specific targeting. There is a need to identify and characterize therapeutic antibodies useful in the treatment of inflammatory disorders, including those that are described herein.

Summary of the Invention

In ~~a first~~one aspect, the invention features an anti-~~FKN~~fractalkine antibody or ~~FKN~~fractalkine-binding fragment thereof, wherein the antibody or fragment thereof ~~includes six CDRs selected from:~~comprises:

(a) a CDR-H1 ~~that includes~~consisting of the amino acid sequence of SEQ ID NO: 28;

(b) a CDR-H2 ~~that includes~~consisting of the amino acid sequence of SEQ ID NO: 29;

(c) a CDR-H3 ~~that includes~~consisting of the amino acid sequence of SEQ ID NO: 30;

(d) a CDR-L1 ~~that includes~~consisting of the amino acid sequence of SEQ ID NO: 31;

(e) a CDR-L2 ~~that includes~~consisting of the amino acid sequence of SEQ ID NO: 32; and

(f) a CDR-L3 ~~that includes~~consisting of the amino acid sequence of SEQ ID NO: 33.

The antibody may be an intact antibody. In one example, the antibody is a humanized antibody. The heavy chain variable domain of the humanized antibody may include the amino acid sequence of SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 42, or SEQ ID NO: 43, and the light chain variable domain of the humanized antibody may include the amino acid sequence of SEQ ID NO: 38, SEQ ID NO: 44, or SEQ ID NO: 45.

In one ~~example~~aspect, ~~the antibody is~~there is provided an anti-fractalkine antibody or ~~FKN~~fractalkine-binding fragment thereof, wherein the antibody or fragment thereof ~~includes~~comprises a heavy chain ~~with~~comprising the amino acid sequence of SEQ ID NO: 37 and a light chain ~~with~~comprising the amino acid sequence of SEQ ID NO: 44.

The antibody may also be a chimeric antibody. In one example, the heavy chain variable domain of the chimeric antibody includes the amino acid sequence of SEQ ID NO: 26, and the light chain variable domain of the chimeric antibody includes the amino acid sequence of SEQ ID NO: 27.

~~FKN~~fractalkine-binding fragments of an antibody are also contemplated. The ~~FKN~~fractalkine-binding fragment may be a Fab, Fab', F(ab')2, or Fv fragment that retains the binding specificity to FKN.

In certain examples, when the antibody or ~~FKN~~fractalkine-binding fragment thereof includes a human constant region, the constant region is of the IgG isotype (e.g., the IgG2 isotype). In other examples, the antibody or ~~FKN~~fractalkine-binding fragment includes a mutated Fc region such that the antibody has reduced ADCC and/or complement activation relative to the Fc region lacking the mutation. For example, the Fc region may be mutated at one or more of amino acid residues V234, G237, C131, or C219.

Desirably, the antibody or ~~FKN~~fractalkine-binding fragment substantially reduces or inhibits binding of ~~FKN~~fractalkine to its receptor, CX3CR1, by at least 50%, 60%, 70%, 80%, 90%, or greater, or substantially inhibits neutralized hFKN in a chemotaxis assay such as the one described herein.

In one aspect, there is provided an anti-fractalkine antibody or fractalkine-binding fragment thereof, wherein the antibody or fragment thereof comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 37 and a light chain comprising the amino acid sequence of SEQ ID NO: 44.

In one aspect, there is provided a pharmaceutical composition comprising the antibody or fractalkine-binding fragment thereof as defined above.

~~The invention also features~~In one example, there is provided a pharmaceutical composition that includes the antibody or ~~FKN~~fractalkine-binding fragment of the present invention and a pharmaceutically acceptable carrier or excipient. In certain ~~embodiments~~examples, the composition includes one or more additional therapeutic agents, such as those described below.

~~Also contemplated is~~In one aspect, there is provided a nucleic acid encoding ~~and~~the antibody or ~~FKN~~fractalkine-binding fragment ~~described above~~thereof of the present invention. In one ~~embodiment~~example, the nucleic acid encodes all or a portion of the heavy chain of the antibody or ~~FKN~~fractalkine-binding fragment thereof. In another ~~embodiment~~example, the nucleic acid encodes all or a portion of the light chain of the antibody or ~~FKN~~fractalkine-binding fragment thereof. ~~The nucleic acid may be in a vector (e.g.,~~In one aspect, there is provided a vector comprising the nucleic acid of the present invention. An example is an expression vector~~)~~.

~~The invention also features~~In one aspect, there is provided a host cell ~~that includes~~comprising one or more vectors of the invention. In one example, the host cell includes two vectors, the first vector including a nucleic acid encoding a heavy chain and the second vector including a nucleic acid encoding a light chain of an antibody or ~~FKN~~fractalkine-binding fragment described herein. The expression of the heavy and light chain in the host cell produces an antibody or ~~FKN~~fractalkine-binding fragment. In one ~~embodiment~~example, the host cell is prokaryotic. In another

~~embodiment~~example, the host cell is eukaryotic. Exemplary mammalian cells useful for producing antibodies and ~~FKN~~fractalkine-binding fragments are CHO cells and NS0 cells.

~~The invention also features~~There is also provided a method for making an anti-~~FKN~~fractalkine antibody or ~~FKN~~fractalkine-binding fragment thereof~~.~~, ~~The~~said method ~~includes~~comprising (a) expressing a vector of the invention in a suitable host cell, and (b) recovering the antibody or fractalkine binding fragment thereof. The antibody or ~~FKN~~fractalkine-binding fragment may be secreted by the host cell into culture media. In one example, the antibody or ~~FKN~~fractalkine-binding fragment thereof is purified to remove at least 95% or greater purity of the non-antibody marterials.

In one aspect, there is provided the antibody or fractalkine-binding fragment thereof as defined above, for use in treating an inflammatory disorder, wherein the inflammatory disorder is ulcerative colitis, Crohn's disease, or rheumatoid arthritis.

In another aspect, there is provided the pharmaceutical composition of the present invention, for use in treating an inflammatory disorder, wherein the inflammatory disorder is ulcerative colitis, Crohn's disease, or rheumatoid arthritis.

In another aspect, there is provided the use of an antibody or fractalkine-binding fragment thereof as defined above in the preparation of a medicament for use in treating an inflammatory disorder, wherein the inflammatory disorder is ulcerative colitis, Crohn's disease, or rheumatoid arthritis.

Methods for treating an inflammatory disorder by administering an effective amount of an anti-~~FKN~~fractalkine antibody or ~~FKN~~fractalkine-binding fragment in accordance with the ~~invention~~disclosure are also contemplated. Exemplary inflammatory disorders are inflammatory bowel disease, Crohn's disease, and ulcerative colitis. For these disorders, the method may further include administering one or more additional therapeutic agents. Exemplary therapeutic agents are opiates, 5-aminosalicylic acid, 6-mercaptopurine, azathioprine, glucocorticoids, methotrexate, cyclosporine, and metronidazole. Other suitable therapeutic agents are described below.

Inflammatory disorders that may be treated by administering an effective amount of an anti-~~FKN~~fractalkine antibody or ~~FKN~~fractalkine-binding fragment in accordance with the ~~invention~~disclosure also include autoimmune hepatobiliary diseases (e.g., autoimmune hepatitis, primary biliary cirrhosis, or primary sclerosing cholangitis). For these disorders, the method may further include administering 6-mercaptopurine, ursodeoxycholic acid, azathioprine, a glucocorticoid, a thiazide diuretic, an anti-aldosterone diuretic, cyclosporine, albumin, or spironolactone.

Rheumatoid arthritis (RA) may also be treated by administering an effective amount of an anti-~~FKN~~fractalkine antibody or ~~FKN~~fractalkine-binding fragment in accordance with the ~~invention~~disclosure. In the treatment of RA, the method may further include administering a non-steroidal anti-inflammatory drug (NSAID), methotrexate, leflunomide, bucillamine, 5-aminosalicylic acid, a glucocorticoid, hydrochloroquine, vitamin D, calcium, or alendronate.

Systemic lupus erythematosus (e.g., lupus of the central nervous system or lupus nephritis) may also be treated by administering an effective amount of an anti-~~FKN~~fractalkine antibody or ~~FKN~~fractalkine-binding fragment, either alone or in combination with one or more additional therapeutic agents. Exemplary additional therapeutic agents are glucocorticoids, cyclophosphamide, methotrexate, cyclosporine, and tacrolimus.

Multiple sclerosis and neuromyelitis optica may also be treated by administering an effective amount of an anti-~~FKN~~fractalkine antibody or ~~FKN~~fractalkine-binding fragment. Treatment may further include administering one or more additional therapeutic agents, such as a glucocorticoid, interferon-β, or Copaxone®.

Demyelinating polyradiculopathy (e.g., Guillain-Barré syndrome or chronic inflammatory demyelinating polyradiculopathy) may also be treated by administering an effective amount of an anti-~~FKN~~fractalkine antibody or ~~FKN~~fractalkine-binding fragment. Again, cotherapy is also contemplated, and treatment may further include administering one or more additional therapeutic agents, such as a glucocorticoid or an intravenous immunoglobulin.

Administering an effective amount of an anti-~~FKN~~fractalkine antibody or ~~FKN~~fractalkine-binding fragment may also be useful for the treatment of neuropathic pain, either alone or in combination with an additional therapeutic agent. Exemplary additional agents for the treatment of neuropathic pain are lamotrigine, topiramate, oxcarbazepine, levetiracetam, fentanyl, tramadol, capsaicin, cloridine, an NSAID, amitriptyline, pregabalin, lidocaine, duloxetine, and carbamazepine.

Alzheimer's disease may also be treated by administering an effective amount of an anti-~~FKN~~fractalkine antibody or ~~FKN~~fractalkine-binding fragment. The method may further include administering one or more additional therapeutic agents, such as tacrine hydrochloride, donepezil hydrochloride, rivastigmine tartrate, galantamine hydrobromide, memantine hydrochloride, paroxetine, risperidone, quetiapine, or perospirone.

Visceral pain associated with cancer may also be treated by administering an effective amount of an anti-~~FKN~~fractalkine antibody or ~~FKN~~fractalkine-binding fragment, either alone or in combination with one or more additional therapeutic agents, such as morphine, an NSAID, phentanyl, lidocaine, pentazocine, or clonidine.

Atherosclerosis may also be treated by administering an effective amount of an anti-~~FKN~~fractalkine antibody or ~~FKN~~fractalkine-binding fragment. The method may further include administering one or more additional therapeutic agents, such as prostacyclin, aspirin, clopidogrel, ticlopidine, limaprost, prostaglandin E1, an HMG CoA reductase inhibitor, bezafibrate, lidocaine, mexiletine, a diuretic, digitalis, dopamine, a β-adrenergic receptor agonist, isosorbide dinitrate, nitroglycerin, a natriuretic peptide, warfarin, heparin, tissue plasminogen activator, urokinase, or procainamide.

Vasculopathies (e.g., age-related macular degeneration, Behcet's disease, Harada's disease, and sarcoidosis-origined uveitis) may also be treated by administering an effective amount of an anti-~~FKN~~fractalkine antibody or ~~FKN~~fractalkine-binding fragment in accordance with the ~~invention~~disclosure. The method may further include administering one or more additional therapeutic agents selected from glucocorticoids, cyclophosphamide, pegaptanib, ranibizumab, NSAIDs, colchicine, chlorambucil, thalidomide, and verteporfin.

Nephropathies (e.g., lupus nephritis, glomerulonephritis, or diabetic nephropathy) may also be treated by administering an effective amount of an anti-~~FKN~~fractalkine antibody or ~~FKN~~fractalkine-binding fragment in accordance with the ~~invention~~disclosure. Glucocorticoids, sulfonylurea, cyclophosphamide, glinides, cyclosporine, tacrolimus, mycophenolate mofetil, mizoribine, diuretics, insulin, biguanide, α-glucosidase inhibitors, angiotensin receptor blockers, thiazolidinedione, angiotensin converting enzyme (ACE) inhibitors, calcium channel blockers, or β-adrenergic receptor inhibitors may be included in the treatment methods. adrenergic receptor inhibitors may be included in the treatment methods.

The ~~invention~~disclosure also relates to the use of an anti-~~FKN~~fractalkine antibody or ~~FKN~~fractalkine-binding fragment in accordance with the invention in the treatment of any of the inflammatory disorders described herein.

The ~~invention~~disclosure also relates to the use of an anti-~~FKN~~fractalkine antibody or ~~FKN~~fractalkine-binding fragment in accordance with the ~~invention~~disclosure in the preparation of a medicament for the treatment of any of the inflammatory disorders described herein.

The ~~invention~~disclosure also features a method of inhibiting the recruitment of leukocytes to an inflammation site in a subject by administering an effective amount of an anti-~~FKN~~fractalkine

antibody or ~~FKN~~fractalkine-binding fragment to a subject in need of such treatment, whereby the recruitment of leukocytes to an inflammation site is inhibited.

An *ex vivo* strategy can also be used for therapeutic applications. *Ex vivo* strategies involve transfecting or transducing cells obtained from the subject with a polynucleotide encoding an antibody or antibody fragment. The transfected or transduced cells are then returned to the subject. The cells can be any of a wide range of types including, without limitation, hemopoietic cells (e.g., bone marrow cells, macrophages, monocytes, dendritic cells, T cells, or B cells), fibroblasts, epithelial cells, endothelial cells, keratinocytes, or muscle cells.

For the purpose of the present ~~invention~~disclosure, the following abbreviations and terms are defined below.

The term "antibody" (used herein interchangeably with "immunoglobulin") includes intact monoclonal and polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments that exhibit the desired biological activity (e.g., the ability to bind FKN and modulate the interaction between FKN and CX3CR1). "Intact antibodies" are heterotetrameric glycoproteins of about 150,000 Daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (VH) followed by a number of constant domains. Each light chain has a variable domain at one end (VL) and a constant domain at its other end. The constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains.

There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. An antibody may be part of a larger fusion molecule, formed