted to evaluate the degree of homology between two nucleic acids; the higher the stringency, the higher percent homology between the two nucleic acids. As used herein, the phrase "hybridizing," or grammatical variations thereof, refers to the binding of a first nucleic acid molecule to a second nucleic acid molecule under low, medium or high stringency conditions, or under nucleic acid synthesis conditions. Hybridization can include instances where a first nucleic acid molecule binds to a second nucleic acid molecule, and where the first and second nucleic acid molecules are complementary.

[0113] Stringent hybridization conditions include, but are not limited to, hybridization to filter-bound DNA in 6X sodium chloride/sodium citrate (SSC) at about 45 degrees Celsius, followed by one or more washes in 0.2X SSC/0.1% SDS at about 50-65 degrees Celsius. Other stringent conditions include

hybridization to filter-bound DNA in 6X SSC at about 45 degrees Celsius, followed by one or more washes in 0.1X SSC/0.2% SDS at about 65 degrees Celsius. Other hybridization conditions of known stringency are familiar to one of skill and are included herein.

[0114] In certain embodiments, a nucleic acid disclosed herein may encode the amino acid sequence of a chain or chains in an antibody or fragment capable of neutralizing CXCR3 activity, or the nucleic acid may hybridize under stringent conditions to a nucleic acid that encodes the amino acid sequence of a chain or chains in the antibody or fragment.

[0115] In certain embodiments, a polynucleotide sequence is disclosed herein, comprising a nucleotide sequence encoding an amino acid sequence of a VH domain of a CXCR3 neutralizing antibody or fragment, and which is at least about 80-100%, (e.g., about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical (or any percentage in between) to the nucleotide sequence encoding the heavy chain of any one of clones 12, 135, 82, 53, and 4 and their chimeric and humanized variants. In certain embodiments, the polynucleotide sequence may comprise a nucleotide sequence having 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mutations (including additions, deletions, and substitutions, such as conservative substitutions) relative to the nucleotide sequence encoding the heavy chain of any one of clones 12, 135, 82, 53, and 4 and their chimeric and humanized variants.

[0116] In certain embodiments, a polynucleotide sequence is disclosed herein, comprising a nucleotide sequence encoding an amino acid sequence of

a VL domain of a CXCR3 neutralizing antibody or fragment, and which is at least about 80-100%, (e.g., about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical (or any percentage in between) to the nucleotide sequence encoding the light chain of any one of clones 12, 135, 82, 53, and 4 and their chimeric and humanized variants. In certain embodiments, the polynucleotide sequence may comprise a nucleotide sequence having 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mutations (including additions, deletions, and substitutions, such as conservative substitutions) relative to the nucleotide sequence encoding the light chain of any one of clones 12, 135, 82, 53, and 4 and their chimeric and humanized variants.

[0117] In particular embodiments, a polynucleotide sequence is disclosed herein, comprising a nucleotide sequence that is at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical (or any percentage in between) to a VH amino acid sequence and at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical (or any percentage in between) to a VL amino acid sequence, where the nucleotide sequences encode the heavy and light chain amino acid sequences from any one of clones 12, 135, 82, 53, and 4 and their chimeric and humanized variants.

[0118] The disclosed polynucleotides may be obtained by any method known in the art. For example, if the nucleotide sequence of an antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides. This would involve, for example, the

synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating those oligonucleotides, and then amplifying the ligated oligonucleotides by PCR. The disclosed polynucleotides can also be generated from any other suitable source of nucleic acids, such as an antibody cDNA library, or a cDNA library isolated from any tissue or cells expressing the antibody (e.g., from hybridoma cells selected to express an antibody).

[0119] In some embodiments, any of the disclosed polynucleotides may be incorporated into an expression vector. Suitable vectors for expression in various human and animal cell types are known in the art. In some embodiments, host cells are provided comprising the vectors. Suitable host cells include, e.g., CHO, COS, SF9, and/or other human or nonhuman cell lines. In some embodiments, the host cells transiently or stably express the nucleic acid on the vector when cultured in culture medium, thereby providing a method for producing the antibodies or fragments disclosed herein.

[0120] *Pharmaceutical Compositions*

[0121] A pharmaceutical composition can comprise any of the antibodies disclosed herein, or fragments thereof. Also disclosed are pharmaceutical compositions comprising nucleic acids encoding the antibodies or fragments thereof, e.g., for use in gene therapy applications and/or for transient or stable expression in host cells (e.g., CHO, COS, SF9, and/or other human or nonhuman cell lines) to produce the proteins or fragments thereof.

[0122] The pharmaceutical compositions disclosed herein can comprise a pharmaceutically acceptable carrier and/or at least one additive such as a solvent, filler, bulking agent, disintegrant, buffer, or stabilizer. Standard pharmaceutical formulation techniques are well known to persons skilled in the art (see, e.g., *2005 Physicians' Desk Reference*[®], Thomson Healthcare: Montvale, NJ, 2004; *Remington: The Science and Practice of Pharmacy*, 20th ed., Gennado et al., Eds. Lippincott Williams & Wilkins: Philadelphia, PA, 2000). Suitable pharmaceutical additives include, e.g., mannitol, 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, ethanol, and the like. In certain embodiments, the pharmaceutical compositions may also contain pH buffering reagents and wetting or emulsifying agents (e.g., phosphate buffered saline, sterile saline for injection, etc.). In further embodiments, the compositions may contain preservatives or other stabilizers.

[0123] In some embodiments, the pharmaceutical compositions comprising any of the antibodies disclosed herein, or fragments thereof, or nucleic acids encoding the antibodies or fragments, may further comprise one or more of the following: mannitol, polysorbate 80, sodium phosphate dibasic heptahydrate, and sodium phosphate monobasic monohydrate. In another embodiment, pharmaceutical compositions may contain 10mM Histidine pH 6.5 with up to 2% glycine, up to 2% mannitol, and up to 0.01% polysorbate 80.

[0124] The formulation of pharmaceutical compositions may vary depending on the intended route of administrations and other parameters (see, e.g., Rowe et al., *Handbook of Pharmaceutical Excipients*, 4th ed., APhA Publications, 2003.) In some embodiments, the pharmaceutical composition may be a lyophilized cake or powder. The lyophilized composition may be reconstituted for administration by intravenous injection, for example with Sterile Water for Injection, USP. In other embodiments, the composition may be a sterile, non-pyrogenic solution.

[0125] The pharmaceutical compositions described herein may comprise an antibody as disclosed herein, or a fragment thereof, or nucleic acids encoding the antibodies or fragments, as the sole active compound, or the pharmaceutical composition may comprise a combination with another compound, composition, or biological material. For example, the pharmaceutical composition may also comprise one or more small molecules or other agents useful for the treatment of a disease or disorder associated with CXCR3, such as T1D. In some embodiments, the pharmaceutical composition can comprise a β -cell stimulating agent, insulin, and/or an insulin-producing cell. In some embodiments, the pharmaceutical composition may also comprise one or more immunosuppressants, mTOR inhibitors or autophagy inhibitors. Examples of immunosuppressants include rapamycin and velcade. Rapamycin is also an mTOR inhibitor.

[0126] Administration and Dosing

[0127] In some embodiments, a method is provided for treating a patient suffering from a disease or disorder associated with CXCR3 comprising administering to the patient one or more of the anti-CXCR3 antibodies disclosed herein, and/or a fragment thereof. In some embodiments, the antibody or fragment is capable of neutralizing CXCR3. In some embodiments, the disease or disorder is an inflammatory disorder. In some embodiments, the disorder is T1D. In some embodiments, administering a composition (e.g., a pharmaceutical composition) comprising the antibody or fragment prevents, treats, reduces the severity, and/or otherwise ameliorates the symptoms of a disease or disorder associated with CXCR3. In some embodiments, the antibody or fragment is administered at a dose and frequency sufficient to prevent, treat, reduce the severity, and/or otherwise ameliorate the symptoms of a disease or disorder associated with CXCR3.

[0128] In certain embodiments, a composition is provided for use in the manufacture of a medicament for treating a disease or disorder, wherein the medicament comprises any of the antibodies disclosed herein, or fragments thereof. For example, the antibody or fragment can comprise the three CDRs from any one of heavy chain variable domains 4.0-4.11 paired with the three CDRs from any one of light chain variable domains 4.0-4.7; the three CDRs from any one of heavy chain variable domains 12.0-12.3 paired with the three CDRs from any one of light chain variable domains 12.0-12.3; the three CDRs from any one of heavy chain variable domains 53.0-53.10 paired with the three

CDRs from any one of light chain variable domains 53.0-53.13; the three CDRs from any one of heavy chain variable domains 82.0-82.3 paired with the three CDRs from any one of light chain variable domains 82.0-82.3; or the three CDRs from any one of heavy chain variable domains 135.0-135.3 paired with the three CDRs from any one of light chain variable domains 135.0-135.3.

[0129] In some embodiments, the antibody or fragment in the medicament can comprise any one of heavy chain variable domains 4.0-4.11 paired with any one of light chain variable domains 4.0-4.7, any one of heavy chain variable domains 12.0-12.3 paired with any one of light chain variable domains 12.0-12.3, any one of heavy chain variable domains 53.0-53.10 paired with any one of light chain variable domains 53.0-53.13, any one of heavy chain variable domains 82.0-82.3 paired with any one of light chain variable domains 82.0-82.3, or any one of heavy chain variable domains 135.0-135.3 paired with any one of light chain variable domains 135.0-135.3.

[0130] Doses of CXCR3 antibody for use in the methods disclosed herein will vary based on numerous parameters familiar to the skilled artisan, such as patient physiology (size or surface area, weight, age, and metabolism) and disease state, as well as pharmacological parameters, such as the mechanism of delivery, formulation, and any concurrent or sequential therapies. An "effective amount" of CXCR3 antibody can produce a desired *in vivo* effect such as one or more of maintenance or decreased HA1bc (haemoglobin A1c) levels (less than about 7%, e.g., less than 7.5, 7.4, 7.3, 7.2, 7.1, 7.0, 6.9, 6.8, 6.7, 6.6, or 6.5%, or any percentage in between), increased endogenous insulin

production and/or circulating insulin levels, maintenance or increased fasting C-peptide levels, improved glucose tolerance, reduced fasting blood glucose levels in the absence of exogenous insulin, reduction in exogenous insulin usage, reduction in β -cell inflammation, and/or increased β -cell population and/or growth. Direct cellular assays for CXCR3 inhibition can also be used, such as a reduction in the blood of CXCR3+ cells (including but not limited to T cells), inhibition of CXCR3 ligand binding, GTP binding, calcium influx and/or mobilization, cell chemotaxis, and/or receptor internalization.

[0131] In certain embodiments, an effective amount of CXCR3 antibody results in about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% (or any percentage in between) or 1, 2, 4, 5, 10, 15, 20, 30, 40, 50, or 100 fold (or any fold in between), or more, improvements in any of the above parameters *in vivo*, relative to controls. In certain embodiments, an improvement can be characterized by a fasting blood glucose level in the absence of exogenous insulin that is reduced to below 100, 110, 120, 130, 140, 150, 160, 180, 200, 220, 240, 250, 300, or 350 mg/dL (or any value in between). In certain embodiments, an improvement can be characterized by an increase in basal serum C-peptide levels to more than 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, or 1.0 nmol/L (or any value in between). In some embodiments, an improvement can be characterized by an increase in fasting integrated serum C-peptide levels during C-peptide challenge (post-oral glucose tolerance test) to greater than about 0.03, 0.033, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.4, 0.5, 0.6, 0.8, or 1.0 nmol/L x min (or any value in between). In certain embodiments, the effective

dose of CXCR3 antibody may be further characterized by reducing the concentration of CD4+ and/or CD8+ cells in the pancreas by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% (or any percentage in between) or at least 1, 2, 3, 4, or 5 fold (or any value in between), relative to control subjects.

[0132] In various embodiments, an effective dose of antibody is also selected to be a safe dose for administration to a human subject. In certain embodiments, a safe dose of anti-CXCR3 antibody may be characterized as one that results in no substantial gross depletion of T cells or T cell activity (other than CXCR3 activity) in the subject (e.g., as measured by T cell concentration or activity in the blood of the subject). In particular embodiments, "no substantial gross depletion of T cells or T cell activity" means a 40%, 30%, 25%, 20%, 15%, 10%, 5% (or any percentage in between) or less reduction in the concentration and/or activity (other than CXCR3 activity) of CD4+ and/or CD8+ cells in the subject treated with CXCR3 neutralizing antibody, relative to control subjects treated with placebo and/or relative to treatment subjects prior to treatment. In some embodiments, the safe dose is further characterized by a 40%, 30%, 25%, 20%, 15%, 10%, 5% (or any percentage in between) or less reduction in the concentration of one or more cell types selected from T regs, B cells, myeloid cells, dendritic cells, and/or granulocytes, relative to control subjects.

[0133] Exemplary, non-limiting doses for a subject, such as a human, include about 0.03, 0.06, 0.12, 0.24, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, or 3.7 mg/kg/dose (or any value in between) for an antibody with an ND₅₀ of about 1-6

µg/mL in an *in vitro* chemotaxis assay. In certain embodiments, the antibody may be administered in a range of about 0.03-3.7 mg/kg/dose, 0.15-0.7 mg/kg/dose, or 0.25-0.5 mg/kg/dose.

[0134] Anti-CXCR3 antibody may be administered in a single administration or in repeat administrations over different periods of time, such as daily, weekly, biweekly, monthly, bimonthly, quarterly, or yearly. Accordingly, in a non-limiting example based on the dosage ranges discussed above, a patient may receive an approximate total dose of 0.16-18 mg/kg of CXCR3 antibody over the course of a treatment regimen.

[0135] Anti-CXCR3 antibodies may be administered by any suitable means known to the skilled artisan, including, for example, intravenously, intraperitoneally, nasally, ocularly, orally, parenterally, subcutaneously, or transdermally. In particular embodiments, the antibody may be administered directly to the pancreas of the subject or proximate to the pancreas or to specific regions of the pancreas, such as the islet cells of the pancreas.

[0136] Effective dosages achieved in one animal may be converted for use in another animal, including humans, using conversion factors known in the art. See, e.g., Reagan-Shaw *et al.*, *FASEB J.* 22:659-61 (2008); Schein *et al.*, *Clin. Pharmacol. Ther.* 11: 3-40 (1970); and Freireich *et al.*, *Cancer Chemother. Reports* 50(4):219-244 (1966). For example, human equivalent dosing (HED) in mg/kg based on animal dosing may be given by the following equation: HED (mg/kg) = animal dose (mg/kg) X (Km^{animal}/Km^{human}), where Km = weight/surface area (kg/m²).

[0137] Exemplary conversion factors based on the above equation are shown in the following table. The exemplary doses provided above for human may be adjusted for other species or other human patients based on these coefficients or other means known to the skilled artisan.

Table 3

To:\nFrom:	Mouse\n(20 g)	Rat\n(150 g)	Monkey\n(3.5 kg)	Dog\n(8 kg)	Human\n(60 kg)
Mouse	1	0.5	0.25	0.17	0.08
Rat	2	1	0.5	0.25	0.14
Monkey	4	2	1	0.6	0.33
Dog	6	4	1.7	1	0.5
Human	12	7	3	2	1

[0138] Subjects

[0139] Subjects to be treated by the methods provided by the invention can include humans or animals, such as livestock, domestic, and wild animals. In some embodiments, animals are avian, bovine, canine, cetacean, equine, feline, ovine, pisces/fish, porcine, primate, rodent, or ungulate. Subjects may be at any stage of development, including adult, youth, fetal, or embryo. In particular embodiments, the patient is a mammal, and in more particular embodiments, a human.

[0140] In various embodiments, a subject can be treated prophylactically or after onset of any condition associated with aberrant CXCR3 activity or any condition in which the disruption of CXCR3 signaling could be therapeutically beneficial. In some embodiments, a subject can be treated prophylactically or after onset of T1D. In some embodiments, a subject can be treated

prophylactically prior to onset of T1D using the methods provided herein, or a subject having new onset T1D can be treated using the methods provided herein.

[0141] "A subject having new onset T1D" is any subject who has diminished, but still detectable, insulin-producing capacity from the β -cells of the pancreas, regardless of the age of the subject when diabetes is clinically diagnosed (e.g., including adult, youth, fetal, or embryo subjects). In certain embodiments, a subject having new onset T1D will receive treatment preferably within about six months (e.g., within about 1 day, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, or any time in between) of the earliest clinical diagnosis of T1D. In other embodiments, the subject may receive treatment more than six months after the earliest clinical diagnosis of T1D, wherein the subject retains minimal but measurable basal serum C-peptide levels of greater than or equal to about 0.2 nmol/L (e.g., at least about 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, or 1.0 nmol/L). In some embodiments, treatment comprises administration of one or more doses comprising one or more of the antibodies disclosed herein. In some embodiments, the antibody is a CXCR3 neutralizing antibody.

[0142] In some embodiments, a subject having new onset T1D retains a fasting integrated serum C-peptide level of at least about 0.03, 0.033, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.4, 0.5, 0.6, 0.8, or 1.0 nmol/L x min, e.g., about 0.03 to 1.0 or 0.033 to 1.0 nmol/L x min during C-peptide stimulation. In particular embodiments, the subject has a fasting integrated serum C-peptide

level of 0.033 to 1.0 nmol/L x min during C-peptide stimulation. In certain embodiments, the C-peptide stimulation is a post-oral glucose test and may comprise measuring integrated serum C-peptide levels for 60-150 minutes following administration of 10.0-13.9 mmol/L glucose. See Keymeulen et al., *Diabetologia* 53: 614-623 (2010). In more particular embodiments, the subject's measureable post-oral glucose tolerance test integrated serum C-peptide level increase is less than 0.8, 0.7, 0.6, 0.54, 0.5, 0.4, 0.3, 0.2, or 0.1 nmol/L x min. In still more particular embodiments, the subject has an increase of 0.54 nmol/L x min, or less, in post-oral glucose tolerance test integrated serum C-peptide level. C-peptide corresponds to residues 57-87 of the insulin precursor peptide (human reference sequence NP_000198), with residues 90-110 and 25-54 corresponding to the A and B chains of insulin, respectively.

[0143] In some embodiments, a subject having new onset T1D has an elevated fasting blood glucose level in the absence of exogenous insulin of greater than about 100, 110, 120, 130, 140, 150, 160, 180, 200, 220, 240, 250, 300, 350 mg/dL (or any value in between), or more. In certain embodiments, the subject may have both an elevated fasting blood glucose level as described above, as well as a reduced fasting integrated serum C-peptide level, as described above.

[0144] In certain embodiments, a subject is treated by the methods disclosed herein shortly after being diagnosed with new onset T1D. In more particular embodiments, the subject is first treated by the methods of the invention within 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 days, or 1, 2, 3, 4, 5, 6, 7, 8, 9, or

10 weeks, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months of clinical diagnosis of new onset T1D (or at any time in between). In more specific embodiments, a subject is first treated by the methods of the invention within 6 months of clinical diagnosis of new onset T1D. In other embodiments, a subject having T1D is treated by the methods disclosed herein at any point, regardless of time since clinical diagnosis, wherein the subject retains residual serum C-peptide levels of at least about 0.2 nmol/L.

[0145] Additional methods

[0146] The methods provided herein may, in certain embodiments, comprise additional treatments that may be administered concurrently or sequentially (before or after) with the administration of an anti-CXCR3 antibody disclosed herein. For example, in some embodiments, methods are disclosed comprising the further step of administering an immunosuppressant to the subject in addition to an anti-CXCR3 antibody. The immunosuppressants can include, but are not limited to, one or more of Azathioprine (Imuran), β interferon 1a, β interferon 1b, basiliximab, corticosteroids, Cyclosporine (Sandimmune), cyclophosphamide, chlorambucil, daclizumab, deoxyspergualin, Etanercept, glatiramer acetate, infliximab, leflunomide, Mercaptopurine (6-MP), methotrexate, mitoxantrone, Muromonab-CD3, Mycophenolate (MFM or CellCept), natalizumab, anakinra, canakinumab, rituximab, belimumab, abatacept, aldesleukin, prednisone, rapamycin, sirolimus, tacrolimus, and Ustekinumab.

[0147] In some embodiments, the methods disclosed herein may comprise, in addition to administering an anti-CXCR3 antibody, the step of administering a β -cell stimulating agent to the subject. The step of administering a β -cell stimulating agent may be concurrent or sequential (before or after) with administering an anti-CXCR3 antibody. Exemplary β -cell stimulating agents include, but are not limited to, one or more of transplanted β -cells (autologous, allogenic, or syngenic), transplanted insulin-producing cells (allogeneic or syngenic), DDP4 (human protein reference sequence NP_001926.2) inhibitors, TM4SF20 peptides (human protein reference sequence NP_079071), TMEM27 peptides (human protein reference sequence NP_065716), exendin 1 or GLP-1 (human protein reference sequence NP_002045) analogs, gp130 and EGF receptor ligands, and those disclosed in paragraphs 8-11 of U.S. Patent Application Publication No. 20100130476. A β -cell stimulating agent may be administered along with an immunosuppressant in the methods of the invention, either concurrently or sequentially (before or after). In certain embodiments, a β -cell stimulating agent, insulin-producing cell, and/or immunosuppressant may be administered by implanting a device capable of delivering the β -cell stimulating agent, insulin-producing cell, and/or immunosuppressant to the targeted tissue or organ.

[0148] Also disclosed herein are methods for detecting and/or quantifying CXCR3 and/or cells expressing CXCR3 (e.g., CXCR3+ T cells). In some embodiments, the methods comprise using one or more of the anti-CXCR3 antibodies disclosed herein to detect and/or quantify CXCR3 and/or cells

expressing CXCR3. For example, one or more antibody can be added to a patient sample (e.g., a blood sample) and detected using detectable label such as a secondary antibody conjugated to a detectable signal (e.g., a fluorescent secondary detection antibody). For example, FACS sorting can be used to quantify the level of CXCR3-expressing cells in a sample following primary and fluorescent secondary antibody binding.

[0149] In some embodiments, the diagnostic methods can be used to diagnose a CXCR3 disorder or a CXCR3-associated disorder (e.g., diabetes, T1D). For example, a disorder can be diagnosed by detecting the presence or absence of CXCR3 in a patient sample, or by comparing the concentration of CXCR3 in a sample to the level in one or more reference standards, wherein a deviation from the level in the standard indicates the presence of a disorder.

[0150] In various embodiments, kits comprising at least one anti-CXCR3 antibody or fragment are also provided. The kits are useful for various research, diagnostic, and therapeutic purposes. For example, the kits can be used to detect CXCR3+ T cells, or to treat type I diabetes by administering the anti-CXCR3 antibody or fragment contained within the kit to a subject. For isolation and purification purposes, the kit may contain an antibody or fragment coupled to a bead (e.g., sepharose beads). In certain embodiments, the kit also comprises instructions for using the anti-CXCR3 antibody or fragment for the desired research, diagnostic, and/or therapeutic purpose.

[0151] In this application, the use of the singular includes the plural unless specifically stated otherwise. Also in this application, the use of "or"

means “and/or” unless stated otherwise. Furthermore, the use of the term “including,” as well as other forms, such as “includes” and “included,” are not limiting. Any range described herein will be understood to include the endpoints and all values between the endpoints.

[0152] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited in this application, including but not limited to patents, patent applications, articles, books, and treatises, are hereby expressly incorporated by reference in their entirety for any purpose. To the extent publications and patents or patent applications incorporated by reference contradict the invention contained in the specification, the specification will supersede any contradictory material.

[0153] All information associated with reference gene sequences disclosed in this application, such as GenelDs or accession numbers, including, for example, genomic loci, genomic sequences, functional annotations, allelic variants, and reference mRNA (including, *e.g.*, exon boundaries) and protein sequences (such as conserved domain structures) are hereby incorporated by reference in their entirety.

EXAMPLES

[0154] The following examples serve to illustrate, and in no way limit, the present disclosure.

Example 1: Materials and Methods

[0155] **Generation of immunogen.** CHO cells were transformed with DNA encoding full-length human CXCR3 and CXCR3 was expressed on the cell surface ("r-CXCR3-CHO cells"). The CXCR3 sequence used to transform the cells was obtained and the CXCR3 open reading frame was placed into an expression vector pcDNA3.1neo_DEST, and then transfected into 300-19 cells (Immunogen). An N-terminal peptide fragment of the CXCR3 extracellular domain (EC domain), with the amino acid sequence, MVLEVSDHQVLNDAEVAALLENFSSSYDYGENESDSC (SEQ ID NO:XXX), was conjugated to KLH by the C terminal cysteine, and used as an immunogen. The cells expressing CXCR3 were maintained at 37° C. under 5% CO₂ in RPMI (Invitrogen, Carlsbad, Calif.) supplemented with 10% dialyzed fetal bovine serum (FBS) (Invitrogen). Cells were prepared for injection by substituting the above culture medium with phosphate-buffered (Ca/Mg-free) saline (CMF-PBS) supplemented with 5 mM EDTA, and harvesting the cells in that buffer. The harvested cells were pelleted by centrifugation at 500×g for about 5 minutes, washed once by re-suspending the pellet in CMF-PBS and centrifuging as before, counted, and adjusted to the appropriate volume (such as 5×10⁶ cells in 0.2 ml) for injection by resuspending the cell pellet in CMF-PBS.

[0156] **Antibody preparation.** The N-terminal 37 amino acids of human CXCR3 were used to generate mouse monoclonal hybridomas for anti-human CXCR3, and five antibody clones (4, 12, 53, 82, and 135) were selected for further characterization. The 37 amino acid N-terminus of human CXCR3 is 65% homologous to the aligned region in mouse CXCR3 and contains residues

important for CXCL9, CXCL10, and CXCL11 binding. BALB/c mice, about 6-8 weeks old (Charles River Laboratories, Wilmington, MA) were immunized with the cells expressing CXCR3 or an extracellular peptide of CXCR3. A group of mice were primed subcutaneously (SC) on day 0 with a 1:1 emulsion of KLH-conjugated peptide mixed with adjuvant (TiterMax Gold, Sigma Aldrich, #T2685-1ML), boosted SC 3-5 times at 2-3 week intervals with a 1:1 emulsion of peptide to adjuvant or intraperitoneal (IP) with cells in PBS without adjuvant, and boosted two consecutive days prior to sacrifice via IP with either peptide and/or cells in PBS all without adjuvant. Another group of mice were primed IP 3-5 times at 2-3 week intervals and boosted via IP with cells in PBS two consecutive days prior to sacrifice. For both groups of mice, each injection contained approximately 2×10^6 cells in a volume of approximately 100 μ l.

[0157] The day after the last injection, mice were sacrificed and the spleen was removed and placed in approximately 10 ml of serum-free DMEM (Gibco) in a Petri dish. Sp2/O mouse myeloma cells (ATCC CRL-1581) were fused with spleen cells from the immunized mouse using 50% (w/w) PEG based on the method of Kohler and Milstein (*Nature*, 256:495-7, (1975)). At the end of the procedure the cells were resuspended in 50ml of ClonaCell-Hy Hybridoma Recovery medium (StemCell Technologies), transferred to a T75cm² flask and incubated for 16-24hrs at 37°C. Following this incubation the cells were harvested and added to 100ml of ClonaCell-HY methylcellulose selection media (StemCell Technologies). This mixture was then aliquoted into ten 100mm² tissue culture dishes and incubated for 10-14 days. Clonal hybridomas were

transferred from the methylcellulose to liquid medium and grown in 96 multi-well plates for assays to identify monoclonal antibodies specific for CXCR3.

[0158] Unless indicated otherwise, reference in these examples to an antibody variant, such as Hu4.1, refers to an antibody containing a heavy chain variant and light chain variant of the same number (e.g., Hu4.1 would contain heavy chain 4.1 and light chain 4.1). All references to antibodies are consistent with the antibody numbering and VH/VK chain pairing shown in Table 2.

[0159] **Animals.** Female NOD/LtJ mice were purchased from the Jackson Laboratory (Bar Harbor, ME) and were maintained under pathogen-free conditions. Mice were screened for glycosuria using an ACCU-CHEK® Compact Plus Blood Glucose Meter (Roche, Indianapolis, IN) by tail vein puncture two times a week starting at 10 weeks of age. Mice were deemed diabetic when blood glucose measured above 250 mg/dL for three consecutive days. Mice were observed for a minimum of 100 days post treatment start. All animal experiments were approved by in-house IACUC.

[0160] **Antibody injections.** For prevention studies, pre-diabetic NOD mice were injected with 100 µg antibody intraperitoneally (i.p.) once a week for 6 weeks starting at 10 weeks of age. For reversal studies, animals were randomly enrolled in treatment groups within 1 week after mice were deemed diabetic, blood glucose was measured twice a day, at least six hours between readings, and insulin was administered by i.p. injection to those mice with a blood glucose above 250 mg/dL for the duration of the study. Mice in the treatment groups that maintained insulin independence for 30 consecutive days

were considered reversed. Five mice from each group were harvested between the fifth and sixth treatments and lymphoid organs, blood, bone marrow, and pancreas were harvested for cellular analysis. At the end of the study, the pancreas was harvested and processed for histological and immunohistochemical analysis. The anti-mouse CXCR3 antibody, clone CXCR3-173 (Uppaluri *et al.* 2008), and the hamster IgG control antibody were purchased from BioLegend (San Diego, CA).

[0161] **FACS analysis.** Single cell suspensions of the spleen, inguinal lymph nodes, pancreatic lymph nodes, and bone marrow were made. The pancreas was snipped into small pieces and incubated in 2 mg/ml collagenase D (Roche Diagnostics, Indianapolis, IN) for 30 minutes at 37 °C, filtered through a 70 micron cell strainer (BD Biosciences, San Jose, CA), and lymphocytes were separated from pancreas tissue using density gradient centrifugation. Cells were stained with APC labeled anti-mouse CD25, FITC labeled anti-mouse CD4 and PE labeled anti-mouse Foxp3 (eBiosciences, San Diego, CA) for regulatory T cells (T regs), PerCP labeled anti-mouse CD8 α , PE labeled anti-mouse CD44, APC labeled anti-mouse CD62L, and PeCy7 labeled anti-mouse CXCR3 for activated/memory T cells, and FITC labeled anti-mouse CD94, PerCP labeled anti-mouse CD4, APC-labeled anti-mouse B220, PeCy7 labeled anti-mouse CD11c, Pacific blue labeled anti-mouse CD11b, and PE labeled anti-mouse NKp46 for B cells, myeloid cells, dendritic cells, NK cells and NKT cells. The cells were incubated for 30 min at 4 °C after blocking with anti-mouse CD16/32 for 20 min on ice. For the T reg stain, surface staining

was performed, the cells were washed, fixed and permeabilized in Cytofix/Perm buffer (eBiosciences) then stained with the anti-mouse Foxp3 antibody for 30 min on ice. After staining, cells were washed twice, fixed in paraformaldehyde and acquired on the LSRII cytometer, and data was analyzed using Flow Jo software (Treestar, Ashland, OR). All antibodies were purchased from BD Biosciences unless stated otherwise.

[0162] **Chemotaxis assay.** The assay for chemotaxis was performed in 24-well plates (Costar) carrying transwell permeable supports with a 5µm membrane. CXCR3- transfected 300.19 cells were placed in the transwell inserts at 1×10^6 cells in 2.5% heat-inactivated fetal bovine serum in RPM1640 (0.2 ml total volume). Media alone or supplemented with recombinant chemokine 300 ng/ml CXCL9 (MIG), 100 ng/ml CXCL10 (IP-10) or 100 ng/ml CXCL11 (I-TAC) was placed in the lower compartment (0.6mls) and the transwell inserts containing the cells were loaded into the lower compartment. The plates were incubated between 4-5 hours in a 5% CO₂ humidified incubator at 37°C. Following the incubation period, transwell inserts were removed and the total media in the lower compartment was pooled and the cells pelleted by centrifugation for 5 min at 1200 RPM. The media was aspirated and the cells were stained with Calcein AM (10 µg/ml final) for 30 minutes at 37°C. The cells were pelleted and washed, media was added (0.1 ml), and the suspension transferred to 96 well black-walled clear bottom plates. The plates were pulsed at 1200 RPM to settle the cells and the fluorescence was measured at 490/520 nm on a Flexstation. All conditions were tested in triplicate. The resulting data

is expressed as mean relative fluorescence units (RFUs) of the migrated cells. See Fig. 14 A-C and Fig. 15A-C.

[0163] For chemotaxis with CXCR3 blockade, the CXCR3 transfected 300.19 cells were pre-treated with various amounts of blocking antibody or control IgG for 20-30 minutes at 37°C prior to being used in the chemotaxis assay. The antibody was not washed out but was present during the assay incubation.

[0164] **Calcium mobilization assay using FLIPR.** Human Embryonic Kidney 293 (HEK) cells expressing hCXCR3 were harvested at 80% confluency by treating with PBS + 2mM EDTA. The cells were suspended in serum free HEK-SFM media at a density of 1×10^6 cells/mL. 15 μ L (15,000 cells) of the suspension was dispensed into each well of a 384 well plate. Cells were dye loaded for 30 minutes at room temperature by adding 15 μ L of reconstituted FLIPR Calcium 4 Dye. Anti-human CXCR3 antibody clones and isotype controls were serially diluted (3 fold) in HBSS + 20 mM HEPES + 1% BSA to generate 10 test concentrations per clone. Each test concentration was tested in duplicate ($n = 2$) on the same plate. 15 μ L of the test concentration was added to the cells in each well and the plate was incubated at room temperature for 1 hour. A fixed concentration of CXCL11 (R & D Systems) representing EC80 for eliciting intracellular calcium mobilization was added on the FLIPR into each well and the change in fluorescence was monitored over time. The maximum response of each well was normalized to the baseline and

the data were fit after averaging to a four parameter equation using GraphPad Prism and the IC₅₀ for each clone was determined. See Fig. 14D and Fig. 21.

[0165] **Biacore analysis for affinity.** *Biacore surface preparation.*

Binding affinities of mouse α -human CXCR3 hybridoma antibody clones 4, 12, 53, 82 and 135 to human CXCR3 peptide were calculated using a Biacore T100 Kinetics/Affinity assay. A Biacore CM5 Series S sensor chip (GE #BR-1006-68) was immobilized with rabbit-anti-mouse-Fc (RAM-Fc) capture antibody (GE# BR-1008-38) using the standard amine coupling program. The chip's carboxymethyl dextran surface was activated using a 1:1 mixture of 0.1M N-hydroxysuccinimide (NHS) and 0.4M N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), allowing the surface to bind reactive amine groups on the capture antibody. Following antibody immobilization, the reactive sensor chip surface was quenched using 1M ethanolamine hydrochloride/NaOH pH 8.5. Immobilization resulted in 8,000 RU of the RAM-Fc capture antibody on one flow cell. Another blank flow cell was used as a surface for reference subtraction during data analysis.

[0166] *Biacore Assay Conditions.* The Biacore T100 instrument sample chamber and assay temperatures were set to 4°C and 25°C respectively. Mouse anti-hCXCR3 antibodies were diluted to 500nM in HBS-EP+ running buffer (10mM HEPES, 150mM NaCl, 0.05% P20 surfactant, 3mM EDTA, pH 7.4), and were captured using a thirty second injection at 10 μ l/min. These conditions resulted in ~1,200 RU stable capture of each mouse anti-hCXCR3 clone tested. hCXCR3 peptides were diluted to 200, 100, 50, 25, 12.5 and 0nM

concentrations in HBS-EP+. Each assay cycle, peptide was injected for five minutes at a 50 μ l/min flow rate to measure association, then washed in HBS-EP+ for ten minutes at 50 μ l/min flow rate to measure dissociation. The capture surface (RAM-Fc) was regenerated between assay cycles using 10mM glycine-HCl pH 1.7 at 50 μ l/min for three minutes. Analysis was performed in Biacore T100 Kinetics Evaluation software v2.0 (GE Healthcare). Sensorgrams fit to a 1:1 binding model with reference flow cell and 0nM concentration subtraction (double-reference subtraction).

[0167] **Biacore whole receptor assay.** Full-length human CXCR3 receptor protein with C-terminal 6xHis and HPC4 tag was expressed in insect Sf9 cells with a baculovirus vector. The receptor protein was then purified via Ni-NTA and HPC4 affinity purifications. The final product was buffer exchanged into 10 mM HEPES, 300 mM NaCl, 0.5% n-Dodecyl β -D-Maltopyranoside and 5% glycerol. The receptor protein was captured on NTA chips via Ni-chelating and further stabilized by amine coupling using 1:10 diluted mixture of the 1:1 mixture of 0.1M N-hydroxysuccinimide (NHS) and 0.4M N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC). The stabilized receptor surface was tested for ligand binding activity by injecting 20 nM of hCXCL10 and hCXCL11 ligands. For kinetics analysis, human CXCR3 ligands (hCXCL9, hCXCL10 and hCXCL11) were diluted to 20, 10, 5, 2.5, 1.25, 0.6125, 0.3125 and 0nM concentrations in HBS-EP+. Anti-CXCR3 antibodies were diluted to 80, 40, 20, 10, 5, 2.5, 1.25 and 0 nM concentrations in HBS-EP+. Analyte was injected for five minutes at a 50 μ l/min flow rate to measure

association, and then washed in HBS-EP+ for ten minutes at 50 μ l/min flow rate to measure dissociation. The receptor surface was regenerated between assay cycles using 10mM glycine-HCl pH 1.7 at 50 μ l/min for 1 minute. Analysis was performed in Biacore T100 Kinetics Evaluation software v2.0 (GE Healthcare). Sensorgrams fit to a 1:1 binding model with reference flow cell and 0nM concentration subtraction (double-reference subtraction).

[0168] **Glucose tolerance testing.** The evening before glucose challenge, non-fasting blood glucose was monitored and insulin treatment of diabetic animals was withheld. Mice were fasted for 12 hours before D-glucose (20%; Sigma) at 2mg/g body weight was injected i.p.. Blood glucose was measured before and 15, 30, 60, and 120 minutes after the injection.

Example 2: Characterization of NOD Mice

[0169] Representative sections of pancreas from 6 to 10 week old pre-diabetic female NOD mice and new-onset diabetes female NOD mice embedded in paraffin were stained for insulin, CXCL10, and the T cell marker CD3. Fig. 1. CXCL10 expression was detected in the pancreas of NOD mice within islets surrounded by infiltrating cells (arrows in central column of Fig. 1). Older pre-diabetic and new-onset diabetic mice had a marked increase in T cell infiltration of the islets (Fig. 1, right column) and a decrease in insulin production within the islets (Fig. 1, left column).

[0170] To further evaluate whether CXCR3+ T cells were present in the pancreas of NOD mice, flow cytometry analysis was conducted on pancreas tissue harvested from female NOD mice with new-onset diabetes. Fig. 2.

CXCR3 expression was evaluated on CD4+/TCR+ and CD8+/TCR+ T cells. Staining with isotype control antibody is represented by the shaded curve. Flow cytometry was performed on single cell suspensions of pooled pancreas tissue harvested from several mice. The data indicate that CXCR3+ cytotoxic and helper T cells were present in the pancreas of NOD mice.

Example 3: Prophylactic Treatment of NOD Mice with Anti-CXCR3 Antibody

[0171] Pre-diabetic female NOD mice were treated once a week for six weeks with 100 µg of a hamster anti-mouse CXCR3 antibody (clone CXCR3-173, purchased from BioLegend, San Diego, CA), or with a control hamster IgG or PBS, starting at 10 weeks of age. Blood glucose was monitored twice a week and an animal was considered diabetic and euthanized after exhibiting three consecutive blood glucose readings above 250 mg/dL. Fig. 3 shows the percentage of mice that developed diabetes over time for each treatment group. Each line represents the combined results from ten mice per group. Results from two independent studies are shown (Fig. 3A and 3B). The plots illustrate that prophylactic treatment with an anti-CXCR3 antibody prevented development of diabetes in pre-diabetic female NOD mice.

[0172] To further evaluate the effects of prophylactic anti-CXCR3 antibody administration, representative pancreas sections from female NOD mice treated with anti-CXCR3 antibody were stained for insulin (Fig. 4, left panel), CD3 and Foxp3 (Fig. 4, center and right panels). The right hand panel in Fig. 4 is an increased magnification image of the section shown in the center panel. The pancreas tissue was harvested from mice at the end of the study

period (26 weeks of age). Fig. 4 demonstrates that insulin-positive islets were present in NOD mice treated with an anti-CXCR3 antibody, while the majority of T cells surrounded and had not invaded the islets.

Example 4: Reversal of New Onset Diabetes in NOD Mice

[0173] Female mice with three consecutive blood glucose readings above 250 mg/dL were deemed diabetic and randomly enrolled in treatment groups. Treatment was started within one week of enrollment. Mice were treated with PBS, anti-mouse CXCR3 antibody (100 µg administered intraperitoneally, clone CXCR3-173) or control IgG (100 µg administered i.p.) once a week for six weeks, or murine anti-thymocyte globulin (mATG; 500 µg administered i.p.) on days 0 and 4. Once enrolled, blood glucose was measured in the morning and afternoon (at least six hours in between), and insulin was administered by i.p. injection only to those mice whose blood glucose was above 250 mg/dL. Daily morning blood glucose values for individual mice are shown (Fig. 5). Data is pooled from four independent reversal studies with 8-10 mice per group per study. The data demonstrates reversal of new-onset diabetes in NOD mice after treatment with anti-CXCR3 antibody.

[0174] To evaluate changes in T cell subsets in the pancreas of mice treated with anti-CXCR3 antibody, single cell suspensions of pancreas from four mice per treatment group (PBS, anti-mouse CXCR3, control IgG or mATG treated mice) were pooled, stained for T cells and analyzed by flow cytometry. Pancreas tissue was harvested a few days after the fifth treatment dose of PBS,

anti-mouse CXCR3, or control IgG, and from age-matched mATG-treated mice. The percentage of CD4⁺ and CD8⁺ T cells in the suspensions are shown in Fig. 6A. Fig. 6B shows the expression of CD44 and CD62L on CD4⁺ T cells in the pancreas from mice treated with PBS (left), control IgG (middle), and anti-mouse CXCR3 antibody (right). The percentage of cells in gate 1 (G1; CD44^{hi}CD62L^{lo}) is indicated for each treatment group (67.3% for PBS, 67.1% for control IgG, and 30.4% for anti-CXCR3 treatment). Fig. 6C is a plot of CXCR3 expression on CD4⁺ T cells in gate 1 (G1) or gate 2 (G2) as defined in Fig. 6B, compared to cells stained with isotype control antibody and gated on lymphocytes.

[0175] To evaluate whether insulin-positive islets are present in NOD mice reversed with anti-CXCR3 treatment, paraffin-embedded pancreas sections were prepared from female NOD mice treated with control IgG (left panels), anti-mouse CXCR3 antibody (middle panels) or murine ATG (right panels) and stained for insulin (top row) or co-stained for CD3 and Foxp3 (bottom row). See Fig. 7. The pancreas tissue was harvested from mice at the end of the study (around 100 days post enrollment). The stained sections demonstrated that insulin-positive islets were present in NOD mice reversed with anti-CXCR3 treatment, and that T cells surrounded the islets but few invaded the islets.

[0176] To evaluate the response to glucose challenge, a glucose tolerance test was performed. Fig. 8. Age-matched female non-diabetic NOD mice (Fig. 8A), diabetic NOD mice that had been treated with PBS (Fig. 8B),

diabetic NOD mice reversed with anti-mouse CXCR3 treatment (Fig. 8C), and diabetic NOD mice that had been treated with IgG (Fig. 8D) were fasted overnight and challenged with glucose by i.p. injection. Blood glucose was measured before (time 0) and after challenge at the indicated times. Representative data from 4-5 mice per treatment group are shown. The data illustrate that anti-CXCR3 antibody treatment improved fasting glucose tolerance 100 days post-enrollment.

Example 5: Adoptive Transfer of T Cells

[0177] To evaluate the ability of T cells from NOD mice treated with anti-CXCR3 antibody (clone CXCR3-173) and exhibiting disease remission to induce diabetes in recipient animals, isolated CD4⁺ and CD8⁺ T cells were adoptively transferred to recipient NOD.scid (non-obese diabetic - severe combined immunodeficiency) mice by intravenous injection. Fig. 9 shows the percentage of non-diabetic mice over time after adoptive transfer of isolated CD4⁺ and CD8⁺ T cells from diabetic mice treated with PBS or control IgG, or mice in disease remission after treatment with murine ATG or anti-mouse CXCR3 antibody. CD4⁺ and CD8⁺ T cells were isolated from spleen, pancreatic lymph nodes, and inguinal lymph nodes harvested 80-90 days post-enrollment from female NOD mice in the different treatment groups. CD4⁺ and CD8⁺ T cells were pooled and 8 million total cells were adoptively transferred to NOD.Scid recipients, and the development of diabetes was monitored by bi-weekly blood glucose measurements. Each line in Fig. 9 represents the combined data from five mice per group. Two representative studies are shown

(Fig. 9A and 9B). Isolated T cells from anti-CXCR3 antibody-treated mice exhibit a delay in disease transfer.

[0178] The isolated donor T cells were further characterized. Fig. 10A shows the percentage of total CD4⁺ and CD8⁺ T cells (left panel) in the donor T cells isolated from mice treated with PBS, anti-mouse CXCR3 antibody, control IgG, or murine ATG as described in the previous paragraph. The right panels of Fig. 10A show the percentage of effector and central memory T cells in the subset of T cells that were CD4⁺ (upper panel) and CD8⁺ (lower panel) for each donor cell suspension, as defined by expression of CD44 and CD62L. Isolated pooled CD4⁺ and CD8⁺ T cells were stained for CD44 and CD62L expression before transfer, acquired on a flow cytometer and analyzed. The percentage of regulatory T cells in the pools of isolated donor T cells was also evaluated. Fig. 10B shows the percentage of regulatory T cells for each treatment group, as defined by CD4 and CD25 expression or by CD4, CD25 and intracellular Foxp3 expression. Fig. 10C shows the percentage of CD8⁺ (left panel) and CD4⁺ (right panel) T cells in the donor cells that also express CXCR3. The data demonstrate that there was a reduced percentage of CXCR3⁺ T cells in donor cells from mice reversed with anti-CXCR3 antibody treatment.

[0179] The effectiveness of CXCR3 treatment following adoptive transfer of OT-1 CD8⁺ donor T cells was evaluated using the RIP-OVA model of type 1 diabetes. RIP-OVA mice are transgenic mice where a transgene encoding ovalbumin protein (OVA) driven by the rat insulin promoter (RIP) has been

introduced into the mouse genome and results in the expression of a membrane form of ovalbumin in islet β cells. The background strain of mice is C57BL/6 and the RIP-OVA mice do not spontaneously develop diabetes. RIP-OVA mice, also called C57BL/6-Tg(Ins2-TFRC/OVA)²⁹⁶Wehi/WehiJ, were purchased from the Jackson Laboratory. Diabetes develops in these mice after adoptive transfer of ovalbumin-specific CD8⁺ T cells from OT-1 TCR (T cell receptor) transgenic mice (Kurts et al. *J Exp Med* 184: 923-930) purchased from the Jackson Laboratory. OT-1 mice contain transgenic inserts for mouse TCR α -V2 and TCR β -V5 genes (Hogquist et al. *Cell* 76:17-27). The transgenic TCR recognizes ovalbumin residues in the context of MHC I H2K^b proteins. Greater than 95% of CD8⁺ T cells in OT-1 mice express the transgenic TCR and recognize and are activated by ovalbumin peptide.

[0180] Fig. 11 shows the percentage of non-diabetic mice over time following adoptive transfer of OT-1 CD8⁺ T cells into RIP-OVA recipient mice that were then left untreated, treated with anti-mouse CXCR3 antibody, or treated with control IgG. Treatment (100 μ g i.p.) was started 1 day (study 1 and study 2) or 7 days (study 2) after adoptive transfer and was given twice a week for 3 weeks. Each line represents the combined data from five mice per group. Results from two studies are shown in Fig. 11A and 11B. The data demonstrate that anti-CXCR3 antibody treatment protected mice from developing diabetes in the RIP-OVA model.

[0181] Fig. 12 provides further data characterizing the effectiveness of different treatments following adoptive transfer of OT-1 T cells into RIP-OVA

recipient mice. Fig. 12A shows CXCR3 expression on donor T cells analyzed by flow cytometry before adoptive transfer into RIP-OVA recipients. Staining with isotype control antibody is represented by the shaded curve. Fig. 12B shows the percentage of donor cells in the blood, spleen, and pancreatic lymph nodes (pLN) at the indicated times following adoptive transfer of OT-1 T cells to RIP-OVA recipient mice treated with anti-mouse CXCR3 antibody or control IgG. Antibody treatment (100 µg i.p.) was started one day after T cell transfer and was given twice a week for two weeks. Each dot represents data from one individual mouse. Fig. 12C indicates the number of donor cells in the blood, spleen and pancreatic lymph nodes (pLN) that were proliferating in response to auto-antigen (OVA) stimulation in RIP-OVA recipient mice treated with anti-mouse CXCR3 antibody or control IgG at the indicated times following adoptive transfer. Antibody treatment (100 µg i.p.) was started one day after OT-1 T cell transfer and was given twice a week for two weeks. Each dot represents data from one individual mouse. Fig. 12D shows the percentage of CXCR3-expressing donor cells in the blood, spleen, and pancreatic lymph nodes (pLN) at the indicated times following adoptive transfer of OT-1 T cells to RIP-OVA recipient mice treated with anti-mouse CXCR3 antibody or control IgG. Antibody treatment (100 µg i.p.) was started one day after OT-1 T cell transfer and was given twice a week for two weeks. Each dot represents data from one individual mouse. Treatment with anti-CXCR3 antibody led to a reduced percentage of CXCR3+ T cells in RIP-OVA mice.

[0182] Fig. 13 shows representative paraffin-embedded pancreas sections from RIP-OVA recipient mice left untreated and stained for insulin (Fig. 13A) or CD3 (Fig. 13B), or treated with anti-mouse CXCR3 antibody and stained for insulin (Fig. 13C) or CD3 (Fig. 13D). Anti-CXCR3 treatment (100 µg i.p.) was started one day after OT-1 T cell transfer and given twice a week for 3 weeks. The pancreas tissue was harvested at the end of the study (around 60 days post T cell transfer). The sections show a lack of T cell infiltration in RIP-OVA mice treated with anti-mouse CXCR3 antibody.

Example 6: Evaluation of anti-human CXCR3 antibody clones

[0183] Anti-human CXCR3 antibody clones Cl 4, 12, 53, 82, and 135 were evaluated for their effect on CXCR3 chemotaxis and calcium mobilization, using the methods described above in the Materials and Methods section (Example 1). For the chemotaxis assay, human CXCR3 transfected 300.19 cells were pre-incubated in media alone or with various concentration of antibody, as indicated in Figures 14 A-C, prior to being added to the chemotaxis assay. Figure 14A-C shows that CXCR3-mediated chemotaxis to CXCL 11 is inhibited by clones Cl 4, 12, 53, 82, and 135. Clone 2 in Figure 14 is identical to clone 4.

[0184] For the calcium flux assay, human CXCR3-transfected HEK cells were pre-incubated in various concentrations of antibody prior to being added to the FLIPR according to the methods described above in the Materials and Methods section (Example 1). The concentration of antibody needed to inhibit calcium mobilization by 50% for each antibody is shown in Fig. 14D. Figure

14D shows that CXCR3-mediated calcium mobilization to CXCL11 is inhibited by clones CI 4, 12, 53, and 135.

[0185] To further assess the effect of clones 4, 12, 53, 82, and 135 on chemotaxis, hCXCR3-transfected 300.19 cells were pre-incubated in media alone or with 50 µg/ml antibody prior to being added to the chemotaxis assay and assessed for migration to CXCL9 (Fig. 15A), CXCL10 (Fig. 15B), and CXCL11 (Fig. 15C). The data demonstrate that clones 4, 12, 53, 82, and 135 inhibit migration to CXCL10 and CXCL11 and partially inhibit migration to CXCL9.

[0186] To evaluate the specificity of clones 4, 12, 53, 82, and 135, the antibodies were assayed for binding to other chemokine receptors. 300.19 cells were transfected with human CXCR1, CXCR5, CXCR2, CXCR4 or CCR5 and antibody binding was analyzed by incubation with the anti-human CXCR3 antibody clones, followed by secondary antibody staining and flow cytometry. Administration of the secondary antibody alone served as the negative control. 300.19 cells transfected with human CXCR3 served as a positive control for staining by the clones. Fig. 16 shows histogram plots of antibody binding to cells expressing the different chemokine receptors, demonstrating that clones 4, 12, 53, 82, and 135 do not bind the other chemokine receptors and are specific for CXCR3.

[0187] Standard flow cytometry procedures were employed in the chemokine receptor binding assay. Briefly, cell lines were harvested by Versene treatment and each cell line was divided into seven samples. Each

sample was incubated on ice with one primary antibody (5 µg/ml) followed by staining with a FITC-conjugated secondary antibody to detect the bound primary antibody. As a negative control, cells were stained with secondary antibody alone (no primary antibody incubation). The primary antibody was an anti-human CXCR3 antibody clone or the anti-human CXCR3 control antibody clone 1C6. After staining, the cells were acquired on a flow cytometer and the data analyzed using FlowJo software. Each line in Fig. 16 represents an individual sample of cells stained with one primary antibody and the secondary antibody, or with the secondary antibody alone.

[0188] Affinity (K_a) and off-rate (K_d) for clones 4, 12, 53, 82, and 135 were analyzed using a Biacore assay according to the methods described above (Example 1). The results are summarized below in Table 4.

Clone #	k_a (1/Ms)	k_d (1/s)	KD (M)
4	108539.245	0.000348	3.2076E-09
53	79557.574	0.000581	7.3085E-09
12	183854.704	0.001473	8.01056E-09
82	195114.396	0.001828	9.36793E-09
135	88939.340	0.001214	1.36548E-08

Table 4

Example 7: Epitope mapping

[0189] Truncated, biotinylated human CXCR3 peptides (16 amino acid N-terminal fragments) from the CXCR3 N-terminal extracellular domain were used to determine epitopes for clones 4, 12, 53, 82, and 135. A series of alanine substituted fragments were generated (see table 5 below, alanine substitution in

bold) and biotinylated. Epitope mapping was evaluated by Octet® (ForteBio, Menlo Park, CA) and Biacore™ (GE Healthcare) analyses.

[0190] For Octet analysis, peptides were re-suspended in 80% DMSO and diluted to 10µg/ml in PBS. Antibody clones 4, 12, 53, 82, 135 and commercial clone 1C6 (BD Biosciences) were diluted to 120nM in PBS. The kinetics assay was performed in 96-well plate format with 300µl/well on an Octet QK system (ForteBio). Each assay plate included N-terminally biotinylated full-length WT hCXCR3 ECD peptide (Abgent) as a positive control, as well as PBS buffer blank for reference subtraction. Octet Streptavidin biosensors (ForteBio) were pre-soaked in PBS for at least five minutes prior to running the assay. Biosensors were first immersed in PBS for five minutes with no shaking for a baseline. For all remaining steps, the shake speed was 1000rpm. The biosensors were dipped in peptide solutions for five minutes to load peptides. Another baseline step in PBS for five minutes was performed. Biosensors were then dipped into antibody solutions for ten minutes to measure association. Finally, the biosensors were transferred into PBS for fifteen minutes for dissociation. Sensorgrams were analyzed using Octet Data Analysis v7.0. Binding activity was expressed as a percentage of each antibody's maximum response level compared to WT full-length hCXCR3 peptide.

[0191] Relative response levels were recorded in an epitope heat map. Maximal sensorgram responses to wild type hCXCR3 ECD peptide ranged between 4-8nm. Each clone screened had a unique epitope. None of the

mutants tested completely abolished binding of clone 1C6. The Valine residue in position 10 and Aspartate in position 13 played a role in binding of all antibodies screened. Antibodies 12 and 1C6 had the most N-terminal epitopes, with position 5 Valine mutations influencing activity of both. Antibody 82 had the most C-terminal epitope, and a reduction in binding activity started with position 9 Glutamine. Based on these data, amino acid epitope boundaries on the CXCR3 sequence were as follows for each antibody; Cl 4: amino acids 7-13; Cl 12: amino acids 5-13; Cl 53: amino acids 7-13; Cl 82: amino acids 9-15; Cl 135: amino acids 7-13; and clone 1C6: amino acids 5-13.

[0192] For Biacore analysis, peptides were diluted to 10ng/ml in HBS-EP+ running buffer (10mM HEPES, 150mM NaCl, 3mM EDTA, 0.005% Polysorbate 20). Using a Biacore T100™, peptides were injected over CM5-SA (GE #BR-1005-31) chips at a rate of 5µl/min until 20 response unit (RU) stable capture was obtained per flow cell. Flow cell 1 remained blank for reference subtraction on each chip. Wild-type 37AA hCXCR3 peptide was included on one flow cell of each chip as a positive control. Mouse anti-hCXCR3 antibodies 4, 12, 53, 82, and 135 were diluted to 50, 25, 12.5, 6.25, and 3.125nM in HBS-EP+. Each cycle, antibodies were injected for three minutes at a flow rate of 50µl/min to measure association, followed by three minutes of buffer at 50µl/min to measure dissociation. The peptide surface was regenerated between cycles using 10mM glycine-HCl pH 2.0 at 50µl/min for sixty seconds. Sensorgrams were fit to a 1:1 binding model and analyzed using double-reference subtraction in BiaEvaluation v2.0.1 and captured on streptavidin biosensors. Typical

response levels ranged between 0-500RU, and cutoffs for 'Strong', 'Moderate', and 'Weak' binding responses were determined. Relative response levels were recorded to generate an epitope map. Off-rates were ranked, and peptides which resulted in fast K_d values (greater than $0.001s^{-1}$) were also recorded.

Table 5

Seq Id No.	Sequence	Seq Id No.	Sequence
	AVLEVSDHQVLNDAEV		MVLEVSDHQLNDAEV
	MALEVSDHQVLNDAEV		MVLEVSDHQLNDAEV
	MVAEVSDHQVLNDAEV		MVLEVSDHQVANDAEV
	MVLAVSDHQVLNDAEV		MVLEVSDHQVLADAEV
	MVLEASDHQVLNDAEV		MVLEVSDHQVLNAAEV
	MVLEVADHQVLNDAEV		MVLEVSDHQVLNDAEV (wild type)
	MVLEVSAHQVLNDAEV		MVLEVSDHQVLNDAEV
	MVLEVSDAQVLNDAEV		MVLEVSDHQVLNDAEA

[0193] All antibodies bound to wild-type hCXCR3 and within the first 16AAs of the hCXCR3 sequence. The binding data is shown in Table 6 for clones 4, 12, 53, 82, and 135, and the corresponding map showing the boundaries of the minimum epitope required for binding activity for each antibody clone is shown in Fig. 18, with important residues marked with an X. All antibody epitopes mapped within human CXCR3 sequence residues 6-15, the region involved in CXCL10 and CXCL11 binding. Amino acids within the epitope were determined by detecting a reduction in binding response after an alanine substitution at a given position in the CXCR3 peptide fragment. Clones 53 and 135 had the most similar epitopes. Clones 4 and 12 had the most N-terminal epitope (binding strength affected starting with the valine at position

five). Clone 82 had the most C-terminal epitope, with a reduction in binding activity starting with position 9 glutamine. The aspartate at position 7 in CXCR3 is required for every clone except 82. The valine at position ten and the aspartate at position thirteen in CXCR3 both play a role in binding of all clones. There were no difference in epitopes between the mouse, Chimeric and humanized versions of the five clones.

Table 6

Sequence	α -hCXCR3 mAb clones				
	4	12	83	82	135
Biotin-AVLEVSDHQVLNDAEV	+++	+++	+++	+++	+++
Biotin-MALEVSDHQVLNDAEV	+++	+++	+++	+++	+++
Biotin-MVAEVSDHQVLNDAEV	+++	+++	+++	+++	+++
Biotin-MVLAVSDHQVLNDAEV	+++	+++	+++	+++	+++
Biotin-MVLEASDHQVLNDAEV	+++ *	+++ *	+++	+++	+++
Biotin-MVLEVADHQVLNDAEV	+++	+	+++	+++	+++
Biotin-MVLEVSAHQVLNDAEV	-	+	+	+++	+
Biotin-MVLEVSLAQVLNDAEV	+++ *	+	+++	+++	+++ *
Biotin-MVLEVSDHQLNDAEV	+++	+++	+++	+++ *	+++
Biotin-MVLEVSDHQALNDAEV	-	++ *	+	++ *	+
Biotin-MVLEVSDHQVANDAEV	+	+++	+++	+++	++
Biotin-MVLEVSDHQVLADAEV	+	+++	+++	-	++ *
Biotin-MVLEVSDHQVLNAAEV	-	++	-	-	-
Biotin-MVLEVSDHQVLNDAEV	+++	+++	+++	+++	+++
Biotin-MVLEVSDHQVLNDAEV	+++ *	+++	+++	++ *	+++ *
Biotin-MVLEVSDHQVLNDAEA	+++	+++	+++	+++	+++

[0194] Key

- +++ Strong binding response (>300RU)
- ++ Moderate binding response (150-300RU)
- +
- Weak binding response (10-150RU)
- No binding (<10RU)

- * Fast off-rate ($K_d > 0.001$) despite stronger binding response (>10RU)

Example 8: Humanization of anti-human CXCR3 clones

[0195] Four variants (chimeric, humanized 1 (Hu1), Hu2, and Hu3) were generated for each of the five anti-CXCR3 clones (clones 4, 12, 53, 82, and 135). The 20. All chimeric variants were produced by "pool expression" for *in vivo* animal model studies. CHO-DXB11 cells (Urlaub and Chasin, *Proc. Natl. Acad. Sci.*, 77:4216-20, (1980)) that were adapted to suspension culture were electroporated with expression vectors containing the heavy and light chains from chimeric CXCR3 antibody clones 4, 12, 53, 82 and 135. These expression vectors also contain the dihydrofolate reductase gene for selecting stable CHO transfectants. Following the 100 nM methotrexate selection, stably transfected CHO pools were used to complete bioproduction runs in shake flasks with OptiCHO medium. The recombinant chimeric antibody was purified from the conditioned medium using standard protein A chromatography.. Hu1 clones were CDR grafted onto human framework regions and were fully humanized, excluding the amino acids of the mouse CDRs and any Vernier position residues. Hu2 clones were "expanded CDR" clones, with backmutated changes from the Hu1 sequences at residues positioned within four amino acids of the mouse CDRs. Hu3 clones included further backmutations from the Hu2 sequences at positions identified as "very dissimilar" between mouse and human using IMGT-based modeling. The twenty total variants were transiently expressed in CHO-NRC cells and purified for *in vitro* assays.

[0196] Additional humanization was performed for clones 4 and 53. Heavy chains 4.7-4.11 included further humanized backmutations outside the CDRs, as well as modifications to remove a deamidation site at positions 58 and 59 (IMGT numbering) in VH CDR2 in an effort to improve clone stability. In particular, the deamidation site in VH 4.2 was replaced with a number of alternative residues. Clones VH 53.4-53.6 and VL 53.4-53.9 included further humanized backmutations outside the CDRs, particularly in an effort to make the chains more similar to VH 4.1 and VL 4.1.

Example 9: Whole receptor binding kinetics

[0197] The binding kinetics of the twenty anti-CXCR3 variants was evaluated using Octet® and Biacore™ with intact CXCR3 peptides. Octet analysis was conducted as described previously. For Biacore analysis, three column step purified human wild type CXCR3 peptides were C-terminal His and HPC4 tagged and captured on NTA chips via nickel chelating and amine coupling. Medium RU (approximately 1200RU) chips were used for better data quality and to minimize bivalent binding effect. 0-80nM of the antibody samples were injected over the receptor. Standard Biacore™ evaluation analysis was conducted using sensorgram plots with local Rmax fit for better curve fitting. The binding curves for the four variants (chimeric (Ch) and humanized (Hu) 1-3) for the five clones (4, 12, 53, 82, and 135) were evaluated. For comparison purposes, the binding kinetics of the human CXCR3 ligands CXCL9, CXCL10, and CXCL11, and mouse CXCL11 (mCXCL11) were also evaluated. The results are shown below in Tables 7A (ranked by KD) and 7B (ranked by Kd).

The top four variants by KD and Kd are highlighted in the tables. When the humanized anti-hCXCR3 mAb variants were ranked by off rates (Kd), most of the variants showed at least 1 digit slower off rates than the most potent human CXCR3 ligand hCXCL11.

Table 7A

Sample	ka (1/Ms)	kd (1/s)	KD (M)
4Hu2	1.59E+05	8.18E-05	5.15E-10
4Ch	2.09E+05	1.15E-04	5.48E-10
4Hu3	1.54E+05	8.62E-05	5.60E-10
4Hu1(low RU)	2.24E+05	1.40E-04	6.34E-10
mCXCL11	3.69E+06	4.26E-03	1.17E-09
82Hu3	3.40E+05	4.82E-04	1.42E-09
53Ch	4.44E+04	7.07E-05	1.60E-09
CXCL11	2.58E+06	4.41E-03	1.72E-09
53Hu3	6.88E+04	1.13E-04	1.72E-09
82Hu2	8.67E+04	2.62E-04	3.02E-09
12Ch	9.66E+04	4.52E-04	6.00E-09
135Hu3	6.68E+04	3.35E-04	6.01E-09
82Ch	1.61E+05	8.92E-04	5.54E-09
53Hu2	3.05E+04	2.04E-04	6.70E-09
135Ch	5.52E+04	3.82E-04	6.93E-09
CXCL10	1.36E+05	1.27E-03	9.76E-09
12Hu3	6.60E+04	1.02E-03	1.56E-08
12Hu2	6.39E+04	1.02E-03	1.60E-08
CXCL9	poor data quality		
135Hu2	No binding		
12Hu1			
53Hu1			
82Hu1			
135Hu1			

Sample	ka (1/Ms)	kd (1/s)	KD (M)
53Ch	4.44E+04	7.07E-05	1.60E-09
4Hu2	1.59E+05	8.18E-05	5.15E-10
4Hu3	1.54E+05	8.62E-05	5.60E-10
53Hu3	6.88E+04	1.13E-04	1.72E-09
4Ch	2.09E+05	1.15E-04	5.48E-10
4Hu1(low RU)	2.24E+05	1.40E-04	6.34E-10

53Hu2	3.05E+04	2.04E-04	6.70E-09
82Hu2	8.67E+04	2.62E-04	3.02E-09
135Hu3	6.68E+04	3.35E-04	5.01E-09
135Ch	5.52E+04	3.82E-04	6.93E-09
12Ch	9.66E+04	4.52E-04	5.00E-09
82Hu3	3.40E+05	4.82E-04	1.42E-09
82Ch	1.61E+05	8.92E-04	5.54E-09
12Hu2	6.39E+04	1.02E-03	1.60E-08
12Hu3	6.60E+04	1.02E-03	1.56E-08
CXCL10	1.36E+05	1.27E-03	9.76E-09
mCXCL11	3.69E+06	4.26E-03	1.17E-09
CXCL11	2.58E+06	4.41E-03	1.72E-09
CXCL9	poor data quality		
135Hu2	No binding		
12Hu1			
53Hu1			
82Hu1			
135Hu1			

Table 7B

[0198] As indicated in Table 7A, the top four variants by KD are chimeric clone 4 (fast on rate, slow off rate), Hu 3 clone 4 (fast on rate, slow off rate), Hu 3 clone 82 (fast on rate, average off rate), and chimeric clone 53 (average on rate, slow off rate).

[0199] Antibody binding was further evaluated in CXCR3-expressing cells. Human CXCR3 transfected 300.19 cells were contacted with purified humanized anti-hCXCR3 antibody variants Hu1, Hu2, Hu3, and the chimeric antibody. As shown in Fig. 19 for each of clones 4, 12, 53, 82, and 135 at 5 µg/ml (black line), 0.5 µg/ml (dark gray line), or 0.1 µg/ml (black dashed line) or 5 µg/ml secondary antibody alone (filled gray histogram). The cells were stained with the unlabeled antibody clones for 30 min on ice followed by two washes with PBS-1%FBS and bound antibody was detected using a FITC-conjugated anti-human IgG1 specific secondary antibody by incubating on ice

for 30 min in the dark. The samples were washed twice, fixed in a 2% paraformaldehyde PBS solution, stored in the dark at 4°C and acquired on a flow cytometer. Histograms of CXCR3 positivity gated on viable cells are shown in Fig. 19.

Example 10: Comparison to antibody 1C6

[0200] Anti-hCXCR3 mAb clone 1C6 (Becton, Dickinson catalog #557183, same clone reported U.S. Patent No. 6,184,358) was compared to mouse anti-hCXCR3 mAb clone 4 and its humanized variants Hu2 and Hu3 using the Biacore whole receptor assay method. Clone 4 exhibited about 2-fold better affinity (KD). The humanized variants exhibited further improved affinity (approximately 4-fold better affinity for both the Hu2 and Hu3 variants). Table 8 lists binding kinetics and affinity for clone 1C6 and for clone 4 and its humanized variants Hu2 and Hu3.

Sample	ka (1/Ms)	kd (1/s)	KD (M)
1C6-Hybridoma	4.11E+04	1.42E-04	3.51E-09
4-Hybridoma	1.18E+05	2.22E-04	1.88E-09
4Hu3	1.54E+05	8.62E-05	5.60E-10
4Hu2	1.59E+05	8.18E-05	5.15E-10

Table 8

Example 11: Functional assays

[0201] The functional effects of the twenty variant antibodies were evaluated. The antibodies were evaluated for their effect on cell chemotaxis in response to CXCL9, CXCL10, and CXCL11, and inhibition of calcium mobilization by FLIPR® calcium assay.

[0202] Chemotaxis of human CXCR3-transfected 300.19 cells to the CXCR3 ligands CXCL9, CXCL10, and CXCL11 was evaluated in the presence or absence of 10 µg/ml humanized anti-human CXCR3 antibody variants of clones 4, 12, 53, 82, and 135. Transfected cells were pre-treated for 20 min at 37°C with 10 µg/ml humanized anti-human CXCR3 antibody variants or the commercial antibody 1C6. Cells with antibody were transferred to 5 micron transwells and inserts were placed in the receiver well containing 100 or 300 ng/ml of recombinant mouse CXCL10 and CXCL11 or CXCL9, respectively. The chemotaxis plates were incubated at 37°C, 5% CO₂ for 4 hr. The transwell inserts were removed and the cells that migrated into the receiver wells were transferred to U-bottom 96 well plates, pelleted and resuspended in calcein AM dye. The cells were incubated for 30 min at 37°C, 5% CO₂, washed once, transferred to a black/clear plate and immediately read on the FlexStation at 490/520 nm. Data is presented as percent inhibition of chemotaxis. Fig. 20 provides representative data showing the ability of the different variants for each of the five clones to inhibit chemotaxis to CXCL9, CXCL10, and CXCL11.

[0203] To evaluate the inhibition of intracellular calcium mobilization, calcium flow in human CXCR3-Gqi4qi4 transfected CHO cells was measured in response to CXCL10 in the presence or absence of various concentrations of anti-CXCR3 antibody variants or the positive control antibody 1C6. Cells were seeded (12,000/well) in 384-black plates and allowed to attach overnight. The next day, cells were loaded with calcium sensitive dye, Fluo-4NW dye, for 40 min prior to the addition of antibody and incubated at 37°C. Antibody was added

and allowed to incubate for 20 min prior to addition of the CXCR3 ligand, recombinant human CXCL10, at the pre-determined EC_{80} concentration of CXCL10. Addition of dye was performed manually using an electronic multichannel pipet but antibody and agonist addition was automated on the FLIPR Tetra® and the plate immediately read at 470-495 nm after the addition of CXCL10. Samples were run in duplicate and the average percent inhibition ($\pm$ Standard Deviation) at each antibody concentration was graphed. Clone 4 Hu1 did not inhibit calcium mobilization and was used as a negative control in these experiments. Fig. 21 shows the ability of the different variants for each of the five clones to inhibit calcium mobilization and compares them to the commercial clone 1C6. Representative IC_{50} values (M) of antibodies against CXCL10 in the calcium mobilization assay are shown in Table 9 below.

	Clone 4	Clone 12	Clone 53	Clone 82	Clone 135	1C6
Hu1	N/A	N/A	N/A	N/A	N/A	-
Hu2	N/A	1.038E-06	1.675E-06	1.772E-06	N/A	-
Hu3	1.146E-06	8.636E-07	1.261E-06	1.169E-06	1.499E-06	-
Chimeric	9.352E-07	1.204E-06	1.87E-06	6.778E-07	1.891E-06	3.135E-06*

Table 9

[0204] (*) in Table 9 indicates that the 1C6 antibody is not a chimeric antibody but a fully mouse IgG1 antibody against human CXCR3.

[0205] As shown in Table 10, the binding kinetic data (see Example 9), correlated well with the functional assay results. Based on the binding kinetic data and the functional assay results, clones 4 and 53 were selected for further evaluation and 4D humanization.

Table 10

Shading Code	Best	Average	Poor								
Clones	Epitope group	Octet Peptide Binding		Biacore Whole Receptor Binding			In Vitro Assays				
		Response	KD (M)	ka (1/Ms)	kd (1/s)	KD (M)	Flow	Chemotaxis CXCL11	Chemotaxis CXCL3	Primary CXCL10	FLPR
4 chimera	2	7.1856	8.57E-11	2.08E+05	1.15E-04	5.88E-10					
4 Hu1	2	3.5388	1.52E-08	2.24E+05	1.48E-04	6.34E-10					
4 Hu2	2	6.7748	3.78E-11	1.58E+05	1.16E-03	1.07E-08					
4 Hu3	2	6.8723	4.88E-11	2.28E+05	1.47E-04	6.48E-10					
12 chimera	1	6.6488	<1.0E-12	8.68E+04	4.52E-04	5.09E-09				<50%	
12 Hu1	1	4.6044	6.54E-09		no binding					inhibition for all variants	
12 Hu2	1	6.87151	3.44E-10	8.38E+04	1.62E-03	1.88E-08					
12 Hu3	1	7.0274	<1.0E-12	8.68E+04	1.02E-03	1.58E-08					
53 chimera	3	6.1888	<1.0E-12	4.44E+04	7.67E-05	1.88E-08					
53 Hu1	3	2.0452	5.27E-08		no binding						
53 Hu2	3	8.0188	2.98E-11	3.85E+04	2.04E-04	8.79E-09					
53 Hu3	3	6.929	1.43E-11	8.88E+04	1.13E-04	1.72E-08					
82 chimera	4	0.8554	<1.0E-12	1.81E+05	8.92E-04	5.84E-09					
82 Hu1	4	2.2811	3.81E-08		no binding						
82 Hu2	4	6.2257	4.89E-12	8.67E+04	2.82E-04	3.02E-09					
82 Hu3	4	6.1277	5.07E-12	3.48E+05	4.82E-04	1.42E-09					
135 chimera	3	7.0743	<1.0E-12	5.52E+04	3.82E-04	8.83E-09				<50%	
135 Hu1	3	0.9288	5.74E-08		no binding					inhibition for all variants	
135 Hu2	3	0.9256	4.59E-08		no binding						
135 Hu3	3	7.1572	2.42E-11	8.58E+04	3.35E-04	5.01E-08					

Example 12: 4D humanization

[0206] 4D humanization of anti-hCXCR3 antibody clones is done as described in WO 2009/032661 (e.g., at paragraphs [0037]-[0044]). Briefly, 4D humanization comprises: a) building a 3-D model of the variable domain that is to be humanized; b) identifying the flexible residues in the variable domain using a molecular dynamics simulation of the 3-D model of the domain; c) identifying the closest human germline by comparing the molecular dynamics trajectory of the 3-D model to the molecular dynamics trajectories of 49 human germlines (rather than a comparison of antibody sequences, as is done in traditional humanization); and d) mutating the flexible residues, which are not part of the CDR, into their human germline counterpart (identified in step c). Heavy chains 4.4-4.6 and light chains 4.4-4.7 were designed using this method. In particular, an initial 4D humanized construct (VH 4.4 and VL 4.4) was

designed and then further modifications to the heavy and light chain were designed (VH 4.5-4.6 and VL 4.5-4.7) to introduce stabilizing and anti-aggregating mutations and to eliminate other unwanted motifs. Similar methods were also used to design 4D humanized constructs VH 53.7-53.10 and VL 53.10-53.13.

[0207] Table 11 indicates the humanizing strategy (and the additional modifications, where applicable) used to prepare the heavy and light chain variants for clones 4, 12, 53, 82, and 135, including the humanized and 4D humanized chains (VH = heavy chain; VK = light chain).

[0208] Several 4D variants of clone 4 (4Hu6, 4Hu7, 4Hu8, 4Hu9, 4Hu10) were evaluated by Biacore whole receptor assay to evaluate binding kinetics and CXCR3 affinity and compared to the clone 4 chimeric antibody. As shown in Table 2, Clone 4Hu6 contained heavy chain 4.4 and light chain 4.4. Clone 4Hu7 contained heavy chain 4.4 and light chain 4.7. Clone 4Hu8 contained heavy chain 4.5 and light chain 4.5. Clone 4Hu9 contained heavy chain 4.5 and light chain 4.6. And clone 4Hu10 contained heavy chain 4.6 and light chain 4.4.

[0209] As shown in Table 12, when the 4D modeling variants of clone 4 were compared to the chimeric variant (4Ch), four out of five variants showed improved affinity (KD). The 4D variant 4Hu10, however, showed nearly 1 order of magnitude decreased affinity.

Clone	heavy/light chain variant	strategy
4	VH1	CDR grafting
4	VH2	CDR grafting
4	VH3	CDR grafting
4	VH4	4D modeling
4	VH5	4D modeling, includes stabilizing mutations
4	VH6	4D modeling, includes mutations to remove unwanted motifs
4	VH7	CDR grafting, modification of VH 4.2 at residues NG>QG to remove CDR2 deamidation site
4	VH8	CDR grafting, modification of VH 4.2 at residues NG>NL to remove CDR2 deamidation site
4	VH9	modification of VH 4.2 at residues NG>NS to remove CDR2 deamidation site
4	VH10	modification of VH 4.2 at residues NG>DG to remove CDR2 deamidation site
4	VH11	modification of VH 4.2 at residues NG>NV to remove CDR2 deamidation site
4	VK1	CDR grafting
4	VK2	CDR grafting
4	VK3	CDR grafting
4	VK4	4D modeling
4	VK5	4D modeling, includes stabilizing mutations
4	VK6	4D modeling, includes other stabilizing mutations
4	VK7	4D modeling, includes anti-aggregation mutations
53	VH1	CDR grafting
53	VH2	CDR grafting
53	VH3	CDR grafting
53	VH4	modification of VH 4.2 at residue T50>V - back mutation to incorporate VH 4.1 residue
53	VH5	modification of VH 4.2 at residue P61>A - back mutation to incorporate VH 4.1 residue
53	VH6	modification of VH 4.2 at residue M93>V - back mutation to incorporate VH 4.1 residue
53	VH7	4D modeling
53	VH8	4D modeling
53	VH9	4D modeling
53	VH10	4D modeling

53	VK1	CDR grafting
53	VK2	CDR grafting
53	VK3	CDR grafting
53	VK4	modification of VK 4.2 at residue I32>L - back mutation to incorporate VK1 residue
53	VK5	modification of VK 4.2 at residue Y33>A - back mutation to incorporate VK1 residue
53	VK6	modification of VK 4.2 at residue N52>T - back mutation to incorporate VK1 residue
53	VK7	modification of VK 4.2 at residue A54>Q - back mutation to incorporate VK1 residue
53	VK8	modification of VK 4.2 at residue P55>S - back mutation to incorporate VK1 residue
53	VK9	modification of VK 4.2 at residue G99>Q - back mutation to incorporate VK1 residue
53	VK10	4D modeling
53	VK11	4D modeling
53	VK12	4D modeling
53	VK13	4D modeling
12	VH1	CDR grafting
12	VH2	CDR grafting
12	VH3	CDR grafting
12	VK1	CDR grafting
12	VK2	CDR grafting
12	VK3	CDR grafting
82	VH1	CDR grafting
82	VH2	CDR grafting
82	VH3	CDR grafting
82	VK1	CDR grafting
82	VK2	CDR grafting
82	VK3	CDR grafting
135	VH1	CDR grafting
135	VH2	CDR grafting
135	VH3	CDR grafting
135	VK1	CDR grafting
135	VK2	CDR grafting
135	VK3	CDR grafting

Table 11

Curve	ka (1/Ms)	kd (1/s)	KD (M)
4Ch	1.71E+05	8.57E-05	5.09E-10
4Hu6	4.76E+05	1.55E-04	3.27E-10
4Hu7	4.12E+05	1.33E-04	3.26E-10
4Hu8	3.27E+05	1.13E-04	3.49E-10
4Hu9	3.48E+05	1.34E-04	3.87E-10
4Hu10	3.55E+05	1.05E-03	2.96E-09

Table 12

Example 13: NSG-PBL mouse model

[0210] NOD-*scid* *IL2r^{null}* (NSG) mice were injected with 2E7 fresh primary human ficoll isolated peripheral blood mononuclear cells (PBMCs) on day 0. Animals (n=8/group) were treated with 5mg/kg anti-human CXCR3 chimeric antibodies or control human IgG1 (Herceptin) twice a week for the entire study starting on day 3 post cell transfer. Blood taken on day 14 post initiation was processed for flow cytometry and stained using standard procedures with antibodies to human CD45 (hCD45), human CD3 (hCD3), human CD4 (hCD4), human CD8 (hCD8), and human CXCR3. Cells in the lymphocyte gate were gated on hCD45+ cells followed by gating on hCD3+ cells. hCD4 and hCD8 expression on hCD45+hCD3+ T cells were evaluated and the percentage of human CD4+CD45+CD3+ T cells and human CD8+CD45+CD3+ T cells was determined. The percentage of CXCR3 expressing human CD4+ T cells and CD8+ T cells was determined. Each dot in Fig. 22 represents a single mouse, with representative data are shown from three experiments. The data show that anti-CXCR3 antibody treatment in the NSG-PBL mouse model of xenogeneic GvHD (graft vs. host disease) resulted

in modulation of CXCR3-expressing T cells, but reveals functional differences between the five clones.

[0211] Table 12 shows the median survival of NSG-PBL mice with xenogeneic GvHD after treatment with chimeric anti-human CXCR3 candidate antibodies.

Treatment	Median survival (days)
PBS	31
hulgG1	33.5
Clone 4 chimeric	43**
Clone 12 chimeric	41
Clone 53 chimeric	44*
Clone 82 chimeric	36.5
Clone 135 chimeric	45***

Table 13

[0212] *, $p = 0.043$; ** $p = 0.016$; *** $p = 0.010$ anti-CXCR3 antibody treatment versus hulgG1 treatment using Log Rank test.

[0213] The preceding examples are intended to illustrate and in no way limit the present disclosure. Other embodiments of the disclosed devices and methods will be apparent to those skilled in the art from consideration of the specification and practice of the devices and methods disclosed herein.

WHAT IS CLAIMED IS:

1. An antibody or antigen binding fragment capable of binding to CXCR3, wherein the antibody or fragment comprises a heavy chain variable region (VH) and a light chain variable region (VL), and wherein:
 - a) the heavy chain variable region comprises three complementarity determining regions (CDRs) that are at least about 95% identical to the three CDRs from any one of SEQ ID NO: 2, 4, 6, and 8; and/or the light chain variable region comprises three CDRs that are at least about 95% identical to the three CDRs from any one of SEQ ID NO: 3, 5, 7, and 9,
 - b) the heavy chain variable region comprises three CDRs that are at least about 95% identical to the three CDRs from any one of SEQ ID NO: 10, 12, 14, and 16; and/or the light chain variable region comprises three CDRs that are at least about 95% identical to the three CDRs from any one of SEQ ID NO: 11, 13, 15, and 17,
 - c) the heavy chain variable region comprises three CDRs that are at least about 95% identical to the three CDRs from any one of SEQ ID NO: 18, 20, 22, 24, and 26-33; and/or the light chain variable region comprises three CDRs that are at least about 95% identical to the three CDRs from any one of SEQ ID NO: 19, 21, 23, 25, and 34-37,
 - d) the heavy chain variable region comprises three CDRs that are at least about 95% identical to the three CDRs from any one of SEQ ID NO:

38, 40, 42, 44, 46-48, and 63-66; and/or the light chain variable region comprises three CDRs that are at least about 95% identical to the three CDRs from any one of SEQ ID NO: 39, 41, 43, 45, 49-54, and 67-70, or

e) the heavy chain variable region comprises three CDRs that are at least about 95% identical to the three CDRs from any one of SEQ ID NO: 55, 57, 59, and 61; and/or the light chain variable region comprises three CDRs that are at least about 95% identical to the three CDRs from any one of SEQ ID NO: 56, 58, 60, and 62.

2. The antibody or fragment of claim 1, wherein the antibody or fragment is chimeric, CDR grafted, mutated, mutated to remove one or more deamidation site, human, humanized, humanized and back-mutated, synthetic, or recombinant.
3. The antibody or fragment of claim 1 or 2, wherein the antibody or fragment is a Fab, Fab', a F(ab')₂, an Fv, a single-chain Fv (scFv), a diabody (Fd), a linear antibody, a nanobody (VHH), a bi-specific antibody, or a multi-specific antibody.
4. The antibody or fragment of any one of claims 1-3, wherein the antibody or fragment is capable of binding to a peptide selected from
 - a) a peptide comprising residues 1-58 of SEQ ID NO:1;
 - b) a peptide comprising residues 1-16 of SEQ ID NO:1; and/or
 - c) a peptide comprising residues 1-37 of SEQ ID NO:1.

5. The antibody or fragment of any one of claims 1-4, wherein the antibody or fragment is capable of binding to a peptide selected from
- a) a peptide comprising the amino acid sequence SDHQVLNDAE;
 - b) a peptide comprising the amino acid sequence SDHQVLND;
 - c) a peptide comprising the amino acid sequence DHQVLND;
 - d) a peptide comprising the amino acid sequence VLNDAE;
 - e) a peptide comprising the amino acid sequence VLND;
 - f) a peptide comprising the amino acid sequence XDXXVXNDXX;
 - g) a peptide comprising the amino acid sequence XDXXVXND;
 - h) a peptide comprising the amino acid sequence DXXVXND;
 - i) a peptide comprising the amino acid sequence VXNDXX;
- and/or
- j) a peptide comprising the amino acid sequence VXND,
- wherein X indicates any amino acid.
6. The antibody or fragment of any one of claims 1-5, wherein:
- a) the heavy chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 2, 4, 6, and 8;
 - b) the heavy chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 10, 12, 14, and 16;
 - c) the heavy chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 18, 20, 22, 24, and 26-33;

- d) the heavy chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 38, 40, 42, 44, 46-48, and 63-66;
 - or
 - e) the heavy chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 55, 57, 59, and 61.
7. The antibody or fragment of any one of claims 1-6, wherein:
- a) the light chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 3, 5, 7, and 9,
 - b) the light chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 11, 13, 15, and 17,
 - c) the light chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 19, 21, 23, 25, and 34-37,
 - d) the light chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 39, 41, 43, 45, 49-54, and 67-70,
 - or
 - e) the light chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 56, 58, 60, and 62.

8. The antibody or fragment of any one of claims 1-7, wherein:
- a) the heavy chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 2, 4, 6, and 8; and the light chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 3, 5, 7, and 9,
 - b) the heavy chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 10, 12, 14, and 16; and the light chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 11, 13, 15, and 17,
 - c) the heavy chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 18, 20, 22, 24, and 26-33; and the light chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 19, 21, 23, 25, and 34-37,
 - d) the heavy chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 38, 40, 42, 44, 46-48, and 63-66; and the light chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 39, 41, 43, 45, 49-54, and 67-70,
- or
- e) the heavy chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 55, 57, 59, and

61; and the light chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 56, 58, 60, and 62.

9. The antibody or fragment of any one of claims 1-8, wherein:
- a) the heavy chain variable region comprises a sequence identical or comprising 1, 2, or 3 amino acid residue mutations relative to the three CDRs in any one of SEQ ID NO: 2, 4, 6, and 8;
 - b) the heavy chain variable region comprises a sequence identical or comprising 1, 2, or 3 amino acid residue mutations relative to the three CDRs in any one of SEQ ID NO: 10, 12, 14, and 16;
 - c) the heavy chain variable region comprises a sequence identical or comprising 1, 2, or 3 amino acid residue mutations relative to the three CDRs in any one of SEQ ID NO: 18, 20, 22, 24, and 26-33;
 - d) the heavy chain variable region comprises a sequence identical or comprising 1, 2, or 3 amino acid residue mutations relative to the three CDRs in any one of SEQ ID NO: 38, 40, 42, 44, 46-48, and 63-66;
- or
- e) the heavy chain variable region comprises a sequence identical or comprising 1, 2, or 3 amino acid residue mutations relative to the three CDRs in any one of SEQ ID NO: 55, 57, 59, and 61.

10. The antibody or fragment of any one of claims 1-9, wherein:
- a) the light chain variable region comprises a sequence identical or comprising 1, 2, or 3 amino acid residue mutations relative to the three CDRs in any one of SEQ ID NO: 3, 5, 7, and 9,
 - b) the light chain variable region comprises a sequence identical or comprising 1, 2, or 3 amino acid residue mutations relative to the three CDRs in any one of SEQ ID NO: 11, 13, 15, and 17,
 - c) the light chain variable region comprises a sequence identical or comprising 1, 2, or 3 amino acid residue mutations relative to the three CDRs in any one of SEQ ID NO: 19, 21, 23, 25, and 34-37,
 - d) the light chain variable region comprises a sequence identical or comprising 1, 2, or 3 amino acid residue mutations relative to the three CDRs in any one of SEQ ID NO: 39, 41, 43, 45, 49-54, and 67-70,
- or
- e) the light chain variable region comprises a sequence identical or comprising 1, 2, or 3 amino acid residue mutations relative to the three CDRs in any one of SEQ ID NO: 56, 58, 60, and 62.
11. The antibody or fragment of any one of claims 1-10, wherein:
- a) the heavy chain variable region comprises a sequence identical or comprising 1, 2, or 3 amino acid residue mutations relative to the three CDRs in any one of SEQ ID NO: 2, 4, 6, and 8; and/or the light chain variable region comprises a sequence identical or comprising 1, 2, or 3

amino acid residue mutations relative to the three CDRs in any one of SEQ ID NO: 3, 5, 7, and 9,

b) the heavy chain variable region comprises a sequence identical or comprising 1, 2, or 3 amino acid residue mutations relative to the three CDRs in any one of SEQ ID NO: 10, 12, 14, and 16; and/or the light chain variable region comprises a sequence identical or comprising 1, 2, or 3 amino acid residue mutations relative to the three CDRs in any one of SEQ ID NO: 11, 13, 15, and 17,

c) the heavy chain variable region comprises a sequence identical or comprising 1, 2, or 3 amino acid residue mutations relative to the three CDRs in any one of SEQ ID NO: 18, 20, 22, 24, and 26-33; and/or the light chain variable region comprises a sequence identical or comprising 1, 2, or 3 amino acid residue mutations relative to the three CDRs in any one of SEQ ID NO: 19, 21, 23, 25, and 34-37,

d) the heavy chain variable region comprises a sequence identical or comprising 1, 2, or 3 amino acid residue mutations relative to the three CDRs in any one of SEQ ID NO: 38, 40, 42, 44, 46-48, and 63-66; and/or the light chain variable region comprises a sequence identical or comprising 1, 2, or 3 amino acid residue mutations relative to the three CDRs in any one of SEQ ID NO: 39, 41, 43, 45, 49-54, and 67-70,

or

e) the heavy chain variable region comprises a sequence identical or comprising 1, 2, or 3 amino acid residue mutations relative to the three

CDRs in any one of SEQ ID NO: 55, 57, 59, and 61; and/or the light chain variable region comprises a sequence identical or comprising 1, 2, or 3 amino acid residue mutations relative to the three CDRs in any one of SEQ ID NO: 56, 58, 60, and 62.

12. An antibody or antigen binding fragment capable of binding to CXCR3, wherein the antibody or fragment comprises a heavy chain variable region (VH) and a light chain variable region (VL), and wherein:
 - a) the heavy chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 2, 4, 6, and 8; and/or the light chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 3, 5, 7, and 9,
 - b) the heavy chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 10, 12, 14, and 16; and/or the light chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 11, 13, 15, and 17,
 - c) the heavy chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 18, 20, 22, 24, and 26-33; and/or the light chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 19, 21, 23, 25, and 34-37,
 - d) the heavy chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 38, 40, 42, 44,

46-48, and 63-66; and/or the light chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 39, 41, 43, 45, 49-54, and 67-70,

or

e) the heavy chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 55, 57, 59, and 61; and/or the light chain variable region comprises a sequence at least about 85%, 90%, 95%, or 99% identical to any one of SEQ ID NO: 56, 58, 60, and 62.

13. The antibody or fragment of claim 12, wherein:

a) the heavy chain variable region comprises a sequence identical or comprising 1-10 amino acid residue mutations relative to any one of SEQ ID NO: 2, 4, 6, and 8; and/or the light chain variable region comprises a sequence identical or comprising 1-10 amino acid residue mutations relative to any one of SEQ ID NO: 3, 5, 7, and 9,

b) the heavy chain variable region comprises a sequence identical or comprising 1-10 amino acid residue mutations relative to any one of SEQ ID NO: 10, 12, 14, and 16; and/or the light chain variable region comprises a sequence identical or comprising 1-10 amino acid residue mutations relative to any one of SEQ ID NO: 11, 13, 15, and 17,

c) the heavy chain variable region comprises a sequence identical or comprising 1-10 amino acid residue mutations relative to any one of SEQ ID NO: 18, 20, 22, 24, and 26-33; and/or the light chain variable region

comprises a sequence identical or comprising 1-10 amino acid residue mutations relative to any one of SEQ ID NO: 19, 21, 23, 25, and 34-37,
d) the heavy chain variable region comprises a sequence identical or comprising 1-10 amino acid residue mutations relative to any one of SEQ ID NO: 38, 40, 42, 44, 46-48, and 63-66; and/or the light chain variable region comprises a sequence identical or comprising 1-10 amino acid residue mutations relative to any one of SEQ ID NO: 39, 41, 43, 45, 49-54, and 67-70,

or

e) the heavy chain variable region comprises a sequence identical or comprising 1-10 amino acid residue mutations relative to any one of SEQ ID NO: 55, 57, 59, and 61; and/or the light chain variable region comprises a sequence identical or comprising 1-10 amino acid residue mutations relative to any one of SEQ ID NO: 56, 58, 60, and 62.

14. The antibody or fragment of claim 12 or 13, wherein:

a) the heavy chain variable region comprises three CDRs that are at least about 95% identical to the three CDRs from any one of SEQ ID NO: 2, 4, 6, and 8; and/or the light chain variable region comprises three CDRs that are at least about 95% identical to the three CDRs from any one of SEQ ID NO: 3, 5, 7, and 9,

b) the heavy chain variable region comprises three CDRs that are at least about 95% identical to the three CDRs from any one of SEQ ID NO: 10, 12, 14, and 16; and/or the light chain variable region comprises three

CDRs that are at least about 95% identical to the three CDRs from any one of SEQ ID NO: 11, 13, 15, and 17,

c) the heavy chain variable region comprises three CDRs that are at least about 95% identical to the three CDRs from any one of SEQ ID NO: 18, 20, 22, 24, and 26-33; and/or the light chain variable region comprises three CDRs that are at least about 95% identical to the three CDRs from any one of SEQ ID NO: 19, 21, 23, 25, and 34-37,

d) the heavy chain variable region comprises three CDRs that are at least about 95% identical to the three CDRs from any one of SEQ ID NO: 38, 40, 42, 44, 46-48, and 63-66; and/or the light chain variable region comprises three CDRs that are at least about 95% identical to the three CDRs from any one of SEQ ID NO: 39, 41, 43, 45, 49-54, and 67-70,
or

e) the heavy chain variable region comprises three CDRs that are at least about 95% identical to the three CDRs from any one of SEQ ID NO: 55, 57, 59, and 61; and/or the light chain variable region comprises three CDRs that are at least about 95% identical to the three CDRs from any one of SEQ ID NO: 56, 58, 60, and 62.

15. The antibody or fragment of any one of claims 1-14, wherein the antibody or fragment comprises any of the combinations of heavy chain and light chain variable regions in Table 2.

16. The antibody or fragment of any one of claims 1-15, wherein the antibody is capable of binding to CXCR3 with an affinity constant of at least about 10^8 M^{-1} .
17. The antibody or fragment of any one of claims 1-16, wherein the antibody or fragment is capable of binding to both the A and B isoforms of CXCR3.
18. The antibody or fragment of any one of claims 1-17, wherein the antibody or fragment is capable of preferentially binding to the A-isoform of CXCR3.
19. The antibody or fragment of any one of claims 1-18, wherein the antibody or fragment is capable of neutralizing CXCR3 activity.
20. An isolated nucleic acid encoding the amino acid sequence of the antibody or fragment of any one of claims 1-19.
21. A vector comprising the isolated nucleic acid of claim 20.
22. An isolated host cell comprising the vector of claim 21.
23. A method of producing the antibody or fragment of any one of claims 1-19, comprising culturing the host cell of claim 19 in culture medium under conditions suitable to produce the antibody or fragment.
24. A kit comprising the antibody or fragment of any one of claims 1-19 or the nucleic acid of claim 20 and instructions for using the antibody or fragment for research, diagnostic, or therapeutic purposes
25. A method of preventing, treating or reducing the progression of new onset type 1 diabetes (T1D) comprising:
 - a) identifying a subject at risk for developing T1D or who has new onset T1D; and

- b) administering an effective amount of the antibody or fragment of any one of claims 1-19 to the subject, thereby prophylactically preventing the development of T1D, or treating or reducing the progression of new onset T1D.
26. The method of claim 25, wherein the subject is a mammal.
27. The method of claim 25 or 26, wherein the subject is human.
28. The method of any one of claims 25-27, wherein the subject has a basal serum C-peptide level of greater than or equal to about 0.2 nmol/L, and/or wherein the patient has a fasting integrated serum C-peptide level during C-peptide stimulation of between about 0.033 and 1.0 nmol/L x min.
29. The method of any one of claims 25-28, wherein the patient has a fasting blood glucose level of greater than about 120 mg/dL in the absence of exogenous insulin.
30. The method of any one of claims 25-29, wherein the antibody or fragment thereof is humanized.
31. The method of any one of claims 25-30, wherein the antibody or fragment thereof is administered at a dose of about 0.03-3.7 mg/kg/dose.
32. The method of any one of claims 25-31, wherein the antibody or fragment thereof is administered at least daily, weekly, biweekly, monthly, bimonthly, quarterly, or yearly.
33. The method of any one of claims 25-32, wherein the antibody or fragment thereof is administered at a total dose over all administrations of about 0.16-18 mg/kg.

34. The method of any one of claims 25-33, wherein the antibody or fragment thereof is administered intravenously, intraperitoneally, nasally, ocularly, orally, parenterally, subcutaneously, or transdermally.
35. The method of any one of claims 25-34, wherein the antibody or fragment thereof is administered directly to the pancreas.
36. The method of claim 35, wherein the antibody or fragment thereof is administered proximate to islet cells in the pancreas.
37. The method of any one of claims 25-36, wherein the method further comprises administering a β -cell stimulating agent, insulin, or an insulin-producing cell.
38. The method of any one of claims 25-37, wherein the method further comprises administering an immunosuppressant.
39. A method of treating or reducing the progression of new onset type 1 diabetes (T1D) comprising:
- a) identifying a human subject having new onset T1D and having a basal serum C-peptide level of greater than or equal to about 0.2nmol/L, and/or having a fasting integrated serum C-peptide level during C-peptide stimulation of between about 0.033 and 1.0 nmol/L x min; and
 - b) administering about 0.03-3.7 mg/kg/dose of an antibody or fragment of any one of claims 1-19,
- thereby treating or reducing the progression of new onset T1D.
40. A CXCR3 neutralizing antibody or fragment thereof for preventing, treating or reducing the progression of new onset type 1 diabetes (T1D) in a

subject, wherein the antibody or fragment thereof comprises the antibody or fragment of any one of claims 1-19.

41. Use of a CXCR3 antibody or fragment thereof selected from the antibodies or fragments of any one of claims 1-19 in the preparation of a medicament for treating a disease or disorder.
42. The use of claim 41, wherein the disease or disorder is a disease or disorder associated with CXCR3.
43. The use of claim 41 or 42, wherein the disease or disorder is new onset type 1 diabetes (T1D).
44. A conjugate comprising the antibody or fragment of any one of claims 1-19 and at least one additional agent.
45. The conjugate of claim 44, wherein the additional agent is a therapeutic agent, a solubilizing agent, a stabilizing agent, an immunosuppressant, a receptor, or an antigen binding peptide.
46. A pharmaceutical composition comprising the antibody or fragment of any one of claims 1-19, or the nucleic acid of claim 20, and a pharmaceutically acceptable carrier.
47. The pharmaceutical composition of claim 46, wherein the composition further comprises at least one additional therapeutic agent.
48. The pharmaceutical composition of claim 47, wherein the at least one additional therapeutic agent comprises an agent for treating a disease or disorder associated with CXCR3.

49. The pharmaceutical composition of claim 47 or 48, wherein the at least one additional therapeutic agent comprises a β -cell stimulating agent, insulin, or an insulin-producing cell.
50. An in vitro method of detecting the presence or concentration of CXCR3 in a test sample, comprising contacting the test sample with the antibody or fragment of any one of claims 1-19 and a detectable label, wherein the presence or concentration of CXCR3 is directly or indirectly correlated with a signal generated by the detectable label.
51. The method of claim 50, wherein the method is used to diagnose a condition associated with CXCR3.
52. The method of claim 50, wherein the condition is T1D.