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(54) Title: NEUTRALIZING ANTI-CCL20 ANTIBODIES

(57) Abstract: The present invention relates to novel humanized, chimeric and murine antibodies that have binding specificity for the human CC chemokine ligand 20 (CCL20). The present invention further relates to heavy chains and light chains of said antibodies. The invention also relates to isolated nucleic acids, recombinant vectors and host cells that comprise a sequence which encodes a heavy chain and/or a light chain of said antibodies, and to a method of preparing said antibodies. The anti-CCL20 antibodies of the invention can be used in therapeutic applications to treat, for example, inflammatory and autoimmune disorders and cancer.

Neutralizing Anti-CCL20 Antibodies

[0001] This application claims priority from U.S. Provisional Patent Application No. 61/415,614, filed November 19, 2010. The disclosure of that application is incorporated by reference herein in its entirety.

Field of the Invention

[0002] The present invention relates to novel humanized, chimeric and murine antibodies specifically directed against the human CC chemokine ligand 20 (CCL20). The humanized antibodies of the invention are particularly well-suited as therapeutics for the treatment of inflammatory and autoimmune diseases.

Background of the Invention

[0003] The immune system is a highly sophisticated bio-circuit used by the body to discriminate non-self (*e.g.*, foreign organisms or substances) from self. The detection of non-self in the body can result in inflammation, in which various cellular and molecular components are orchestrated to respond to potentially harmful events caused by the non-self organism or substance. Although the inflammatory process helps to protect the body from foreign attack, de-regulation of the immune system can lead to negative consequences such as self attack, *e.g.*, autoimmune disease. By altering the function of inflammatory molecules such as chemokines, it may be possible to reduce the initiation and progression of disorders relating to immune/inflammatory responses.

[0004] Chemokines are a family of small (8-10 kDa) proteins that play a pivotal role in inflammation. During the inflammatory process, chemokines are produced locally at the site of the noxious stimulus and work as central players to recruit immune cells that express their cognate receptors, seven trans-membrane G protein—coupled receptors (GPCRs). CCL20, alternatively named liver and activation-regulated chemokine (LARC), macrophage inflammatory protein-3 alpha

(MIP-3α), or Exodus-1, is a soluble chemokine that is expressed by epithelial cells. Epithelial keratinocytes and synovium-lining cells are known to produce large amounts of CCL20 during homeostatic as well as inflammatory and pathological conditions such as cancer, psoriasis, and rheumatoid arthritis. The cognate receptor for CCL20 is CC chemokine receptor 6 (CCR6); CCL20 is the only chemokine known to interact with CCR6. In response to the CCL20 signal, immune cells possessing CCR6, such as immature dendritic cells (DC), effector/memory T-cells, and B-cells, migrate and infiltrate the surrounding tissues, thus activating the inflammatory cascade.

[0005] Because CCL20 expression is significantly enhanced in inflammation induced by inflammatory cytokines such as interleukin 1β (IL-1β) and tumor necrosis factor α (TNF-α), the CCL20-CCR6 interaction is thought to play a role in pathological inflammatory processes.

[0006] Rheumatoid Arthritis (RA) is one of the most common autoimmune diseases. The first sign of RA is often synovitis, which manifests as a swollen, painful joint. Although the specific factors that initiate synovitis remain unknown, synovium lining epithelial cells and synovial fibroblasts are thought to be primary inducers of the inflammatory reaction. Synovial fluid from RA patients effectively chemo-attracts human monocytes and pro-inflammatory T helper 17 (Th17) cells, which then induce and exacerbate the RA inflammatory process. Because reactive synovial cells are capable of producing large amounts of CCL20 (particularly under the influence of IL-1β and TNF-α), while CCR6 is the major receptor of Th17 cells, the CCL20-CCR6 interaction is thought to play a key role in the inflammatory process.

[0007] The CCL20-CCR6 interaction may also play an important role in certain types of dermatitis. Psoriasis, for example, initiates with a noxious psoriatic event in the skin (induced by environmental and/or genetic factors) followed by infiltration of Th17 cells. Because CCR6 is expressed on the surface of Th17 cells, B cells, dendritic cells, and tissue damaging effector T cells, CCL20 may represent the main chemoattractant for these cell types in psoriasis. Further evidence for the importance of the CCL20-CCR6 interaction can be found in studies using an interleukin 23 (IL-23)-induced mouse model of psoriasis (Hedrick *et al., J. Clin. Invest.* 119:2317-2329 (2009)). In this model, injection of IL-23 causes interleukin 22 (IL-22)-dependent psoriatic inflammation. However, *Ccr6*$^{-/-}$ mice did not exhibit psoriasis-like symptoms when injected with IL-23, indicating that CCR6 is required for the development of psoriasis.

[0008] Human keratinocytes can produce large amounts of CCL20, especially under the influence of the Th17-derived cytokines interleukin 17 (IL-17), IL-22, and TNF-α. While CCL20 and CCR6 are rarely detected in normal skin, both exhibit increased expression levels in atopic dermatitis and pustular psoriasis. Strong induction of CCL20 and accumulation of CCR6+ cells can be observed in microscopic immunohistochemical analysis of human dermatitis lesions. These observations provide additional evidence for the role of CCL20 and CCR6 in the dermatitis inflammatory process.

[0009] Currently available MAb biologics for treating immune disorders can be roughly classified into three groups: inhibitors of immunostimulatory cytokines (*e.g.*, anti-TNF-α MAbs), immune cell eliminators (*e.g.*, anti-CD20 MAbs), and blockers of accessory molecules (*e.g.* Abatacept). These biologics may be useful in the treatment of inflammatory diseases; however, due to primary non-responsiveness or a gradual decline in response rate to these treatments, there is an urgent need for alternative biologics with novel mechanisms of action to meet the medical needs of patients with, *e.g.*, CCL20/CCR6-mediated disorders. The antibodies of the subject invention represent such alternative biologics.

Summary of the Invention

[0010] The present invention relates to neutralizing anti-CCL20 antibodies, or antigen-binding portions thereof. In certain embodiments, the anti-CCL20 antibody is a humanized anti-human CCL20 antibody, which may comprise the complementarity determining regions (CDRs) of mouse anti-human CCL20 antibodies. In some embodiments, the anti-CCL20 antibody is a mouse or chimeric anti-human CCL20 antibody or an antigen-binding portion thereof.

[0011] In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof binds to human CCL20. In some embodiments, the antibody or portion does not bind to human CCL16.

[0012] The antibodies of the invention specifically bind CCL20. In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof binds to cynomolgus and/or rhesus CCL20 as well as human CCL20. In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof does not bind to mouse and/or rat CCL20. In one embodiment, the anti-CCL20 antibody or antigen-binding portion binds to human, cynomolgus, and rhesus CCL20, but not to mouse and rat CCL20.

[0013] In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof has a binding affinity (K_D) for human CCL20 of less than:

- 1 nM, 500 pM, 100 pM, 90 pM, 80 M, 70 pM, 60 pM, or 50 pM using a monovalent surface plasmon resonance assay, or
- 1 nM, 500 pM, 100 pM, 75 pM, 50 pM, 25 pM, 20 pM, 15 pM, 14 pM, 13 pM, 12 pM, 11 pM, 10 pM, 9 pM, 8 pM, 7 pM, 6 pM, or 5 pM using a bivalent surface plasmon resonance assay.

In certain embodiments, the antibody or portion has a binding affinity for human CCL20 greater than that of human CCR6.

[0014] In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof has a k_a for human CCL20 of less than 100, 90, 80, 70, 60, 50, 40, or 30 ($x10^5$ M^{-1} sec^{-1}), as determined by bivalent surface plasmon resonance. In some embodiments, the anti-CCL20 antibody or antigen-

binding portion thereof has a k_d for human CCL20 of less than 10, 9, 8, 7, 6, 5, 4, or 3 ($x10^{-5}$ sec^{-1}), as determined by bivalent surface plasmon resonance.

[0015] In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof has a k_a for rhesus CCL20 of less than 100, 90, 80, 70, 60, 50, 40, or 30 ($x10^5$ M^{-1} sec^{-1}), as determined by bivalent surface plasmon resonance. In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof has a k_d for rhesus CCL20 of less than 50, 40, 30, 20, 10, 9, 8, 7, 6, 5, 4, 3, or 2 ($x10^{-5}$ sec^{-1}), as determined by bivalent surface plasmon resonance. In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof has a K_D for rhesus CCL20 of less than 100, 90, 80, 70, 60, 50, 40, 30, 20, or 10 pM, as determined by bivalent surface plasmon resonance.

[0016] In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof has a k_a for cynomolgus CCL20 of less than 500, 400, 300, 200, 100, 90, 80, 70, 60, 50, 40, or 30 ($x10^5$ M^{-1} sec^{-1}), as determined by bivalent surface plasmon resonance. In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof has a k_d for cynomolgus CCL20 of less than 50, 40, 30, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, or 6 ($x10^{-5}$ sec^{-1}), as determined by bivalent surface plasmon resonance. In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof has a K_D for cynomolgus CCL20 of less than 100, 90, 80, 70, 60, 50, 40, 30, 20, 19, 18, 17, 16, or 15 pM, as determined by bivalent surface plasmon resonance.

[0017] In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof binds to human CCL20 with an EC_{50} of less than 100, 90, 80, 70, 60, 50, 40, 39, 38, 37, 36, 35, or 34 pM. In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof binds to rhesus CCL20 with an EC_{50} of less than 500, 400, 300, 200, 150, 140, 130, 120, 110, 100, 90, 80, 70, 65, 60, 59, 58, 57, 56, 55, 54, 53, 52, 51, or 50 pM. In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof binds to cynomolgus CCL20 with an EC_{50} of less than 500, 400, 350, 300, 250, 200, 190, 180, 170, 160, 150, 140, 130, 120, 110, 109, 108, 107, 106, 105, 104, 103, or 102 pM.

[0018] In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof has a selectivity for human CCL20 over other human chemokines, including but not limited to CX3CL1, CXCL1, CXCL2, CXCL4, CXCL8, CXCL9, CXCL10, CXCL12, CXCL13, CXCL16, CCL1, CCL2, CCL3, CCL4, CCL5, CCL7, CCL11, CCL13, CCL16, CCL17, CCL19, CCL21, CCL22, CCL24, CCL25, CCL27, CCL28, and/or XCL1. In certain embodiments, the anti-CCL20 antibody or antigen-binding portion has a selectivity for human CCL20 over all of said chemokines.

[0019] In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof reduces human CCL20-induced chemotaxis of human or mouse CCR6+ cells with an IC_{50} of less than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1.7, 1.6, 1.5, 1.4, 1.3, 1.2, or 1.1 nM. In certain embodiments, the anti-CCL20 antibody or antigen-binding portion thereof reduces human CCL20-

induced chemotaxis of human or mouse CCR6+ cells with an IC_{50} of less than 1.7, 1.6, 1.5, 1.4, 1.3, 1.2, or 1.1 nM. In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof reduces human CCL20-induced chemotaxis of human or mouse CCR6+ cells with an IC_{90} of less than 45, 40, 35, 30, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5.5, 5.4, 5.3, 5.2, 5.1, 4.9, 4.8, 4.7, or 4.6 nM. In certain embodiments, the anti-CCL20 antibody or antigen-binding portion thereof reduces human CCL20-induced chemotaxis of human or mouse CCR6+ cells with an IC_{90} of less than 6, 5.5, 5.4, 5.3, 5.2, 5.1, 4.9, 4.8, 4.7, or 4.6 nM. In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof reduces human CCL20-induced chemotaxis of human or mouse CCR6+ cells with an IC_{95} of less than 70, 65, 60, 55, 50, 45, 40, 35, 30, 25, 20, 15, 10, 9, 8, or 7 nM. In certain embodiments, the anti-CCL20 antibody or antigen-binding portion thereof reduces human CCL20-induced chemotaxis of human or mouse CCR6+ cells with an IC_{95} of less than 10, 9.9, 9.8, 9.7, 9.6, 9.5, 9.4, 9.3, 9.2, 9.1, or 9 nM.

[0020] In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof reduces human CCL20-induced chemotaxis of human or mouse CCR6+ cells *in vitro*.

[0021] In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof reduces human CCL20-induced chemotaxis of human or mouse CCR6+ cells *in vivo*.

[0022] In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof reduces the progression of arthritis symptoms (such as articular lesions of the extremities distal to the elbow or knee and degree of erythema and swelling) in a subject, *e.g.*, in a collagen-induced arthritis (CIA) and/or a glucose-6-phosphate isomerase (G6PI)-induced arthritis mouse model. In some embodiments, the antibody or antigen-binding portion thereof reduces bone lesions in a subject, including osteoporosis, bone erosion, and/or new bone formation, e.g., in a CIA model. In some embodiments, the antibody or antigen-binding portion reduces cartilage oligomeric matrix protein (COMP) serum levels in a subject, *e.g.*, in a CIA model. In some embodiments, the antibody or antigen-binding portion thereof reduces mRNA levels of receptor activator for nuclear factor κB ligand (RANKL), receptor activator for nuclear factor κB (RANK), tartrate resistant acid phosphatase (TRAP), and/or cathepsin K in a subject, *e.g.*, in mouse paws in a CIA model. In some embodiments, the antibody or antigen-binding portion thereof reduces atopic dermatitis symptoms (*e.g.*, dryness, scale, erythema, oozing/crusting, and/or excoriation) in a subject, for example, symptoms of oxazolone-induced atopic dermatitis in NC/Nga strain mice. In some embodiments, the antibody or antigen-binding portion reduces allergic contact dermatitis symptoms in a subject, for example, symptoms of dinitrofluorobenzene (DNFB)-induced allergic contact dermatitis in a mouse model.

[0023] In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof comprises a heavy chain whose CDR3 (H-CDR3) comprises the sequence of SEQ ID NO: 67 or 68.

[0024] In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof comprises a light chain whose CDR3 (L-CDR3) comprises the sequence of SEQ ID NO 75.

[0025] In certain embodiments, the anti-CCL20 antibody or antigen-binding portion thereof comprises an H-CDR3 whose sequence comprises SEQ ID NO: 67 and an L-CDR3 whose sequence comprises SEQ ID NO: 75; or an H-CDR3 whose sequence comprises SEQ ID NO: 68 and an L-CDR3 whose sequence comprises SEQ ID NO: 75.

[0026] In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof comprises a heavy chain whose CDR1 (H-CDR1), CDR2 (H-CDR2), and CDR3 (H-CDR3) respectively comprise the sequences of:

- SEQ ID NOS: 60, 63, and 67;
- SEQ ID NOS: 60, 64, and 67;
- SEQ ID NOS: 61, 65, and 68;
- SEQ ID NOS: 77, 79, and 67; or
- SEQ ID NOS: 78, 80, and 68.

[0027] In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof comprises a light chain whose CDR1 (L-CDR1), CDR2 (L-CDR2), and CDR3 (L-CDR3) respectively comprise the sequences of:

- SEQ ID NOS: 70, 73, and 75; or
- SEQ ID NOS: 71, 73, and 75.

[0028] In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof comprises:

- an H-CDR1 comprising a sequence selected from the group consisting of SEQ ID NOS: 60-62, 77, and 78;
- an H-CDR2 comprising a sequence selected from the group consisting of SEQ ID NOS: 63-66 and 79-81;
- an H-CDR3 comprising the sequence of SEQ ID NO: 67 or 68;
- an L-CDR1 comprising a sequence selected from the group consisting of SEQ ID NOS: 70-72;
- an L-CDR2 comprising the sequence of SEQ ID NO: 73 or 74; or
- an L-CDR3 comprising SEQ ID NO: 75;

or any combination thereof.

[0029] In certain embodiments, the anti-CCL20 antibody or antigen-binding portion thereof comprises an H-CDR1, H-CDR2, H-CDR3, L-CDR1, L-CDR2, and L-CDR3 respectively comprising the sequences of:

- SEQ ID NOS: 60, 63, 67, 70, 73, and 75;

- SEQ ID NOS: 60, 64, 67, 70, 73, and 75;
- SEQ ID NOS: 61, 65, 68, 70, 73, and 75;
- SEQ ID NOS: 77, 79, 67, 70, 73, and 75;
- SEQ ID NOS: 78, 80, 68, 70, 73, and 75;
- SEQ ID NOS: 60, 63, 67, 71, 73, and 75;
- SEQ ID NOS: 60, 64, 67, 71, 73, and 75;
- SEQ ID NOS: 61, 65, 68, 71, 73, and 75;
- SEQ ID NOS: 77, 79, 67, 71, 73, and 75; or
- SEQ ID NOS: 78, 80, 68, 71, 73, and 75.

[0030] In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof comprises a heavy chain variable domain comprising a sequence selected from the group consisting of SEQ ID NOS: 9-14. In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof comprises a heavy chain variable domain comprising a sequence selected from the group consisting of SEQ ID NOS: 39, 41, and 43.

[0031] In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof comprises a light chain variable domain comprising the sequence of SEQ ID NO: 15 or 16. In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof comprises a light chain variable domain comprising a sequence selected from the group consisting of SEQ ID NOS: 40, 42, and 44.

[0032] In certain embodiments, the anti-CCL20 antibody or antigen-binding portion thereof comprises a heavy chain variable domain and a light chain variable domain respectively comprising the sequences of:

- SEQ ID NO: 9 and SEQ ID NO: 15;
- SEQ ID NO: 10 and SEQ ID NO: 15;
- SEQ ID NO: 11 and SEQ ID NO: 15;
- SEQ ID NO: 12 and SEQ ID NO: 15;
- SEQ ID NO: 13 and SEQ ID NO: 15;
- SEQ ID NO: 14 and SEQ ID NO: 15;
- SEQ ID NO: 9 and SEQ ID NO: 16;
- SEQ ID NO: 10 and SEQ ID NO: 16;
- SEQ ID NO: 11 and SEQ ID NO: 16;
- SEQ ID NO: 12 and SEQ ID NO: 16;
- SEQ ID NO: 13 and SEQ ID NO: 16; or
- SEQ ID NO: 14 and SEQ ID NO: 16.

[0033] In certain embodiments, the anti-CCL20 antibody or antigen-binding portion thereof comprises a heavy chain variable domain and a light chain variable domain respectively comprising the sequences of:

- SEQ ID NO: 39 and SEQ ID NO: 40;
- SEQ ID NO: 39 and SEQ ID NO: 42;
- SEQ ID NO: 39 and SEQ ID NO: 44;
- SEQ ID NO: 41 and SEQ ID NO: 40;
- SEQ ID NO: 41 and SEQ ID NO: 42;
- SEQ ID NO: 41 and SEQ ID NO: 44;
- SEQ ID NO: 43 and SEQ ID NO: 40;
- SEQ ID NO: 43 and SEQ ID NO: 42; or
- SEQ ID NO: 43 and SEQ ID NO: 44.

[0034] In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof comprises a heavy chain having a sequence selected from the group consisting of SEQ ID NOS: 1-6 and 108 without the signal sequence (if present), and optionally without the C-terminal lysine. In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof comprises a light chain having a sequence selected from the group consisting of SEQ ID NOS: 7, 8, 110, and 112 without the signal sequence (if present). In certain embodiments, the anti-CCL20 antibody comprises a heavy chain and a light chain respectively having the sequences of:

- SEQ ID NO: 1 and SEQ ID NO: 7;
- SEQ ID NO: 2 and SEQ ID NO: 7;
- SEQ ID NO: 3 and SEQ ID NO: 7;
- SEQ ID NO: 4 and SEQ ID NO: 7;
- SEQ ID NO: 5 and SEQ ID NO: 7;
- SEQ ID NO: 6 and SEQ ID NO: 7;
- SEQ ID NO: 1 and SEQ ID NO: 8;
- SEQ ID NO: 2 and SEQ ID NO: 8;
- SEQ ID NO: 3 and SEQ ID NO: 8;
- SEQ ID NO: 4 and SEQ ID NO: 8;
- SEQ ID NO: 5 and SEQ ID NO: 8;
- SEQ ID NO: 6 and SEQ ID NO: 8;
- SEQ ID NO: 108 and SEQ ID NO: 110; or
- SEQ ID NO: 108 and 112;

both of said sequences without the signal sequence, SEQ ID NOS: 1-6 and 108 optionally without the C-terminal lysine.

[0035] The present invention also provides an anti-CCL20 antibody or an antigen-binding portion thereof that comprises a heavy chain variable domain encoded by a sequence selected from the group consisting of SEQ ID NOS: 25-30. In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof comprises a heavy chain variable domain encoded by a sequence selected from the group consisting of SEQ ID NOS: 51, 53, and 55.

[0036] Further, the present invention provides an anti-CCL20 antibody or an antigen-binding portion thereof that comprises a light chain variable domain encoded by the sequence of SEQ ID NO: 31 or 32. In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof comprises a light chain variable domain encoded by a sequence selected from the group consisting of SEQ ID NOS: 52, 54, and 56.

[0037] In certain embodiments, the anti-CCL20 antibody or antigen-binding portion thereof comprises a heavy chain variable domain and a light chain variable domain respectively encoded by the sequences of:

- SEQ ID NO: 25 and SEQ ID NO: 31;
- SEQ ID NO: 26 and SEQ ID NO: 31;
- SEQ ID NO: 27 and SEQ ID NO: 31;
- SEQ ID NO: 28 and SEQ ID NO: 31;
- SEQ ID NO: 29 and SEQ ID NO: 31;
- SEQ ID NO: 30 and SEQ ID NO: 31;
- SEQ ID NO: 25 and SEQ ID NO: 32;
- SEQ ID NO: 26 and SEQ ID NO: 32;
- SEQ ID NO: 27 and SEQ ID NO: 32;
- SEQ ID NO: 28 and SEQ ID NO: 32;
- SEQ ID NO: 29 and SEQ ID NO: 32; or
- SEQ ID NO: 30 and SEQ ID NO: 32.

[0038] In certain embodiments, the anti-CCL20 antibody or antigen-binding portion thereof comprises a heavy chain variable domain and a light chain variable domain respectively encoded by the sequences of:

- SEQ ID NO: 51 and SEQ ID NO: 52;
- SEQ ID NO: 51 and SEQ ID NO: 54;
- SEQ ID NO: 51 and SEQ ID NO: 56;
- SEQ ID NO: 53 and SEQ ID NO: 52;
- SEQ ID NO: 53 and SEQ ID NO: 54;
- SEQ ID NO: 53 and SEQ ID NO: 56;
- SEQ ID NO: 55 and SEQ ID NO: 52;

- SEQ ID NO: 55 and SEQ ID NO: 54; or
- SEQ ID NO: 55 and SEQ ID NO: 56.

[0039] In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof comprises a heavy chain encoded by a sequence selected from the group consisting of SEQ ID NOS: 17-22 and 109, said sequence lacking the sequence encoding a signal sequence (if present) and optionally lacking the sequence encoding the C-terminal lysine. In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof comprises a light chain encoded by a sequence selected from the group consisting of SEQ ID NOS: 23, 24, 111, and 113, said sequence lacking the sequence encoding a signal sequence (if present). In certain embodiments, the anti-CCL20 antibody comprises a heavy chain and a light chain respectively encoded by the sequences of:

- SEQ ID NO: 17 and SEQ ID NO: 23;
- SEQ ID NO: 18 and SEQ ID NO: 23;
- SEQ ID NO: 19 and SEQ ID NO: 23;
- SEQ ID NO: 20 and SEQ ID NO: 23;
- SEQ ID NO: 21 and SEQ ID NO: 23;
- SEQ ID NO: 22 and SEQ ID NO: 23;
- SEQ ID NO: 17 and SEQ ID NO: 24;
- SEQ ID NO: 18 and SEQ ID NO: 24;
- SEQ ID NO: 19 and SEQ ID NO: 24;
- SEQ ID NO: 20 and SEQ ID NO: 24;
- SEQ ID NO: 21 and SEQ ID NO: 24;
- SEQ ID NO: 22 and SEQ ID NO: 24;
- SEQ ID NO: 109 and SEQ ID NO: 111; or
- SEQ ID NO: 109 and SEQ ID NO: 113;

both of said sequences lacking the sequence encoding a signal sequence (if present), SEQ ID NOS: 17-22 and 109 optionally lacking the sequence encoding the C-terminal lysine.

[0040] In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof is humanized or chimeric. In some embodiments, the framework regions of the heavy chain of the humanized anti-CCL20 antibody or portion utilize an IGHV1-46*03 human germline gene (See, *e.g.*, Altschul et al., "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402 (1997)). In certain embodiments, the heavy chain framework regions have at least 50% homology to the framework regions of the IGHV1-46*03 human germline gene. For example, the heavy chain framework regions may be at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or even 100% identical to the framework regions of the IGHV1-46*03 human germline gene. In some

embodiments, the framework regions of the light chain of the humanized anti-CCL20 antibody or portion utilize an IGKV1D-39*01 human germline gene (see Altschul et al., *supra*). In certain embodiments, the light chain framework regions have at least 50% homology to the framework regions of the IGKV1D-39*01 human germline gene. For example, the light chain framework regions may be at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or even 100% identical to the framework regions of the IGKV1D-39*01 human germline gene.

[0041] In some embodiments, the anti-CCL20 antibody or antigen-binding portion thereof (to the extent that said portion comprises at least part of a heavy chain constant region) is an IgG, an IgM, an IgE, an IgA, or an IgD molecule. In certain embodiments, the anti-CCL20 antibody or antigen-binding portion thereof is of the IgG1, IgG2, IgG3, or IgG4 subtype.

[0042] In some embodiments, the anti-CCL20 antibody is a monoclonal antibody.

[0043] The present invention also provides an anti-CCL20 antibody or an antigen-binding portion thereof that binds to an epitope of human CCL20 located in the N-loop and/or β2-β3 hairpin region of the molecule. In some embodiments, said anti-CCL20 antibody or portion binds to the same epitope on human CCL20 as an antibody or portion as described herein. Further, the present invention provides an anti-CCL20 antibody or an antigen-binding portion thereof that competes or cross-competes for binding to human CCL20 with an antibody or portion described herein.

[0044] In some embodiments, the human CCL20 epitope bound by the antibody or antigen-binding portion thereof of the invention comprises one or more amino acid sequences selected from the group consisting of:

- residues 7-9 of SEQ ID NO: 84;
- residues 10-19 of SEQ ID NO: 84;
- residues 20-21 of SEQ ID NO: 84; and
- residues 20-22 of SEQ ID NO: 84,

or the residues of wild-type human CCL20 corresponding to said residues of SEQ ID NO: 84.

[0045] In some embodiments, said human CCL20 epitope further comprises one or more amino acid sequences selected from the group consisting of:

- residues 39-55 of SEQ ID NO: 84;
- residues 39-57 of SEQ ID NO: 84;
- residues 56-67 of SEQ ID NO: 84; and
- residues 61-70 of SEQ ID NO: 84,

or the residues of wild-type human CCL20 corresponding to said residues of SEQ ID NO: 84.

[0046] In certain embodiments, said human CCL20 epitope comprises any combination of the amino acid sequences consisting of residues 7-9, 10-19, 20-22, 39-55, 56-57, and 61-70 of SEQ ID NO: 84, or the residues of wild-type human CCL20 corresponding to said residues of SEQ ID NO: 84.

[0047] In certain embodiments, said human CCL20 epitope comprises residues 7-9, 10-19, and 20-22 of SEQ ID NO: 84, or the residues of wild-type human CCL20 corresponding to said residues of SEQ ID NO: 84. In certain embodiments, said human CCL20 epitope comprises residues 7-9, 10-19, 20-22, 39-55, 56-57, and 61-70 of SEQ ID NO: 84, or the residues of wild-type human CCL20 corresponding to said residues of SEQ ID NO: 84. In certain embodiments, said human CCL20 epitope comprises residues 7-9, 10-19, 20-22, 39-57, and 61-70 of SEQ ID NO: 84, or the residues of wild-type human CCL20 corresponding to said residues of SEQ ID NO: 84.

[0048] In certain embodiments, said human CCL20 epitope comprises residues 7-9, 10-19, and 20-21 of SEQ ID NO: 84, or the residues of wild-type human CCL20 corresponding to said residues of SEQ ID NO: 84. In certain embodiments, said human CCL20 epitope comprises residues 7-9, 10-19, 20-21, 39-55, 56-67, and 61-70 of SEQ ID NO: 84, or the residues of wild-type human CCL20 corresponding to said residues of SEQ ID NO: 84. In certain embodiments, said human CCL20 epitope comprises residues 7-9, 10-19, 20-21, 39-57, and 61-70 of SEQ ID NO: 84, or the residues of wild-type human CCL20 corresponding to said residues of SEQ ID NO: 84.

[0049] In some embodiments, the human CCL20 epitope bound by the antibody or antigen-binding portion thereof comprises one or more amino acid residues of SEQ ID NO: 84 (or the residues of wild-type human CCL20 corresponding to said residues of SEQ ID NO: 84) selected from residues 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, and 70, or any combination thereof.

[0050] The present invention also provides an antigen-binding portion of any of the anti-CCL20 antibodies described herein. Said antigen-binding portion may be, *e.g.*, a single chain antibody, Fv, Fab, Fab', $F(ab')_2$, Fd, single chain Fv molecule (scFv), bispecific single chain Fv dimer, polyvalent single chain Fv multimer, diabody, domain-deleted antibody, or single domain antibody (dAb).

[0051] The present invention also provides a variant of an antibody or antigen-binding portion as described herein, wherein said variant differs from the antibody or portion by 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions.

[0052] In one aspect, the present invention provides an antibody directed against human CCL20, wherein the heavy chain variable domain and the light chain variable domain of said antibody respectively comprise the sequences of:

- SEQ ID NOS: 9 and 15;
- SEQ ID NOS: 9 and 16;

- SEQ ID NOS: 10 and 16;
- SEQ ID NOS: 11 and 15;
- SEQ ID NOS: 11 and 16;
- SEQ ID NOS: 12 and 16;
- SEQ ID NOS: 13 and 16; or
- SEQ ID NOS: 14 and 16.

[0053] In one aspect, the present invention provides an antibody whose heavy chain comprises SEQ ID NO: 1 and whose light chain comprises SEQ ID NO: 7, wherein said amino acid sequences lack signal sequences.

[0054] In another aspect, the present invention provides an antibody whose heavy chain comprises SEQ ID NO: 1 without the C-terminal lysine and whose light chain comprises SEQ ID NO: 7, wherein said amino acid sequences lack signal sequences.

[0055] In one aspect, the present invention provides an antibody whose heavy chain comprises SEQ ID NO: 1 and whose light chain comprises SEQ ID NO: 8, wherein said amino acid sequences lack signal sequences.

[0056] In another aspect, the present invention provides an antibody whose heavy chain comprises SEQ ID NO: 1 without the C-terminal lysine and whose light chain comprises SEQ ID NO: 8, wherein said amino acid sequences lack signal sequences.

[0057] In one aspect, the present invention provides an antibody whose heavy chain comprises SEQ ID NO: 2 and whose light chain comprises SEQ ID NO: 8, wherein said amino acid sequences lack signal sequences.

[0058] In another aspect, the present invention provides an antibody whose heavy chain comprises SEQ ID NO: 2 without the C-terminal lysine and whose light chain comprises SEQ ID NO: 8, wherein said amino acid sequences lack signal sequences.

[0059] In one aspect, the present invention provides an antibody whose heavy chain comprises SEQ ID NO: 3 and whose light chain comprises SEQ ID NO: 7, wherein said amino acid sequences lack signal sequences.

[0060] In another aspect, the present invention provides an antibody whose heavy chain comprises SEQ ID NO: 3 without the C-terminal lysine and whose light chain comprises SEQ ID NO: 7, wherein said amino acid sequences lack signal sequences.

[0061] In one aspect, the present invention provides an antibody whose heavy chain comprises SEQ ID NO: 3 and whose light chain comprises SEQ ID NO: 8, wherein said amino acid sequences lack signal sequences.

[0062] In another aspect, the present invention provides an antibody whose heavy chain comprises SEQ ID NO: 3 without the C-terminal lysine and whose light chain comprises SEQ ID NO: 8, wherein said amino acid sequences lack signal sequences.

[0063] In one aspect, the present invention provides an antibody whose heavy chain comprises SEQ ID NO: 4 and whose light chain comprises SEQ ID NO: 8, wherein said amino acid sequences lack signal sequences.

[0064] In another aspect, the present invention provides an antibody whose heavy chain comprises SEQ ID NO: 4 without the C-terminal lysine and whose light chain comprises SEQ ID NO: 8, wherein said amino acid sequences lack signal sequences.

[0065] In one aspect, the present invention provides an antibody whose heavy chain comprises SEQ ID NO: 5 and whose light chain comprises SEQ ID NO: 8, wherein said amino acid sequences lack signal sequences.

[0066] In another aspect, the present invention provides an antibody whose heavy chain comprises SEQ ID NO: 5 without the C-terminal lysine and whose light chain comprises SEQ ID NO: 8, wherein said amino acid sequences lack signal sequences.

[0067] In one aspect, the present invention provides an antibody whose heavy chain comprises SEQ ID NO: 6 and whose light chain comprises SEQ ID NO: 8, wherein said amino acid sequences lack signal sequences.

[0068] In another aspect, the present invention provides an antibody whose heavy chain comprises SEQ ID NO: 6 without the C-terminal lysine and whose light chain comprises SEQ ID NO: 8, wherein said amino acid sequences lack signal sequences.

[0069] In one aspect, the present invention provides an antibody whose heavy chain comprises SEQ ID NO: 108 and whose light chain comprises SEQ ID NO: 110.

[0070] In another aspect, the present invention provides an antibody whose heavy chain comprises SEQ ID NO: 108 without the C-terminal lysine and whose light chain comprises SEQ ID NO: 110.

[0071] In one aspect, the present invention provides an antibody whose heavy chain comprises SEQ ID NO: 108 and whose light chain comprises SEQ ID NO: 112.

[0072] In another aspect, the present invention provides an antibody whose heavy chain comprises SEQ ID NO: 108 without the C-terminal lysine and whose light chain comprises SEQ ID NO: 112.

[0073] In another aspect, the present invention provides one or more nucleic acid molecules, *e.g.*, isolated nucleic acid molecules, encoding a heavy chain or an antigen-binding portion thereof or a light chain or an antigen-binding portion thereof, wherein said heavy chain and light chain or antigen-binding portions thereof may associate to form an anti-CCL20 antibody or an antigen-binding portion thereof. In some embodiments, the nucleic acid molecule encoding the heavy chain or an antigen-binding portion thereof comprises a nucleotide sequence selected from the group consisting of SEQ

ID NOS: 17-22, 25-30, and 109, or said nucleotide sequence without the sequence encoding a signal sequence, if present. The nucleotide sequences of SEQ ID NOS: 17-22 and 109 may also optionally lack the sequence encoding the C-terminal lysine, if present. In some embodiments, the nucleic acid molecule encoding the light chain or an antigen-binding portion thereof comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS: 23, 24, 31, 32, 111, and 113, or said nucleotide sequence without the sequence encoding a signal sequence, if present. In some embodiments, the one or more nucleic acid molecules encode the heavy chain or an antigen-binding portion thereof and the light chain or an antigen-binding portion thereof of an antibody or portion as described herein. In certain embodiments, one nucleic acid molecule encodes both said heavy chain or an antigen-binding portion thereof and said light chain or an antigen-binding portion thereof.

[0074] In one embodiment, the nucleic acid molecule encoding the heavy chain or an antigen-binding portion thereof, the nucleic acid molecule encoding the light chain or an antigen-binding portion thereof, or nucleic acid molecule(s) encoding the heavy chain and the light chain or antigen-binding portions thereof, are used for treating a subject in need thereof.

[0075] In another aspect, the present invention provides a vector comprising nucleic acid sequence(s) encoding the heavy chain or an antigen-binding portion thereof, the light chain or an antigen-binding portion thereof, or both, of an antibody or portion as described herein.

[0076] In another aspect, the present invention provides a cell expressing the heavy chain or an antigen-binding portion thereof, the light chain or an antigen-binding portion thereof, or both, of an antibody or portion as described herein.

[0077] In another aspect, the present invention provides a method for making an antibody or portion as described herein, comprising maintaining a cell as described herein under conditions appropriate for expression of the antibody or portion. In some embodiments, the method comprises the step of isolating the antibody or portion.

[0078] The present invention also provides a method of producing a hybridoma that secretes an anti-CCL20 antibody. In certain embodiments, the hybridoma produces an antibody that binds to wild-type human CCL20. The invention also provides a hybridoma produced by the methods of the invention. Optionally, the monoclonal antibody secreted by the hybridoma is collected and can be further purified (*e.g.*, substantially purified, isolated). In other embodiments, the method further comprises determining the nucleotide sequence of the monoclonal antibody secreted by the hybridoma.

[0079] In another aspect, the present invention provides a composition comprising an antibody or portion as described herein and a pharmaceutically acceptable vehicle or carrier.

[0080] In one aspect, the antibody or portion as described herein is used as a medicament. In certain embodiments, the antibody or portion as described herein is used for treating a subject in need thereof.

In particular embodiments, the antibody or portion is used to treat a CCR6-associated condition, a CCL20 associated condition or both. Such conditions include but are not limited to inflammatory and auto-immune diseases, particularly arthritis, especially rheumatoid arthritis, atopic or allergic dermatitis, and psoriasis. In another particular embodiment, the antibody or portion is used to treat cancer. The antibody or portion may be used to treat, *e.g.*, Grave's disease, vitiligo, hyperthyroidism, rheumatoid arthritis, psoriasis, atopic dermatitis, contact dermatitis, Crohn's disease, inflammatory bowel disease, B-cell malignancies, breast adenocarcinoma, chronic hepatitis, contact dermatitis, glioblastoma, hepatocellular carcinoma, human papillomavirus infection of the cervix, mycosis fungoides, pancreatic adenocarcinoma, periodontal disease, thyroid papillary carcinoma, pustulosis palmaris et plantaris, conditions associated with maculopapular exanthema, epidermolysis bullosa, alopecia areata, multiple sclerosis, polymyositis, dermatomyositis, Behcet's disease, acute generalized exanthematous pustulosis, vasculitides, juvenile idiopathic arthritis, sarcoidosis, bronchial asthma, allergic rhinitis, renal allograft rejection, graft-versus-host disease, liver allograft rejection, chronic obstructive pulmonary disease, cystic fibrosis, glomerulonephritis, respiratory syncytial virus infection, multiple myeloma, and/or Langerhans cell histiocytosis.

[0081] In some embodiments, the anti-CCL20 antibody or portion is used to reduce CCL20-mediated chemotaxis of CCR6+ cells in a subject in need thereof.

[0082] In some embodiments, the present invention provides:

1. A monoclonal anti-human CCL20 antibody or an antigen-binding portion thereof, wherein said antibody comprises a heavy chain whose complementarity determining region 3 (CDR3) comprises SEQ ID NO: 67 or 68.

2. The monoclonal antibody or antigen-binding portion of embodiment 1, wherein the complementarity determining region 1 (CDR1), complementarity determining region 2 (CDR2), and complementarity determining region 3 (CDR3) of said heavy chain respectively comprise amino acid sequences selected from the group consisting of:
   a) SEQ ID NOS: 60, 64, and 67;
   b) SEQ ID NOS: 60, 63, and 67;
   c) SEQ ID NOS: 61, 65, and 68;
   d) SEQ ID NOS: 77, 79, and 67; and
   e) SEQ ID NOS: 78, 80, and 68.

3. A monoclonal anti-human CCL20 antibody or an antigen-binding portion thereof, wherein said antibody comprises a light chain whose complementarity determining region 3 (CDR3) comprises SEQ ID NO: 75.

4. The monoclonal antibody or antigen-binding portion of embodiment 3, wherein the complementarity determining region 1 (CDR1), complementarity determining region 2 (CDR2), and complementarity determining region 3 (CDR3) of said light chain respectively comprise amino acid sequences selected from the group consisting of:

a) SEQ ID NOS: 70, 73, and 75; and

b) SEQ ID NOS: 71, 73, and 75.

5. The antibody or antigen-binding portion of embodiment 1 or 3, wherein the heavy chain of said antibody comprises a CDR3 comprising SEQ ID NO: 67 or 68 and the light chain of said antibody comprises a CDR3 comprising SEQ ID NO: 75.

6. The antibody or antigen-binding portion according to embodiment 2 or 4, wherein said heavy chain CDR1, CDR2, and CDR3 and said light chain CDR1, CDR2, and CDR3 respectively comprise amino acid sequences selected from the group consisting of:

a) SEQ ID NOS: 60, 64, 67, 70, 73, and 75;

b) SEQ ID NOS: 60, 64, 67, 71, 73, and 75;

c) SEQ ID NOS: 60, 63, 67, 70, 73, and 75;

d) SEQ ID NOS: 60, 63, 67, 71, 73, and 75;

e) SEQ ID NOS: 61, 65, 68, 70, 73, and 75;

f) SEQ ID NOS: 61, 65, 68, 71, 73, and 75;

g) SEQ ID NOS: 77, 79, 67, 70, 73, and 75;

h) SEQ ID NOS: 77, 79, 67, 71, 73, and 75;

i) SEQ ID NOS: 78, 80, 68, 70, 73, and 75; and

j) SEQ ID NOS: 78, 80, 68, 71, 73, and 75.

7. A monoclonal anti-human CCL20 antibody or an antigen-binding portion thereof, wherein said antibody comprises a heavy chain whose variable domain comprises an amino acid sequence selected from SEQ ID NOS: 9-14.

8. A monoclonal anti-human CCL20 antibody or an antigen-binding portion thereof, wherein said antibody comprises a light chain whose variable domain comprises SEQ ID NO: 15 or 16.

9. The antibody or antigen-binding portion according to embodiment 7 or 8, wherein said heavy chain variable domain and said light chain variable domain respectively comprise amino acid sequences selected from the group consisting of:

a) SEQ ID NOS: 9 and 15;
b) SEQ ID NOS: 9 and 16;
c) SEQ ID NOS: 10 and 16;
d) SEQ ID NOS: 11 and 15;
e) SEQ ID NOS: 11 and 16;
f) SEQ ID NOS: 12 and 16;
g) SEQ ID NOS: 13 and 16; and
h) SEQ ID NOS: 14 and 16.

10. A monoclonal anti-human CCL20 antibody whose heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-6 without the signal sequence and SEQ ID NO: 108.

11. A monoclonal anti-human CCL20 antibody whose heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-6 without the signal sequence and SEQ ID NO: 108, wherein said amino acid sequence lacks the C-terminal lysine.

12. A monoclonal anti-human CCL20 antibody whose light chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 7 and 8 without the signal sequence and SEQ ID NOS: 110 and 112.

13. The antibody according to any one of embodiments 10-12, wherein said heavy chain and said light chain respectively comprise amino acid sequences selected from the group consisting of:

a) SEQ ID NOS: 1 and 7;
b) SEQ ID NOS: 1 and 8;
c) SEQ ID NOS: 2 and 8;
d) SEQ ID NOS: 3 and 7;
e) SEQ ID NOS: 3 and 8;
f) SEQ ID NOS: 4 and 8;
g) SEQ ID NOS: 5 and 8;
h) SEQ ID NOS: 6 and 8;
i) SEQ ID NOS: 108 and 110; and

j) SEQ ID NOS: 108 and 112;

wherein said amino acid sequences lack signal sequences, if present, and wherein SEQ ID NOS: 1-6 optionally lack the C-terminal lysine.

14. A monoclonal antibody whose heavy chain comprises SEQ ID NO: 108 and whose light chain comprises SEQ ID NO: 110.

15. A monoclonal antibody whose heavy chain comprises SEQ ID NO: 108 without the C-terminal lysine and whose light chain comprises SEQ ID NO: 110.

16. A monoclonal antibody whose heavy chain comprises SEQ ID NO: 108 and whose light chain comprises SEQ ID NO: 112.

17. A monoclonal antibody whose heavy chain comprises SEQ ID NO: 108 without the C-terminal lysine and whose light chain comprises SEQ ID NO: 112.

18. A monoclonal anti-human CCL20 antibody, or an antigen-binding portion thereof, that binds to the same epitope of human CCL20 as the monoclonal antibody or antigen-binding portion of any one of embodiments 1-17.

19. A monoclonal anti-human CCL20 antibody, or an antigen-binding portion thereof, that competes for binding to human CCL20 with the monoclonal antibody or antigen-binding portion of any one of embodiments 1-17.

20. A monoclonal anti-human CCL20 antibody, or an antigen-binding portion thereof, that cross-competes for binding to human CCL20 with the monoclonal antibody or antigen-binding portion of any one of embodiments 1-17.

21. The monoclonal antibody or antigen-binding portion of any one of embodiments 1-12, wherein the antibody is a humanized antibody.

22. The monoclonal antibody or antigen-binding portion of any one of embodiments 1-6, wherein the framework regions of said heavy chain utilize a IGHV1-46*03 human germline sequence, and wherein the framework regions of said light chain utilize a IGKV1D-39*01 human germline sequence.

23. The monoclonal antibody of any one of embodiments 1-9 and 18-22, wherein said antibody comprises a human IgG1, IgG2, IgG3, or IgG4 constant domain.

24. A monoclonal anti-human CCL20 antibody, or an antigen-binding portion thereof, whose heavy chain comprises a variable domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 39, 41, and 43.

25. A monoclonal anti-human CCL20 antibody, or an antigen-binding portion thereof, whose light chain comprises a variable domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 40, 42, and 44.

26. The antibody or antigen-binding portion of embodiment 24 or 25, wherein said heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 39, 41, and 43 and wherein said light chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 40, 42, and 44.

27. The antibody of embodiment 26, wherein said heavy chain and said light chain respectively comprise amino acid sequences selected from the group consisting of:

a) SEQ ID NOS: 39 and 40;

b) SEQ ID NOS: 41 and 42; and

c) SEQ ID NOS: 43 and 44.

28. A monoclonal anti-human CCL20 antibody, or an antigen-binding portion thereof, wherein said antibody comprises a heavy chain comprising SEQ ID NO: 39 and a light chain comprising SEQ ID NO: 40.

29. The monoclonal antibody or antigen-binding portion of any one of embodiments 24-28, wherein said antibody is a chimeric antibody.

30. The antigen-binding portion of any one of embodiments 1-9, 18-22, and 24-28, wherein said portion is a single chain antibody, Fv, Fab, Fab', $F(ab')_2$, Fd, single chain Fv molecule (scFv), bispecific single chain Fv dimer, diabody, domain-deleted antibody or single domain antibody (dAb).

31. The monoclonal antibody or antigen-binding portion according to any one of embodiments 1-30, wherein said antibody or antigen-binding portion has one or more properties selected from the group consisting of:

a) does not bind to human CCL16;

b) binds to cynomolgus or rhesus CCL20, but not to mouse or rat CCL20;

c) has a binding affinity for human CCL20 of 70 pM or less using a monovalent surface plasmon resonance assay;

d) has a binding affinity for human CCL20 of 12 pM or less using a bivalent surface plasmon resonance assay;

e) has a binding affinity for human CCL20 greater than that of human CCR6;

f) has a selectivity for human CCL20 over human CX3CL1, CXCL1, CXCL2, CXCL4, CXCL8, CXCL9, CXCL10, CXCL12, CXCL13, CXCL16, CCL1, CCL2, CCL3, CCL4, CCL5, CCL7, CCL11, CCL13, CCL16, CCL17, CCL19, CCL21, CCL22, CCL24, CCL25, CCL27, CCL28, or XCL1;

g) reduces human CCL20-induced chemotaxis of CCR6+ cells with an IC_{50} of 1.7 nM or less;

h) reduces human CCL20-induced chemotaxis of CCR6+ cells *in vivo*;

i) reduces human CCL20-induced chemotaxis of CCR6 + cells *in vitro*;

j) reduces progression of arthritis symptoms in a subject;

k) reduces osteoporosis, bone erosion, or new bone formation in a subject;

l) reduces cartilage oligomeric matrix protein (COMP) serum levels in a subject;

m) reduces mRNA levels of RANKL, RANK, TRAP, or cathepsin K in a subject;

n) reduces progression of atopic dermatitis in a subject; and

o) reduces progression of allergic contact dermatitis in a subject..

32. A monoclonal anti-human CCL20 antibody, or an antigen-binding portion thereof, that binds to an epitope of human CCL20 comprising one or more amino acid sequences selected from the group consisting of:

a) residues 7-9 of SEQ ID NO: 84;

b) residues 10-19 of SEQ ID NO: 84; and

c) residues 20-22 of SEQ ID NO: 84.

33. The monoclonal antibody or antigen-binding portion of embodiment 32, wherein said epitope comprises residues 7-9, 10-19, and 20-22 of SEQ ID NO: 84.

34. The monoclonal antibody or antigen-binding portion of embodiment 32, wherein said epitope further comprises one or more amino acid sequences selected from the group consisting of:

a) residues 39-55 of SEQ ID NO: 84;

b) residues 56-67 of SEQ ID NO: 84; and

c) residues 61-70 of SEQ ID NO: 84.

35. The monoclonal antibody or antigen-binding portion of embodiment 34, wherein said epitope comprises residues 7-9, 10-19, 20-22, 39-55, 56-67, and 61-70 of SEQ ID NO: 84.

36. An isolated nucleic acid molecule encoding the heavy chain or an antigen-binding portion thereof of an antibody or portion according to any one of embodiments 1-35.

37. An isolated nucleic acid molecule encoding the light chain or an antigen-binding portion thereof of an antibody or portion according to any one of embodiments 1-35.

38. An isolated nucleic acid molecule encoding the heavy chain or an antigen-binding portion thereof, and the light chain or an antigen-binding portion thereof, of an antibody or portion according to any one of embodiments 1-35.

39. An isolated nucleic acid molecule encoding the heavy chain or an antigen-binding portion thereof of a monoclonal anti-human CCL20 antibody, wherein said nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS: 17-22, 25-30, and 109, or said nucleotide sequence without the sequence encoding a signal sequence, if present.

40. An isolated nucleic acid molecule encoding the light chain or an antigen-binding portion thereof of a monoclonal anti-human CCL20 antibody, wherein said nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS: 23, 24, 31, 32, 111, and 113, or said nucleotide sequence without the sequence encoding a signal sequence, if present.

41. The isolated nucleic acid molecule of embodiment 39 or 40, wherein said nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS: 17-22, 25-30, and 109, or said nucleotide sequence without the sequence encoding a signal sequence if present; and wherein said nucleic acid molecule further comprises a

nucleotide sequence selected from the group consisting of SEQ ID NOS: 23, 24, 31, 32, 111, and 113, or said nucleotide sequence without the sequence encoding a signal sequence if present.

42. Use of (1) a nucleic acid sequence encoding the heavy chain or an antigen-binding portion thereof, (2) a nucleic acid sequence encoding the light chain or an antigen-binding portion thereof, or (3) both, of an antibody or portion according to any one of embodiments 1-35, as a medicament.

43. A recombinant vector comprising (1) a nucleic acid sequence encoding the heavy chain or an antigen-binding portion thereof, (2) a nucleic acid sequence encoding the light chain or an antigen-binding portion thereof, or (3) both, of an antibody or portion according to any one of embodiments 1-35.

44. A host cell comprising a first nucleic acid sequence encoding the heavy chain or an antigen-binding portion thereof of an antibody or portion according to any one of embodiments 1-35, said first nucleic acid sequence operably linked to an expression control element, and a second nucleic acid sequence encoding the light chain or an antigen-binding portion thereof of said antibody or portion, said second nucleic acid sequence operably linked to an expression control element.

45. A method of making an anti-human CCL20 antibody or an antigen-binding portion thereof, comprising maintaining the host cell of embodiment 44 under conditions appropriate for expression of the antibody or portion.

46. The method of embodiment 45, further comprising isolating the antibody or portion.

47. A composition comprising the monoclonal antibody or antigen-binding portion of any one of embodiments 1-35 and a pharmaceutically acceptable vehicle or carrier.

48. A method for treating a subject in need thereof, comprising administering to the subject an effective amount of the antibody or antigen-binding portion of any one of embodiments 1-35 or the composition of embodiment 47.

49. A method for treating a condition in a subject in need thereof, comprising administering to the subject an effective amount of the antibody or antigen-binding portion of any one of embodiments 1-35 or the composition of embodiment 47.

50. The method of embodiment 49, wherein said condition is a CCR6-associated condition.

51. The method of embodiment 49, wherein said condition is an autoimmune or inflammatory condition.

52. The method of embodiment 49, wherein said condition is rheumatoid arthritis, psoriasis, atopic dermatitis, contact dermatitis, Crohn's disease, inflammatory bowel disease, Grave's disease, vitiligo, hyperthyroidism, chronic hepatitis, human papillomavirus infection of the cervix, mycosis fungoides, osteoporosis, or periodontal disease.

53. A method for treating cancer, comprising administering to a subject an effective amount of the antibody or antigen-binding portion of any one of embodiments 1-35 or the composition of embodiment 47.

54. The method of embodiment 53, wherein said cancer is a B-cell malignancy, breast adenocarcinoma, glioblastoma, hepatocellular carcinoma, pancreatic adenocarcinoma, or thyroid papillary carcinoma.

55. A method for reducing CCL20-mediated chemotaxis of CCR6+ cells in a subject in need thereof, comprising administering to the subject the antibody or antigen-binding portion of any one of embodiments 1-35 or the composition of embodiment 47.

56. A method for reducing CCL20-mediated chemotaxis of CCR6+ cells *in vitro*, using the antibody or antigen-binding portion of any one of embodiments 1-35 or the composition of embodiment 47.

57. A method for treating a condition in a subject, comprising administering to the subject the antibody or antigen-binding portion of any one of embodiments 1-35 or the composition of embodiment 47, wherein said condition is selected from the group consisting of:

a) articular lesions of extremities distal to the elbow or knee;

b) erythema;

c) swelling;

d) increased cartilage oligomeric matrix protein (COMP) serum levels;

e) increased mRNA levels of receptor activator for nuclear factor κB ligand (RANKL), receptor activator for nuclear factor κB (RANK), tartrate resistant acid phosphatase (TRAP), or cathepsin K;

f) atopic dermatitis; and

g) allergic contact dermatitis.

58. Use of the antibody or antigen-binding portion of any one of embodiments 1-35 or the composition of embodiment 47 for the manufacture of a medicament.

59. Use of the antibody or antigen-binding portion of any one of embodiments 1-35 or the composition of embodiment 47 as a medicament.

Brief Description of the Drawings

[0083] FIG. 1 is a graph showing that CCR6 deficient mice are less sensitive to collagen-induced arthritis than wild-type mice. Wild type, $CCR6^{+/+}$ mice; Het, $CCR6^{+/-}$ mice; Knockout, $CCR6^{-/-}$ mice. See Example 1.

[0084] FIG. 2 is a graph showing that CCR6 deficient mice exhibit impaired infiltration of T-cells (as indicated by CD3 levels) in collagen-induced arthritic lesions in comparison to wild-type mice. Data shown are means ± SEM from one experiment (n=7 mice per group). *$p<0.05$, **$p<0.01$. See Example 1.

[0085] FIG. 3 is a graph showing that CCR6 deficient mice exhibit impaired infiltration of macrophages (as indicated by F4/80 levels) in collagen-induced arthritic lesions in comparison to wild-type mice. Data shown are means ± SEM from one experiment (n=7 mice per group). **$p<0.01$. See Example 1.

[0086] FIG. 4 is a graph showing that in comparison to wild-type mice, CCR6 deficient mice are resistant to increased ear thickness in a dinitrofluorobenzene (DNFB)-induced allergic contact dermatitis model. WT, wild-type mice; KO, CCR6 deficient mice. See Example 1.

[0087] FIG. 5 is a graph showing that 2F5-5 MAb inhibits CCL20-induced chemotaxis. See Example 2.

[0088] FIGS. 6A-C depict sequence alignments of variable domains of antibodies of the invention (SEQ ID NOS: 9-16) with those of mouse anti-human antibodies 36F7C10 (VH: SEQ ID NO: 39; VL: SEQ ID NO: 40) and 42G5B10 (VH: SEQ ID NO: 43) and with germline sequences IGHV1-

46*03 (SEQ ID NO: 57), JH4 (SEQ ID NO: 58), and IGKV1D-39*01 (SEQ ID NO: 59). Kabat and Chothia definitions of each CDR are indicated. ABM67212 (SEQ ID NO: 82) and BAH04867.1 (SEQ ID NO: 83) are human antibodies from which the framework regions of the shown humanized antibody chains were derived. See Example 3.

[0089] FIGS. 7A-C are tables depicting the inhibition of *in vitro* chemotaxis by different combinations of humanized anti-human CCL20 antibody chains. Data represent three trials, except where indicated. See Example 4.

[0090] FIGS. 8A-C depict three independent trials performed to assess the inhibitory effect of the humanized HC2/LK3 anti-human CCL20 antibody. The HC2/LK3 antibody is shown to inhibit CCL20-induced chemotaxis. Values for IC_{50}, IC_{90}, and IC_{95} are shown for each experiment. See Example 4.

[0091] FIGS. 9A-C depict a series of graphs showing that humanized antibodies B) HC2/LK3 and C) HC2/LC3 demonstrate dose dependent inhibition of cell migration comparable to that of A) mouse antibody 36F7C10 in a transendothelial migration assay. See Example 4.

[0092] FIGS. 10A and B depict Biacore™ sensograms demonstrating that the A) HC2/LC3 and B) HC2/LK3 humanized antibodies bind to human CCL20 in a concentration-dependent manner. See Example 5.

[0093] FIGS. 11A-D demonstrate that anti-human CCL20 antibodies specifically bind to human CCL20. An ELISA assay with plate-bound CCL20 and other chemokines (at 1 μg/ml) was used to detect binding of A) 36F7C10 and chimeric antibodies, and B) humanized HC2LK3 and HC2LC3 antibodies. C) An ELISA assay with His-tag anchored CCL20 was used to detect binding. D) Biacore™ experiments confirm that anti-human CCL20 antibodies bind to human CCL20, and exhibit negligible binding to CXCL4. See Example 6.

[0094] FIG. 12 shows an amino acid sequence alignment between a56-residue overlap portion of CCL20 (SEQ ID NO: 114) and CCL16 (SEQ ID NO: 115). See Example 6.

[0095] FIGS. 13A and B are a series of graphs showing that A) chimeric and B) humanized anti-human CCL20 antibodies react specifically with CCL20 and do not react with CCL16. See Example 6.

[0096] FIG. 14 shows amino acid sequence alignments between human (SEQ ID NO: 85), rhesus (SEQ ID NO: 86), cynomolgus (SEQ ID NO: 87), and mouse (SEQ ID NO: 88) CCL20 orthologs. See Example 7.

[0097] FIG. 15 is a schematic representation of anti-human CCL20 antibody detection in ELISA assays. See Example 7.

[0098] FIGS. 16A-C depict a series of graphs demonstrating that A) chimeric and B), C) humanized anti-human CCL20 antibodies bind effectively to human, rhesus, and cynomolgus CCL20, but not to mouse CCL20. See Example 7.

[0099] FIGS. 17A and B depict graphs demonstrating that humanized and chimeric anti-human CCL20 antibodies bind effectively to human, rhesus, and cynomolgus CCL20, but not to rat or mouse CCL20. The B) humanized and A) chimeric antibodies retain the binding specificity of A) mouse antibody 36F7C10, from which they are derived. See Example 7.

[0100] FIGS. 18A and B show the results of epitope mapping of human CCL20 by hydrogen/deuterium exchange. This experiment utilized a variant of human CCL20 (SEQ ID NO: 84) from R&D Systems in which the second to last residue is a D instead of the N shown in the wild-type sequence. A) The deuteration level of each residue of CCL20 is indicated at four time points (from top: 150 s, 500 s, 1500 s, and 5000 s). B) Structure of human CCL20, indicating an epitope bound by antibodies of the invention. See Example 9.

[0101] FIG. 19 is a pair of graphs demonstrating that mouse CCR6-transduced B300 cells migrate toward both mouse and human CCL20. See Example 10.

[0102] FIG. 20 is a graph showing that mouse, chimeric, and humanized anti-human CCL20 antibodies inhibit mouse T-cell migration toward human CCL20 *in vivo* in a dose-dependent fashion. See Example 10.

[0103] FIG. 21 is a graph showing that hamster anti-mouse CCL-20 MAb 2F5-5 reduces collagen-induced arthritis symptoms in mice. See Example 11.

[0104] FIG. 22 is a table of individual animal clinical scores demonstrating that 2F5-5 MAb reduces progression of collagen-induced arthritis symptoms. See Example 11.

[0105] FIG. 23 is a graph depicting the grading of mouse paws by X-ray scoring. 2F5-5 MAb is shown to reduce bone pathology in mice with collagen-induced arthritis. Cont: control IgG. See Example 11.

[0106] FIG. 24 depicts examples of scored X-rays indicating bone pathology in the paws of mice with collagen-induced arthritis. O: osteoporosis, E: bone erosion, N: new bone formation. See Example 11.

[0107] FIGS. 25A-C show measurements of a marker of cartilage destruction, serum cartilage oligomeric matrix protein (COMP), in a collagen-induced arthritic mouse model. A) Calibrator data with a COMP standard. X axis, units/liter; Y axis, optical density at 450 nm. B) Plate template and raw data for mice treated with a hamster control IgG or 2F5-5 MAb. C) Data from individual animals demonstrates reduced COMP levels in animals treated with 2F5-5 MAb compared to animals treated with control IgG antibodies. See Example 11.

[0108] FIG. 26 is a graph showing that 2F5-5 MAb decreases serum levels of COMP in mice. Ctrl IgG: isotype-matched antibody; CCL20 mAb: 2F5-5 MAb. See Example 11.

[0109] FIGS. 27A and B are graphs showing that 2F5-5 MAb reduces mRNA expression of osteoclast markers A) RANKL and RANK, and B) TRAP and Cathepsin K. Ctrl IgG: isotype-matched antibody; CCL20 mAb: 2F5-5MAb; Y axis: quantitative PCR units. See Example 11.

[0110] FIG. 28 is a graph showing that 2F5-5 MAb has a prophylactic effect on glucose-6-phosphate isomerase-induced arthritis in mice.. See Example 12.

[0111] FIG. 29 is a graph showing that 2F5-5 MAb suppresses the progression of oxazolone-induced atopic dermatitis in mice. See Example 13.

[0112] FIG. 30 is a graph showing that 2F5-5 MAb inhibits dinitrofluorobenzene-induced allergic contact dermatitis (as measured by ear thickness) in mice. See Example 14.

Detailed Description of the Invention

[0113] This invention is directed to antibodies that specifically bind to CCL20 or a portion thereof (*e.g.*, an antigenic portion thereof). The invention is also directed to antigen-binding portions of said antibodies. In one embodiment, the antibodies neutralize one or more activities of CCL20. An antibody is said to bind specifically to CCL20 if it does not substantially bind to non-CCL20 molecules. Substantial binding is, for example, binding with a K_D of $\leq$100 nM, preferably $\leq$ than 10 nM, 1 nM, 100 pM, 50 pM, 40 pM, or 35 pM as determined by Biacore™ (in bivalent format). In one embodiment, the antibodies or portions bind specifically to human CCL20 or a portion thereof, to some sequence variants of human CCL20 such as allelic variants, and may also cross-react with CCL20 from other species. In one embodiment, the antibodies or portions have binding specificity for a wild-type (also referred to as naturally occurring or endogenous) human CCL20. Unless otherwise indicated, "human CCL20" refers to wild-type human CCL20. The amino acid sequence of a wild-type human CCL20 with the signal sequence is shown in FIG. 14 (SEQ ID NO: 85). The amino acid sequence of a wild-type human CCL20 without the signal sequence (residues 1-26 of SEQ ID NO: 85) is found in SEQ ID NO: 99 (see Table 18). FIG. 18 depicts a variant of human CCL20 without the signal sequence (SEQ ID NO: 84) wherein the next to last residue is a D instead of the N as in the wild-type sequence (SEQ ID NO: 85). In some embodiments, the antibodies or portions bind to wild-type human CCL20, or human CCL20 wherein the next to last residue is a D instead of the N shown in the wild-type sequence, either sequence with or without the signal sequence (*e.g.*, SEQ ID NO: 84, 85, or 99). In a particular embodiment, the antibodies or portions bind specifically to human, rhesus, and cynomolgus CCL20 but do not bind to mouse or rat CCL20.

[0114] The antibodies and antigen-binding portions thereof described herein can be purified and/or isolated using known techniques. Antibodies or portions that are "purified" or "isolated" have been

separated away from molecules (*e.g.*, peptides) of their source of origin (*e.g.*, the supernatant of cells; in a mixture such as in a mixture of antibodies in a library; etc.), and include antibodies obtained by methods described herein or other suitable methods. Isolated antibodies include substantially pure (*e.g.*, essentially pure) antibodies, as well as antibodies produced by chemical synthesis, recombinant techniques and a combination thereof.

[0115] More specifically, the invention relates to anti-human CCL20 antibodies, antigen-binding portions (*i.e.*, portions) of the antibodies, the light chains of the antibodies, the heavy chains of the antibodies, and portions of these light chains or heavy chains. The invention also relates to antibodies lacking the heavy and/or light chain signal sequences and glycosylated antibodies. The invention also relates to precursor antibodies, nonglycosylated antibodies, and antibodies whose heavy and/or light chains comprise signal sequences. The invention also relates to nucleic acid molecules that encode any of the antibody heavy chains and/or light chains, or portions thereof described herein; to vectors and host cells that comprise such nucleic acids; to methods of producing any of the antibody heavy and/or light chains or portions thereof described herein; and to methods of using the antibodies, antibody chains, or portions.

[0116] The antibodies and antigen-binding portions thereof of this invention can be used to treat a subject in need thereof (*e.g.*, a human patient) to reduce CCL20 binding to CCR6, CCL20-mediated inflammation, and/or CCL20-mediated chemotaxis of CCR6+ cells as needed.

[0117] Antibodies of the invention include traditional antibodies comprising two heavy chains and two light chains. In some embodiments, one or more of the heavy and/or light chains comprises a variable domain (also referred to herein as a "variable region") and a constant domain (also referred to herein as a "constant region"). Complete variable domains, where present, comprise four framework regions (FRs) and three complementarity determining regions (CDRs), arranged, proceeding from the amino terminus, in the order FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Visual inspection and sequence analysis can be carried out to identify the CDR boundaries. For this invention, the CDR sequences are defined by using the Kabat system (Kabat, E.A. *et al.*, *Sequences of Proteins of Immunological Interest*, Fifth Edition, U.S. Department of Health and Human Services, U.S. Government Printing Office (1991)) and/or the Chothia system (Chothia & Lesk, *Canonical Structures for the Hypervariable Regions of Immunoglobulins*, *J. Mol. Biol.* 196:901-917 (1987)), as indicated in, *e.g.*, FIG. 6.

[0118] Embodiments of the present invention that comprise a human heavy chain constant region may comprise a human constant region of any isotype, including IgG, IgM, IgA, IgD and IgE, in which the heavy chains are of the gamma (γ), mu (μ), alpha (α), delta (δ) or epsilon (ε) type, respectively, and any subclasses including IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2, in which the

heavy chains are of the γ1, γ2, γ3, γ4, α1 and α2 type, respectively. Embodiments comprising human light chains may comprise a human kappa (κ) or a human lambda (λ) light chain.

[0119] As used herein, the term "antibody" refers to a complete antibody (comprising two full-length heavy chains and two full-length light chains). The term "antigen-binding fragment" is used interchangeably herein with the term "antigen-binding portion" unless otherwise indicated. Antigen-binding portions of antibodies can be in the format of, for example, single chain antibodies, Fv fragments, Fab fragments, Fab' fragments, $F(ab')_2$ fragments, Fd fragments, single chain Fv molecules (scFv), bispecific single chain Fv dimers (PCT/US92/09665), diabodies, domain-deleted antibodies and single domain antibodies (dAbs). See, *e.g.*, Jespers et al., *Nature Biotechnology* 22(9):1161-1165 (2004)). Also within the invention are antigen-binding molecules comprising a VH and/or a VL. In the case of a VH, the molecule may also comprise one or more of a CH1, hinge, CH2 and CH3 region.

[0120] Antibody portions can be produced by enzymatic cleavage or by recombinant techniques. For instance, papain or pepsin cleavage can be used to generate Fab or $F(ab')_2$ fragments, respectively. Antibodies also can be produced in a variety of truncated forms using antibody genes in which one or more stop codons have been introduced upstream of the natural stop site. For example, a recombinant construct encoding the heavy chain of an $F(ab')_2$ fragment can be designed to include DNA sequences encoding the CH_1 domain and hinge region of the heavy chain.

[0121] In another embodiment, a fusion antibody or immunoadhesin may be made that comprises all or a portion of an anti-CCL20 antibody of the invention linked to another polypeptide. In one embodiment, only the variable domains of the anti-CCL20 antibody are linked to the polypeptide. In another embodiment, the V_H domain of an anti-CCL20 antibody is linked to a first polypeptide, while the V_L domain of an anti-CCL20 antibody is linked to a second polypeptide that associates with the first polypeptide in a manner such that the V_H and V_L domains can interact with one another to form an antigen binding site. In yet another embodiment, the V_H domain is separated from the V_L domain by a linker such that the V_H and V_L domains can interact with one another (see below under Single Chain Antibodies). The V_H-linker-V_L antibody is then linked to the polypeptide of interest. In addition, fusion antibodies can be created in which two (or more) single-chain antibodies are linked to one another, to create a divalent or polyvalent antibody on a single polypeptide chain, or to create a bispecific antibody.

[0122] To create a single chain antibody of the invention, the V_H- and V_L-encoding DNA fragments are operatively linked to another fragment encoding a flexible linker, *e.g.*, encoding the amino acid sequence $(Gly_4\text{-}Ser)_3$, such that the V_H and V_L sequences can be expressed as a contiguous single-chain protein, with the V_L and V_H domains joined by the flexible linker. See, *e.g.*, Bird *et al.*, *Science* 242:423-426 (1988); Huston *et al.*, *Proc. Natl. Acad. Sci. USA* 85:5879-5883 (1988); McCafferty *et*

al., *Nature* 348:552-554 (1990); etc. In some embodiments, the single chain antibody is monovalent (only a single V_H and V_L are used), bivalent (two V_H and V_L are used), or polyvalent (more than two V_H and V_L are used). The invention also contemplates bispecific or polyvalent antibodies that bind specifically to human CCL20 and to another molecule.

[0123] In other embodiments, other modified antibodies may be prepared using anti-CCL20 antibody-encoding nucleic acid molecules. For instance, "Kappa bodies" (Ill *et al.*, *Protein Eng.* 10:949-57 (1997)), "Minibodies" (Martin *et al.*, *EMBO J.* 13:5303-9 (1994)), "Diabodies" (Holliger *et al.*, *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993)), or "Janusins" (Traunecker *et al.*, *EMBO J.* 10:3655-3659 (1991) and Traunecker *et al.*, *Int. J. Cancer* (Suppl.) 7:51-52 (1992)) may be prepared using standard molecular biological techniques following the teachings of the specification.

[0124] In another aspect, the invention provides a variant of an antibody exemplified herein, or an antigen-binding portion of said variant antibody, wherein said variant antibody binds to human CCL20 specifically but differs in sequence from the exemplified antibody by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more amino acid substitutions (for example, in a CDR region, a FR region, and/or a constant domain). According to the invention, the variant antibody may be at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97% at least 98% or at least 99% identical to the reference antibody in the heavy chain, the heavy chain variable domain, the light chain, the light chain variable domain, the six CDRs, or the eight FRs.

[0125] As used herein, sequence similarity for polypeptides, which is also referred to as sequence identity, is typically measured using sequence analysis software, which matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, the Genetics Computer Group (GCG) Sequence Analysis Package contains programs such as "Gap" and "Bestfit" which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. See, *e.g.*, GCG Version 6.1. Polypeptide sequences can also be compared with FASTA, a program in GCG Version 6.1, using default or recommended parameters. FASTA (*e.g.*, FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, Methods Enzymol. 183:63-98 (1990); Pearson, Methods Mol. Biol. 132:185-219 (2000)). Another algorithm used when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially blastp or tblastn, using default parameters. See, *e.g.*, Altschul *et al.*, J. Mol. Biol. 215:403-410 (1990); Altschul *et al.*, Nucleic Acids Res. 25:3389-402 (1997); herein incorporated by reference.

[0126] According to the invention, one or more cysteines in the antibody, which may be chemically reactive, may be changed to another residue, such as, without limitation, alanine or serine. In one embodiment, a non-canonical cysteine is substituted. The substitution can be made in a CDR or framework region of a variable domain or in the constant domain of an antibody. In some embodiments, the cysteine is canonical. In some embodiments, potential proteolytic sites in the antibody are removed. Such sites may occur in a CDR or framework region of a variable domain or in the constant domain of an antibody. Substitution of cysteine residues and removal of proteolytic sites may decrease the risk of heterogeneity in the antibody product and thus increase its homogeneity. In some embodiments, asparagine-glycine pairs, which form potential deamidation sites, are eliminated by altering one or both of the residues. In some embodiments, the antibody is deimmunized to reduce its immunogenicity. Techniques for reducing the immunogenicity of an antibody are well known in the art. See, *e.g.*, PCT Publication Nos. WO 98/52976 and WO 00/34317.

[0127] In some embodiments, the antibody has one or more conservative amino acid substitutions when compared with an exemplified antibody of the invention. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain R group) with similar chemical properties (*e.g.*, charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. See *e.g.*, Pearson, Methods Mol. Biol. 243:307-31 (1994).

[0128] Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine, and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side chains: aspartic acid and glutamic acid; and 7) sulfur-containing side chains: cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.*, *Science* 256:1443-45 (1992). A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[0129] In certain embodiments, amino acid substitutions to an antibody or antigen-binding portion of the invention are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, for example, to enhance ADCC

and CDC activity of the antibody, (4) confer or modify other physicochemical or functional properties of such analogs, but still retain specific binding to human CCL20, (5) remove C-terminal lysine, and (6) add or remove glycosylation sites.

[0130] In one aspect, the invention provides a new and novel polypeptide that is the heavy or light chain of an antibody of this invention, or that is a variable domain-containing portion of the heavy or light chain. Such a polypeptide is useful because it can partner with a light or heavy antibody chain, respectively, to form an anti-CCL20 antibody.

[0131] Described herein are novel humanized neutralizing anti-CCL20 antibodies comprising the CDRs of novel mouse anti-human CCL20 antibodies, and antigen-binding portions of said humanized antibodies. The term "humanized anti-CCL20 antibody" as used herein refers to an antibody that comprises one or more CDRs (CDR1, CDR2 and CDR3) of an anti-CCL20 antibody of non-human origin, also referred to herein as the donor antibody (*e.g.*, a mouse anti-CCL20 antibody), and at least a portion from a human sequence. The human antibody portion may be one or more framework regions (*e.g.*, all of the framework regions), and/or all or part of a constant region. In some embodiments, the human sequence framework region comprises a germline sequence, but may include non-germline mutations. A CDR-grafted anti-CCL20 antibody in which the six CDRs of a non-human anti-CCL20 antibody are grafted into a human framework is an example of a humanized anti-CCL20 antibody of the invention. See, *e.g.*, Cabilly *et al.*, U.S. Patent No. 4,816,567; Cabilly *et al.*, European Patent No. 0,125,023 B1; Boss *et al.*, U.S. Patent No. 4,816,397; Boss *et al.*, European Patent No. 0,120,694 B1; Neuberger, M.S. *et al.*, WO 86/01533; Neuberger, M.S. *et al.*, European Patent No. 0,194,276 B1; Winter, U.S. Patent No. 5,225,539; Winter, European Patent No. 0,239,400 B1; Padlan, E.A. *et al.*, European Patent Application No. 0,519,596 A1. See also, Ladner *et al.*, U.S. Patent No. 4,946,778; Huston, U.S. Patent No. 5,476,786; and Bird *et al.*, *Science* 242:423-426 (1988)). In some embodiments, humanized antibodies are de-immunized antibodies. See, *e.g.*, Carr *et al.*, U.S. Patent No. 7,264,806, regarding de-immunized antibodies that have been modified to reduce the number of potential T-cell epitopes, thereby reducing the propensity for the antibody to elicit an immune response upon administration to a human.

[0132] In particular embodiments, the humanized antibody comprises one or more light chain CDRs and/or one or more heavy chain CDRs of one or more of the following murine monoclonal anti-human CCL20 antibodies: 36F7C10, 42G5B10, and 40-1C10B9, wherein the CDRs are identified according to the Kabat system, the Chothia system, or any combination thereof. In some embodiments, the humanized antibody comprises all three heavy chain CDRs and all three light chain CDRs of antibody 36F7C10, 42G5B10, or 40-1C10B9.

[0133] In another embodiment, the humanized antibodies have the binding specificity of a murine anti-human CCL20 antibody of the invention (*e.g.*, specificity for human CCL20, the same or similar

epitopic specificity) and/or have a neutralizing activity. The humanized antibodies can have the binding specificity, epitopic specificity, and/or neutralizing activity of a murine, chimeric, or humanized anti-human CCL20 antibody described herein. For example, a humanized antibody of the invention can compete with the murine, chimeric, or humanized anti-human CCL20 antibody for binding to human CCL20, and/or it can have the neutralizing function of the murine, chimeric, or humanized anti-human CCL20 antibody. In a particular embodiment, the humanized antibody has the binding specificity, epitopic specificity and/or neutralizing activity of any one of mouse antibodies 36F7C10, 42G5B10, and 40-1C10B9.

[0134] The human sequence portion of the humanized antibody (*e.g.*, framework region, constant region) can be from any suitable human antibody. For example, a human constant region or portion thereof in a humanized or chimeric antibody can be encoded by a human κ or λ light chain gene, and/or by a human γ (*e.g.*, γ1, γ2, γ3, γ4), μ, α (*e.g.*, α1, α2), δ or ε heavy chain gene, including allelic variants. A particular human constant region isotype (*e.g.*, IgG1, IgG2), variant or portion thereof can be selected to tailor effector function. For example, a mutated constant region (*i.e.*, a variant) can be incorporated into the antibody to reduce binding to an Fc receptor and/or ability to fix complement. (See *e.g.*, Winter *et al.*, GB 2,209,757 B; Morrison *et al.*, WO 89/07142; Morgan *et al.*, WO 94/29351).

[0135] As used herein, the term "germline" refers to the nucleotide sequences and amino acid sequences of the antibody genes and gene segments as they are passed from parents to offspring via the germ cells. This germline sequence is distinguished from the nucleotide sequences encoding a particular antibody in a B cell, which has been altered by recombination and hypermutation events during the course of affinity maturation. An antibody that "utilizes" a particular germline has a nucleotide or amino acid sequence that most closely aligns with the germline nucleotide sequence or with the amino acid sequence that it specifies as compared to other germline sequences. Such antibodies may be encoded by or comprise a sequence that is mutated compared with the germline sequence.

[0136] In some embodiments, the human framework has minimal variation from germline sequence, for example, less than 3, 4, 5, 6, 7, 8, 9, or 10 acceptor framework residues have been replaced to improve one or more properties of the antibody. In some embodiments, acceptor framework residues are replaced with donor framework residues, *e.g.*, to improve binding affinity (see, *e.g.*, Queen *et al.*, U.S. Patent No. 5,530,101). In a particular embodiment, a limited number of amino acids in the framework of a humanized antibody chain (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids) are chosen to be the same as the amino acids at those positions in the donor sequence (*i.e.*, "back-mutated"), rather than in the acceptor sequence, to increase the affinity of an antibody comprising the humanized antibody chain for human CCL20.

[0137] Human framework regions (*e.g.*, of the heavy and/or light chain variable regions) are preferably obtained or derived from a human germline sequence having sequence similarity to the analogous or equivalent region (*e.g.*, heavy or light chain variable regions) of the antigen-binding region of the donor antibody (*e.g.*, murine anti-CCL20 antibody). Other sources of framework regions for human sequence portions of a humanized antibody include human variable region consensus sequences (See *e.g.*, Kettleborough *et al.*, *Protein Engineering* 4:773-783 (1991); Carter *et al.*, WO 94/04679; Carter U.S. Patent No. 6,407,213)). For example, the region of the donor sequence of the antibody (*e.g.*, the sequence of the variable region) used to obtain the nonhuman portion can be compared to human sequences as described in Kabat, E. A. *et al. Sequences of Proteins of Immunological Interest*, Fifth Edition, U.S. Department of Health and Human Services, U.S. Government Printing Office (1991) to select a particular source of the human portions of the humanized antibody, *e.g.*, a source of the framework regions.

[0138] In one embodiment, the framework regions of the humanized antibody chains are obtained, or derived, from a human Ig variable region having at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% or at least about 95% overall sequence identity, with the variable region of the nonhuman donor. In a particular embodiment, the framework regions of the humanized antibody chains are obtained or derived from human variable region framework regions having at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, or at least about 95% overall sequence identity with the framework regions of the variable region of the nonhuman donor antibody.

[0139] In one embodiment, at least one of the framework regions (FR) of the humanized antibody is obtained or derived from one or more chains of a human sequence. Thus, the FR can include a FR1 and/or FR2 and/or FR3 and/or FR4 obtained or derived from one or more human sequence antibodies (*e.g.*, from a human antibody chain, from a human consensus sequence).

[0140] It will be appreciated by one of skill in the art that in some cases residues flanking the one or more CDRs of the murine anti-CCL20 antibod(ies) may contribute, and in some cases, may be essential, either directly or indirectly, to function (*e.g.*, binding). Accordingly, in some embodiments, one or more amino acids flanking one or more CDRs (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more flanking amino acids) of the murine framework are also included in the humanized antibody.

[0141] In some embodiments, the human heavy chain framework regions of the humanized antibodies of this invention utilize the human IGHV1-46*03 germline sequence. In some embodiments, the human light chain framework regions of the humanized antibodies of this invention utilize the human IGKV1D-39*01 germline sequence. Mutations (*e.g.*, back mutations) may

optionally be made in these FR regions, *e.g.*, at one or more of the residues as described in the Examples below to improve CCL20-binding affinity of the humanized antibody.

[0142] "Affinity" is a term of art that describes the strength of a binding interaction and typically refers to the overall strength of binding of the antibody to the antigen. The affinity of the antibody for the antigen is typically expressed as the binding affinity equilibrium constant (K_D) of a particular antibody-antigen interaction.

[0143] In some embodiments, the antibody binds to human CCL20 with an affinity (K_D; $K_D=K_{off}$ (kd)/Kon (ka)) of 500 pM or less, 400 pM or less, 300 pM or less, 200 pM or less, 100 pM or less, 90 pM or less, 80 pM or less, 70 pM or less, 60 pM or less, 50 pM or less, or 45 pM or less as determined by monovalent surface plasmon resonance; or 12 pM or less, 10 pM or less, 8 pM or less, 6 pM or less, or 5 pM or less as determined by bivalent surface plasmon resonance. In some embodiments, the antibody binds to human CCL20 with a k_a of 1000 x 10^5 M^{-1} sec^{-1} or less, 900 x 10^5 M^{-1} sec^{-1} or less, 800 x 10^5 M^{-1} sec^{-1} or less, 700 x 10^5 M^{-1} sec^{-1} or less, 600 x 10^5 M^{-1} sec^{-1} or less, 500 x 10^5 M^{-1} sec^{-1} or less, 400 x 10^5 M^{-1} sec^{-1} or less, 300 x 10^5 M^{-1} sec^{-1} or less, 240 x 10^5 M^{-1} sec^{-1} or less, 200 x 10^5 M^{-1} sec^{-1} or less, 190 x 10^5 M^{-1} sec^{-1} or less, 180 x 10^5 M^{-1} sec^{-1} or less, 170 x 10^5 M^{-1} sec^{-1} or less, 160 x 10^5 M^{-1} sec^{-1} or less, or 150 x 10^5 M^{-1} sec^{-1} or less, as determined by monovalent surface plasmon resonance (Biacore™). In some embodiments, the antibody binds to human CCL20 with a k_d of 1000 x 10^{-5} sec^{-1} or less, 900 x 10^{-5} sec^{-1} or less, 800 x 10^{-5} sec^{-1} or less, 700 x 10^{-5} sec^{-1} or less, 600 x 10^{-5} sec^{-1} or less, 500 x 10^{-5} sec^{-1} or less, 400 x 10^{-5} sec^{-1} or less, 300 x 10^{-5} sec^{-1} or less, 240 x 10^{-5} sec^{-1} or less, 200 x 10^{-5} sec^{-1} or less, 190 x 10^{-5} sec^{-1} or less, 180 x 10^{-5} sec^{-1} or less, 170 x 10^{-5} sec^{-1} or less, 160 x 10^{-5} sec^{-1} or less, 150 x 10^{-5} sec^{-1} or less, 140 x 10^{-5} sec^{-1} or less, 130 x 10^{-5} sec^{-1} or less, 120 x 10^{-5} sec^{-1} or less, 100 x 10^{-5} sec^{-1} or less, 90 x 10^{-5} sec^{-1} or less, 80 x 10^{-5} sec^{-1} or less, 70 x 10^{-5} sec^{-1} or less, or 65 x 10^{-5} sec^{-1} or less as determined by monovalent surface plasmon resonance (Biacore™).

[0144] As is apparent to one of skill in the art, a variety of methods can be used to confirm that antibodies and antigen-binding portions thereof produced according to methods provided herein and known in the art have the requisite specificity (*e.g.*, binding specificity, epitopic specificity). For example, the binding function of a humanized anti-CCL20 antibody or portion of the invention having binding specificity for human CCL20 can be detected using any suitable method, *e.g.*, assays which monitor formation of a complex between the humanized antibody or portion and human CCL20 (or, *e.g.*, a peptide having an amino acid sequence of CCL20 or a solid support comprising human CCL20).

[0145] The ability of an antibody or an antigen-binding portion thereof of the invention (*e.g.*, a humanized antibody or portion of the invention) to bind to the same epitope on human CCL20 as a particular murine, chimeric, or humanized monoclonal antibody disclosed herein, or to bind to an epitope on human CCL20 which overlaps with the epitope on human CCL20 to which a particular

murine, chimeric, or humanized monoclonal antibody disclosed herein binds, can be readily determined using a variety of techniques known to those of skill in the art, including *e.g.*, competitive binding assays. These may involve the use of a labeled form of said particular antibody, and a measurement of the binding of that labeled antibody to human CCL20 in the presence and in the absence of an antibody of the invention.

[0146] An "epitope" as used herein includes any protein determinant capable of specific binding to an antibody. Methods for characterizing the epitope to which an antibody binds are known in the art. One method of characterizing an epitope bound by an anti-CCL20 antibody of the invention is described in Example 9. Once a desired epitope on an antigen is determined, it is possible to generate antibodies to that epitope, *e.g.*, using the techniques described in the present invention. Alternatively, during the discovery process, the generation and characterization of antibodies may elucidate information about desirable epitopes. From this information, it is then possible to competitively screen antibodies for binding to the same epitope. For example, the skilled worker may conduct competition studies to find antibodies that competitively bind with one another, *i.e.*, antibodies that compete for binding to the antigen.

[0147] In one embodiment, to determine if a test antibody or antigen-binding portion thereof binds to the same or overlapping epitope as a humanized antibody of this invention, one allows the anti-CCL20 antibody of the invention to bind to CCL20 under saturating conditions and then measures the ability of the test antibody to bind to CCL20. If the test antibody is able to bind to CCL20 at the same time as the reference anti-CCL20 antibody, then the test antibody may bind to a different epitope than the reference anti-CCL20 antibody. However, if the test antibody is not able to bind to CCL20 at the same time, then the test antibody may bind to the same epitope, an overlapping epitope, or an epitope that is in close proximity to the epitope bound by the anti-CCL20 antibody of the invention. This experiment can be performed using, *e.g.*, ELISA, RIA, Biacore™, or flow cytometry. To test whether an anti-CCL20 antibody cross-competes with another anti-CCL20 antibody, one may use the competition method described above in two directions, *i.e.*, determining if the reference antibody blocks the test antibody and vice versa. In some embodiments, the experiment is performed using Biacore™.

[0148] Epitope binning also can be useful to characterize the antibodies of this invention. The term "binning" refers to a method to group antibodies based on their antigen binding characteristics. A high throughput process for "binning" antibodies based upon their cross-competition is described in International Patent Application No. WO 03/48731. The "epitope binning" can be investigated by allowing an unlabeled form of an anti-CCL20 antibody "A" to bind to a synthetic peptide corresponding to the sequence of CCL20 or to CCL20 positive cells. Subsequently a labeled second anti-CCL20 antibody "B" is added and one can assess the amount of labeled antibody that can bind

relative to a control sample where the cells or synthetic peptide have not been exposed previously to anti-CCL20 antibody "A." Alternatively, anti-CCL20 antibodies "A" and "B" can both be labeled with different fluorochromes or chemicals enabling detection, and one can measure the quantities of both labeled antibodies that can engage the CCL20 peptide at the same time using a device capable of detecting the label or measure the amounts of both antibodies that simultaneously engage CCL20 positive cells by flow cytometry. Biacore™ and Octet technologies enable one to investigate the competitive binding of unlabelled forms of antibodies. This use of unlabelled forms of antibodies is desired as the chemical modification of some antibodies can compromise the binding activity. See also the technology described in Jia *et al., J. Immunol. Methods* 288:91-98 (2004), which is useful in performing epitope binning as well.

[0149] Also provided herein are portions of the anti-CCL20 antibodies of the invention, such as light chains, heavy chains, and portions of light and heavy chains. These antibody portions can be obtained or derived from antibodies (*e.g.*, by reduction and/or cleavage), or produced or expressed by nucleic acids encoding a portion of an antibody or chain thereof having the desired property (*e.g.*, binds human CCL20, sequence similarity). They can also be prepared by *e.g.*, *de* novo synthesis of the relevant portion. Humanized antibodies comprising the desired portions (*e.g.*, antigen-binding region, CDR, FR, C region) of human and nonhuman origin can be produced using synthetic and/or recombinant nucleic acids to prepare constructs (*e.g.*, cDNA) encoding the desired humanized chain. For example, to prepare a portion of an antibody (*e.g.*, a portion of a chain), one or more stop codons can be introduced at the desired position in the nucleic acid sequence. Nucleic acid (*e.g.*, DNA) sequences coding for humanized variable regions can be constructed using PCR mutagenesis methods to alter existing DNA sequences (see *e.g.*, Kamman *et al.*, *Nucl. Acids Res.* 17:5404 (1989)). PCR primers coding for the new CDRs can be hybridized to a DNA template of a previously humanized variable region which is based on the same, or a very similar, human variable region (Sato *et al.*, *Cancer Research* 53:851-856 (1993)). If a similar DNA sequence is not available for use as a template, a nucleic acid comprising a sequence encoding a variable region sequence can be constructed from synthetic oligonucleotides (see *e.g.*, Kolbinger, *Protein Engineering* 8:971-980 (1993)). A sequence encoding a signal peptide ("signal sequence") can also be incorporated into the nucleic acid (*e.g.*, on synthesis, upon insertion into a vector). If a signal peptide sequence is unavailable (*e.g.*, not typically present), a signal peptide sequence from another antibody can be used (see, *e.g.*, Kettleborough, *Protein Engineering* 4:773-783 (1991)). Using these methods, methods described herein or other suitable methods, variants can readily be produced.

[0150] As used herein, the acronym "mAb" (or "MAb") refers to a monoclonal antibody, which may be, *e.g.*, an antibody synthesized by a clonal population of cells or a humanized antibody. A clonal population that produces a monoclonal antibody can be a clonal population of immortalized

cells. In some embodiments, the immortalized cells in the clonal population are hybrid cells - hybridomas - typically produced by the fusion of individual B lymphocytes from an immunized animal with individual cells from a lymphocytic tumour.

[0151] The invention relates in part to a humanized antibody or antigen-binding portion thereof that has binding specificity for human CCL20 and comprises a humanized light chain and a humanized heavy chain and/or portions thereof. In one embodiment, the humanized antibody comprises a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 7 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 1; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 7 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 2; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 7 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 3; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 7 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 4; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 7 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 5; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 7 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 6; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 8 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 1; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 8 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 2; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 8 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 3; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 8 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 4; a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 8 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 5; or a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 8 and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 6.

[0152] In one embodiment, a humanized antibody of the invention comprises heavy chain (H)-CDR1, H-CDR2, H-CDR3, light chain (L)-CDR1, L-CDR2, and L-CDR3 whose amino acid sequences are:

a) SEQ ID NOS: 60, 64, 67, 70, 73, and 75, respectively;

b) SEQ ID NOS: 60, 64, 67, 71, 73, and 75, respectively;

c) SEQ ID NOS: 60, 63, 67, 70, 73, and 75, respectively;

d) SEQ ID NOS: 60, 63, 67, 71, 73, and 75, respectively;

e) SEQ ID NOS: 61, 65, 68, 70, 73, and 75, respectively;

f) SEQ ID NOS: 61, 65, 68, 71, 73, and 75, respectively;

g) SEQ ID NOS: 77, 79, 67, 70, 73, and 75, respectively;

h) SEQ ID NOS: 77, 79, 67, 71, 73, and 75, respectively;

i) SEQ ID NOS: 78, 80, 68, 70, 73, and 75, respectively; and

j) SEQ ID NOS: 78, 80, 68, 71, 73, and 75, respectively.

[0153] In another embodiment, a humanized antibody of this invention comprises an H-CDR3 whose sequence is SEQ ID NO: 67 or 68. In certain embodiments, a humanized antibody of this invention comprises H-CDR3 and L-CDR3 whose sequences are SEQ ID NOS: 67 and 75, respectively; or SEQ ID NOS: 68 and 75, respectively.

[0154] In another embodiment, the humanized antibody has binding specificity for human CCL20 and comprises a light chain comprising one or more CDRs selected from the group consisting of SEQ ID NOS: 70 or 71; 73; and 75, or a combination thereof; and a heavy chain comprising one or more CDRs selected from the group consisting of SEQ ID NOS: 60, 61, 77, or 78; 63, 64, 65, 79, or 80; and 67 or 68; or a combination thereof.

[0155] In another embodiment, the humanized antibody that has a binding specificity for human CCL20 comprises a light chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 7, 8, 110, or 112, and a heavy chain comprising one or more CDRs (*e.g.*, all three CDRs) of SEQ ID NO: 1, 2, 3, 4, 5, 6, or 108.

[0156] The invention also relates to a humanized antibody light chain of the humanized antibody described herein. In one embodiment, the humanized antibody light chain comprises one or more CDRs selected from the group consisting of 70 or 71; 73; and 75, or a combination thereof. For example, the humanized antibody has L-CDR1, L-CDR2, and L-CDR3 whose amino acid sequences are 70, 73, and 75 respectively; or 71, 73, and 75, respectively.

[0157] The invention also relates to a humanized antibody heavy chain of the humanized antibody described herein. In one embodiment, the humanized antibody heavy chain comprises one or more CDRs selected from the group consisting of 60, 61, 77, or 78; 63, 64, 65, 79, or 80; and 67 or 68; or a combination thereof. For example, the humanized antibody has H-CDR1, H-CDR2, and H-CDR3 whose amino acid sequences are:

a) SEQ ID NOS: 60, 63, and 67;

b) SEQ ID NOS: 60, 64, and 67;

c) SEQ ID NOS: 61, 65, and 68;

d) SEQ ID NOS: 77, 79, and 67; or

e) SEQ ID NOS: 78, 80, and 68.

[0158] In one embodiment, a humanized antibody of this invention comprises a light chain comprising a variable domain (V_L) sequence of SEQ ID NO: 15 or 16. In a related embodiment, the humanized antibody comprises a light chain whose amino acid sequence comprises or consists of one of SEQ ID NOS: 7, 8, 110, and 112. In one embodiment, the humanized antibody comprises a light chain whose amino acid sequence comprises or consists of SEQ ID NO: 7 or 8 without the signal sequence.

[0159] In one embodiment, a humanized antibody of this invention comprises a heavy chain comprising a variable domain (V_H) sequence of one of SEQ ID NOS: 9-14. In a related embodiment, the humanized antibody comprises a heavy chain whose amino acid sequence comprises or consists of one of SEQ ID NOS: 1-6 and 108. In one embodiment, the humanized antibody comprises a heavy chain whose amino acid sequence comprises or consists of one of SEQ ID NOS: 1-6 without the signal sequence, and optionally without the C-terminal lysine. In one embodiment, the humanized antibody comprises a heavy chain whose amino acid sequence comprises or consists of SEQ ID NO: 108 without the C-terminal lysine.

[0160] In some embodiments, a humanized antibody of this invention comprises a V_H and a V_L whose amino acid sequences comprise or consist of

a) SEQ ID NO: 9 and SEQ ID NO: 15;
b) SEQ ID NO: 10 and SEQ ID NO: 15;
c) SEQ ID NO: 11 and SEQ ID NO: 15;
d) SEQ ID NO: 12 and SEQ ID NO: 15;
e) SEQ ID NO: 13 and SEQ ID NO: 15;
f) SEQ ID NO: 14 and SEQ ID NO: 15;
g) SEQ ID NO: 9 and SEQ ID NO: 16;
h) SEQ ID NO: 10 and SEQ ID NO: 16;
i) SEQ ID NO: 11 and SEQ ID NO: 16;
j) SEQ ID NO: 12 and SEQ ID NO: 16;
k) SEQ ID NO: 13 and SEQ ID NO: 16; or
l) SEQ ID NO: 14 and SEQ ID NO: 16.

[0161] In one embodiment, a humanized antibody of this invention comprises a light chain (LC) and a heavy chain (HC) whose amino acid sequences comprise or consist of

a) SEQ ID NO: 1 and SEQ ID NO: 7;
b) SEQ ID NO: 2 and SEQ ID NO: 7;
c) SEQ ID NO: 3 and SEQ ID NO: 7;
d) SEQ ID NO: 4 and SEQ ID NO: 7;
e) SEQ ID NO: 5 and SEQ ID NO: 7;

f) SEQ ID NO: 6 and SEQ ID NO: 7;
g) SEQ ID NO: 1 and SEQ ID NO: 8;
h) SEQ ID NO: 2 and SEQ ID NO: 8;
i) SEQ ID NO: 3 and SEQ ID NO: 8;
j) SEQ ID NO: 4 and SEQ ID NO: 8;
k) SEQ ID NO: 5 and SEQ ID NO: 8;
l) SEQ ID NO: 6 and SEQ ID NO: 8,
m) SEQ ID NO: 108 and SEQ ID NO: 110; and
n) SEQ ID NO: 108 and SEQ ID NO: 112;

wherein the amino acid sequences lack the signal sequence, if present, and wherein SEQ ID NOS: 1-6 and SEQ ID NO: 108 optionally lack the C-terminal lysine.

[0162] The present invention provides any combination comprising an exemplified humanized heavy chain or an antigen-binding portion thereof, and an exemplified humanized light chain or an antigen-binding portion thereof, of the invention; in other words, the heavy and light chains can be "mixed and matched." It is understood that any such combination is likely to retain binding to human CCL20 as well as chemotaxis neutralizing activity. These functional properties can readily be tested using methods described herein. Indeed, FIG. 7 demonstrates the neutralizing activity of several combinations of exemplified humanized heavy and light chains of the invention.

[0163] This invention also provides anti-human CCL20 antibodies or antigen-binding portions thereof that bind to the same epitope as, and/or compete or cross-compete with, an antibody exemplified herein. These antibodies can be, for example, humanized, chimeric, or mouse antibodies. For example, the invention provides anti-human CCL20 antibodies and portions that bind to the same epitope as, and/or compete or cross-compete with, one of mouse anti-CCL20 antibodies 36F7C10, 40-1C10B9, and 42G5B10, or humanized or chimeric versions of these mouse antibodies. The ability of an antibody to bind to the same epitope as, or compete or cross-compete with, a reference antibody, can be determined as described herein. By way of nonlimiting example, any antibody or portion comprising the three heavy chain CDRs and the three light chain CDRs from mouse antibody 36F7C10 would be expected to bind to the same epitope as, compete with, and cross-compete with mouse antibody 36F7C10. Such antibodies may include, *e.g.*, antibodies whose heavy chain comprises any one of SEQ ID NOS: 9-11 and whose light chain comprises SEQ ID NO: 15 or 16. In some embodiments, such antibodies may further include, *e.g.*, antibodies whose heavy chain comprises any one of SEQ ID NOS: 12-14 and whose light chain comprises SEQ ID NO: 15 or 16.

[0164] If desired, for example, for diagnostic or assay purposes (*e.g.*, imaging to allow, for example, monitoring of therapies), the humanized antibody (or an antigen-binding portion thereof) can comprise a detectable label. Suitable detectable labels and methods for labeling a humanized

antibody or antigen-binding portion thereof are well known in the art. Suitable detectable labels include, for example, a radioisotope (*e.g.*, as Indium-111, Technnetium-99m or Iodine-131), positron emitting labels (*e.g.*, Fluorine-19), paramagnetic ions (*e.g.*, Gadlinium (III), Manganese (II)), an epitope label (tag), an affinity label (*e.g.*, biotin, avidin), a spin label, an enzyme, a fluorescent group or a chemiluminescent group. When labels are not employed, complex formation (*e.g.*, between a humanized antibody and human CCL20) can be determined by surface plasmon resonance, ELISA, FACS, or other suitable methods.

[0165] Anti-CCL20 antibodies or antigen-binding portions thereof used in the invention also may be conjugated, for example, via chemical reactions or genetic modifications, to other moieties (*e.g.*, pegylation moieties) that improve the antibodies' pharmacokinetics such as half-life. In some embodiments, the anti-CCL20 antibodies used in this invention can be linked to a suitable cytokine, *e.g.*, via chemical conjugation or genetic modifications (*e.g.*, appending the coding sequence of the cytokine in frame to an antibody coding sequence, thereby creating an antibody:cytokine fusion protein).

[0166] The invention also relates to immunoconjugates in which the humanized antibody (or an antigen-binding portion thereof) of the invention is coupled to another therapeutic agent, such as a bioactive compound (*e.g.*, a cytokine, a superantigen, a cytotoxic agent or a toxin). For example, the humanized antibody that has binding specificity for human CCL20 (or an antigen binding portion thereof) can be coupled to a biological protein, a molecule of plant or bacterial origin (or derivative thereof), an interleukin-2 antibody or diptheria toxin antibodies.

[0167] As described herein, mouse monoclonal antibodies having binding specificity for human CCL20 have been produced. Humanized and chimeric antibodies of this invention can be derived from the mouse monoclonal antibodies of this invention. That is, in some embodiments, humanized and chimeric anti-CCL20 antibodies of the invention comprise sequences taken from a mouse monoclonal antibody of the invention, such as one or more CDR sequences (*e.g.*, all six CDR sequences) or one or more variable domains (*e.g.*, the heavy chain variable domain and the light chain variable domain).

[0168] As used herein, the term "mouse monoclonal antibody" refers to an antibody containing light chain CDRs (L-CDR1, L-CDR2 and L-CDR3) and heavy chain CDRs (H-CDR1, H-CDR2 and H-CDR3) of a murine antibody, and framework and constant regions of murine origin.

[0169] The invention relates to the mouse monoclonal antibodies described herein, as well as antigen-binding portions of the mouse monoclonal antibodies, the light chains of the mouse monoclonal antibodies, the heavy chains of the mouse monoclonal antibodies, and portions of these heavy and light chains. In a particular embodiment, the mouse monoclonal antibody is 36F7C10, 40-1C10B9, or 42G5B10. The invention relates to mouse monoclonal antibodies lacking the heavy and

light chain signal sequences and mouse monoclonal antibodies that are glycosylated. The invention also relates to precursor antibodies, nonglycosylated antibodies, and antibodies whose heavy and/or light chains comprise signal sequences. The invention also relates to nucleic acid molecules that encode any of the above mouse antibody heavy chains, light chains, or portions thereof; to vectors and host cells that comprise such nucleic acids; to methods of producing any of the above mouse heavy or light chains or portions thereof; and to methods of using the mouse antibodies or antigen-binding portions thereof.

[0170] The binding function of a mouse monoclonal antibody or antigen-binding portion thereof having binding specificity for human CCL20 can be detected using any suitable method, for example using assays which monitor formation of a complex between a mouse monoclonal antibody or portion and human CCL20 (or, *e.g.*, a peptide having an amino acid sequence of CCL20 or a solid support comprising human CCL20).

[0171] Also provided herein are portions of the murine antibodies which include light chains, heavy chains and portions of light and heavy chains. These antibody portions can be obtained or derived, *e.g.*, by means described herein for humanized antibody portions.

[0172] In one embodiment, a mouse monoclonal antibody of this invention comprises a light chain comprising SEQ ID NO: 40, 42, or 44 and further comprises a heavy chain comprising SEQ ID NO: 39, 41, or 43. In a certain embodiment, the mouse monoclonal antibody comprises a light chain comprising SEQ ID NO: 40 and a heavy chain comprising SEQ ID NO: 39; a light chain comprising SEQ ID NO: 42 and a heavy chain comprising SEQ ID NO: 41; or a light chain comprising SEQ ID NO: 44 and a heavy chain comprising SEQ ID NO: 43.

[0173] In another embodiment, the invention also relates to a mouse monoclonal antibody that has binding specificity for human CCL20, comprising the light chain variable region in a sequence selected from the group consisting of SEQ ID NOS: 40, 42, and 44; and a heavy chain variable region in a sequence selected from the group consisting of SEQ ID NOS: 39, 41, and 43.

[0174] The invention also relates to a mouse monoclonal antibody whose light chain comprises the variable region in SEQ ID NO: 40, 42, or 44.

[0175] The invention also relates to a mouse monoclonal antibody whose heavy chain comprises the variable region in SEQ ID NO: 39, 41, or 43.

[0176] If desired, for example, for diagnostic or assay purposes (*e.g.*, imaging), the mouse monoclonal antibody or antigen binding portion thereof can comprise a detectable label *e.g.*, as described herein for humanized antibodies. All suitable methods and techniques described herein for humanized antibodies of this invention can also be used for mouse monoclonal antibodies of the invention.

[0177] As described herein, chimeric antibodies having binding specificity for human CCL20 have been produced. As used herein, the term "chimeric antibody" refers to a recombinant protein that contains the variable domains of an antibody derived from one species while the constant domains of the antibody are derived from a different species. In one embodiment, the chimeric antibody with binding specificity for human CCL20 comprises variable domains from a mouse anti-human CCL20 monoclonal antibody. In one embodiment, the chimeric antibody with binding specificity for human CCL20 comprises constant domains from a human antibody. In a particular embodiment, the chimeric antibody comprises variable domains from a mouse anti-human CCL20 monoclonal antibody and constant domains from a human antibody.

[0178] The invention relates to the chimeric antibodies described herein, as well as antigen-binding portions of the chimeric antibodies, the light chains and heavy chains of the chimeric antibodies, and portions of these light and heavy chains. The invention relates to chimeric antibodies lacking the heavy and light chain signal sequences and chimeric antibodies that are glycosylated. The invention also relates to precursor antibodies, nonglycosylated antibodies, and antibodies whose heavy and/or light chains comprise signal sequences. The invention also relates to nucleic acid molecules that encode any of the above chimeric antibody heavy chains, light chains, or portions thereof; to vectors and host cells that comprise such nucleic acids; to methods of producing these any of the above chimeric antibody heavy or light chains or portions thereof; and to methods of using the chimeric antibodies.

[0179] The binding function of a chimeric antibody having binding specificity for human CCL20 can be detected using any suitable method, for example using assays which monitor formation of a complex between a chimeric antibody and human CCL20 (or, *e.g.*, a peptide having an amino acid sequence of CCL20 or a solid support comprising human CCL20).

[0180] Also provided herein are portions of the chimeric antibodies which include light chains, heavy chains and portions of light and heavy chains. These antibody portions can be obtained or derived, *e.g.*, by means described herein for humanized antibody portions.

[0181] In one embodiment, a chimeric antibody of this invention comprises the light chain variable region of SEQ ID NO: 40 and the heavy chain variable region of SEQ ID NO: 39; the light chain variable region of SEQ ID NO: 42 and the heavy chain variable region of SEQ ID NO: 41; or the light chain variable region of SEQ ID NO: 44 and the heavy chain variable region of SEQ ID NO: 43.

[0182] The invention also relates to a chimeric antibody that has binding specificity for human CCL20, comprising a light chain variable region sequence selected from the group consisting of: the light chain variable region in SEQ ID NO: 40, 42, or 44; and further comprising a heavy chain variable region sequence selected from the group consisting of: the heavy chain variable region in SEQ ID NO: 39, 41, or 43.

[0183] The invention also relates to a chimeric light chain comprising the variable region in SEQ ID NO: 40, 42, or 44.

[0184] The invention also relates to a chimeric heavy chain comprising the variable region in SEQ ID NO: 39, 41, or 43.

[0185] If desired, for example, for diagnostic or assay purposes (*e.g.*, imaging), the chimeric antibody or antigen-binding portion thereof can comprise a detectable label, *e.g.*, as described herein for humanized antibodies. All suitable methods and techniques described herein for humanized antibodies of this invention can also be used for chimeric antibodies of the invention.

[0186] In some embodiments, the anti-CCL20 antibody of the invention is a fully human antibody. As used herein, the term "human antibody" means any antibody in which the variable and constant domain sequences are human sequences. The term encompasses antibodies with sequences derived from human genes, but which have been changed, *e.g.* to decrease possible immunogenicity, increase affinity, eliminate cysteines that might cause undesirable folding, etc. The term also encompasses such antibodies produced recombinantly in non-human cells, which might impart glycosylation not typical of human cells. Methods for preparing fully human antibodies are known in the art. For example, human anti-CCL20 antibodies may be identified through in vitro methods, such as phage display, ribosome display (CAT), yeast display, and the like, or may be produced from a human B-cell or a human hybridoma cell. Alternatively, human antibodies may be produced by immunizing with a CCL20 antigen any of a number of non-human, transgenic animals comprising within their genomes some or all of human immunoglobulin heavy chain and light chain loci. In some embodiments, the non-human animal comprising human immunoglobulin genes is an animal that has a human immunoglobulin "minilocus" (*e.g.*, GenPharm International, Inc.). In some embodiments, human anti-CCL20 antibodies are produced using a XENOMOUSE® (Abgenix, Inc., Fremont, CA), a HuMAb- Mouse® (Medarex, Inc.), a VelocImmune® mouse (Regeneron Pharmaaceuticals, Inc.), an AlivaMab Mouse (Ablexis, LLC), a KM™ mouse (Kirin Pharma USA, Inc.), or the like.

[0187] The present invention also relates to isolated and/or recombinant nucleic acids comprising sequences which encode a humanized antibody or a light chain or heavy chain thereof, mouse monoclonal antibody or a light chain or heavy chain thereof, chimeric antibody or a light chain or heavy chain thereof, or antigen-binding portions of any of the above, of the present invention. In some embodiments, the present invention provides a nucleic acid sequence selected from the group consisting of SEQ ID NOS: 17-32, 51-56, 109, 111, and 113.

[0188] In some embodiments, the nucleic acid molecules of the invention include nucleic acid sequences that hybridize under highly stringent conditions, or that are at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% identical, to one or more of the nucleic acid sequences recited herein or

to a nucleic acid sequence encoding the amino acid sequence of any of the provided V_H or V_L sequences.

[0189] Nucleic acids referred to herein as "isolated" or "purified" are nucleic acids which have been separated away from the nucleic acids of the genomic DNA or cellular RNA of their source of origin (*e.g.*, as they exist in cells or in a mixture of nucleic acids such as a library), and include nucleic acids obtained by methods described herein or other suitable methods. In some embodiments, the isolated nucleic acids are essentially pure nucleic acids, nucleic acids produced by chemical synthesis, nucleic acids produced by combinations of biological and chemical methods, or recombinant nucleic acids which are isolated (see, *e.g.*, Daugherty *et al., Nucleic Acids Res.* 19(9):2471-2476 (1991); Lewis and Crowe, *Gene* 101:297-302 (1991)).

[0190] A reference to a nucleotide sequence encompasses its complement unless otherwise specified. Thus, a reference to a nucleic acid having a particular sequence should be understood to encompass its complementary strand, with its complementary sequence. The term "polynucleotide" as referred to herein means a polymeric, possibly isolated, form of nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms.

[0191] Nucleic acids referred to herein as "recombinant" are nucleic acids which have been produced by recombinant DNA methodology, including those nucleic acids that are generated by procedures which rely upon a method of artificial recombination, such as the polymerase chain reaction (PCR) and/or cloning into a vector using restriction enzymes. In some embodiments, the recombinant nucleic acids result from recombination events that occur through the natural mechanisms of cells, but are selected for after the introduction into the cells of nucleic acids designed to allow and make probable a desired recombination event.

[0192] The present invention also relates more specifically to isolated and/or recombinant nucleic acids comprising a nucleotide sequence which encodes a humanized antibody, mouse antibody or chimeric antibody, or an antigen-binding portion of said antibody, that has binding specificity for human CCL20. In some embodiments, the antibody is a mouse antibody of the present invention, a humanized antibody of the present invention in which the nonhuman portion(s) are derived from a murine anti-CCL20 monoclonal antibody; or a chimeric antibody of the present invention in which the nonhuman portion(s) are derived from a murine anti-CCL20 monoclonal antibody.

[0193] In some embodiments, nucleic acids of the invention are used to produce humanized antibodies having binding specificity for human CCL20, mouse antibodies having binding specificity for human CCL20, and chimeric antibodies having binding specificity for human CCL20. For example, a nucleic acid (*e.g.*, DNA (such as cDNA) or RNA) or one or more nucleic acids encoding a humanized antibody, mouse antibody or chimeric antibody of the present invention can be

incorporated into a suitable construct (*e.g.*, a recombinant vector) for further manipulation of sequences or for production of the encoded antibodies in suitable host cells.

[0194] Constructs or vectors suitable for the expression of a humanized antibody having binding specificity for human CCL20, mouse antibody having binding specificity for human CCL20 or chimeric antibody having binding specificity for human CCL20 are also provided. A variety of vectors are available, including vectors which are maintained in single copy or multiple copies in a host cell, or which become integrated into the chromosome(s) of a host cell. The constructs or vectors can be introduced into a suitable host cell, and cells which express a humanized antibody, mouse antibody or chimeric antibody of the present invention, can be produced and maintained in culture. A single vector or multiple vectors can be used for the expression of a humanized antibody, mouse antibody or chimeric antibody having binding specificity for human CCL20.

[0195] Suitable expression vectors, for example mammalian cell expression vectors, can also contain a number of components, including, but not limited to, one or more of the following: an origin of replication; a selectable marker gene; one or more expression control elements such as a transcriptional control element (*e.g.*, a promoter, an enhancer, a terminator), one or more translation signals; and/or a signal sequence or leader sequence (encoding a "signal peptide") for membrane targeting or secretion. In a construct or vector, a signal sequence can be provided by the construct or vector or other source. For example, transcriptional and/or translational signals can be used to direct expression.

[0196] In some embodiments, a promoter is provided for expression of an antibody or antibody chain of the invention in a suitable host cell. In some embodiments, the promoter is constitutive. In some embodiments, the promoter is inducible. The promoter may be operably linked to a nucleic acid encoding an antibody or antibody chain, or an antigen-binding portion of said antibody or chain, such that it directs expression of the encoded polypeptide. A variety of suitable promoters for prokaryotic (*e.g.*, lac, tac, T3, T7 promoters for *E. coli)* and eukaryotic (*e.g.*, yeast alcohol dehydrogenase (ADH1), SV40, CMV) hosts are available. Those of skill in the art will be able to select the appropriate promoter for expressing an anti-CCL20 antibody or antigen-binding portion thereof of the invention.

[0197] In some embodiments the vector encoding an antibody or antibody chain of the invention comprises a selectable marker for selection of host cells carrying the vector. In some embodiments, the selectable marker is a gene encoding a product that confers antibiotic or drug resistance that may be used in prokaryotic cells (*e.g.*, β-lactamase gene (ampicillin resistance), Tet gene (tetracycline resistance), etc.) and eukaryotic cells (*e.g.*, neomycin (G418 or geneticin), gpt (mycophenolic acid), ampicillin, or hygromycin resistance genes). In some embodiments, the selectable marker is dihydrofolate reductase, permitting selection with methotrexate in a variety of hosts. In some embodiments, the selectable marker is a gene encoding an auxotrophic marker of the host (*e.g.*, *LEU2, URA3, HIS3)*, *e.g.*, for use

in yeast. In some embodiments, the vector is a viral (*e.g.*, baculovirus) or phage vector. In one embodiment, the vector is capable of integrating into the genome of the host cell (*e.g.*, retroviral vector). In some embodiments, the vector is a replicable vector and comprises an origin of replication.

[0198] The invention thus relates to isolated nucleic acid molecules that encode the humanized antibody, humanized light chain, humanized heavy chain, mouse antibody, mouse antibody light chain, mouse antibody heavy chain, chimeric antibody, chimeric light chain, or chimeric heavy chain of this invention. The invention also relates to isolated nucleic acid molecules that encode an antigen-binding portion of any of these antibodies or their chains. Polypeptide sequences encoded by the nucleic acids of this invention are described above and in the following Examples.

[0199] In some embodiments, a nucleic acid or vector of this invention encodes a heavy chain (or an antigen-binding portion thereof) or a light chain (or an antigen-binding portion thereof) of the invention. A host cell containing both the heavy chain-encoding nucleic acid and the light chain-encoding nucleic acid, or nucleic acids encoding antigen-binding portions of said heavy chain and said light chain, can be used to make an antibody comprising a heavy and a light chain (or an antigen-binding portion of the antibody). The heavy chain-encoding nucleic acid and the light chain-encoding nucleic acid can be placed on separate expression vectors. They can also be placed on a single expression vector under the same or different expression control. *See, e.g.*, U.S. Patent Nos. 6,331,415 and 7,662,623.

[0200] Another aspect of the invention relates to a method of making an anti-human CCL20 antibody or an antigen-binding portion thereof of the invention. The antibody or portion can be produced, for example, by the expression of one or more recombinant nucleic acids encoding the antibody or portion in a suitable host cell. The host cell can be produced using any suitable method. For example, one or more expression constructs described herein can be introduced into a suitable host cell, and the resulting cell can be maintained under conditions suitable for expression of the construct(s) or vector(s). In some embodiments, the resulting cell is maintained in culture, in an animal, or in a plant. Suitable host cells can be prokaryotic, including bacterial cells such as *E. coli* (*e.g.*, strain DH5α™ (Invitrogen, Carlsbad, CA)), *B. subtilis* and/or other suitable bacteria; eukaryotic cells, such as fungal or yeast cells (*e.g.*, *Pichia pastoris*, *Aspergillus sp.*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Neurospora crassa*), or other lower eukaryotic cells, and cells of higher eukaryotes such as those from insects (*e.g.*, *Drosophila* Schneider S2 cells, Sf9 insect cells (WO 94/26087 (O'Connor), TN5B1-4 (HIGH 5) insect cells (Invitrogen), mammals (*e.g.*, COS cells, such as COS-1 (ATCC Accession No. CRL-1650) and COS-7 (ATCC Accession No. CRL-1651), CHO (*e.g.*, ATCC Accession No. CRL-9096), CHO DG44 (Urlaub and Chasin., *Proc. Natl. Acac. Sci. USA* 77(7):4216-4220 (1980)), 293 (ATCC Accession No. CRL-1573), HeLa (ATCC Accession No. CCL-2), CV1 (ATCC Accession No. CCL-70), WOP (Dailey *et al.*, *J. Virol.* 54:739-749 (1985)), 3T3, 293T (Pear *et al.*, *Proc. Natl.*

Acad. Sci. U.S.A. 90:8392-8396 (1993)), NS0 cells, SP2/0 cells, HuT 78 cells and the like)), or plants (*e.g.*, tobacco, lemna (duckweed), and algae). (See, for example, Ausubel *et al.*, eds. *Current Protocols in Molecular Biology*, Greene Publishing Associates and John Wiley & Sons Inc. (1993)). In some embodiments, the host cell is not part of a multicellular organism (*e.g.*, plant or animal). In certain embodiments, the host cell is an isolated host cell or is part of a cell culture.

[0201] The present invention also relates to cells comprising a nucleic acid or a vector of the invention. In some embodiments, the vector is an expression vector. In some embodiments, one or more nucleic acids encoding the heavy and light chains of a humanized antibody or antigen-binding portion thereof, the heavy and light chains of a mouse antibody or antigen-binding portion thereof, or the heavy and light chains of a chimeric antibody or antigen-binding portion thereof, said antibody or portion having binding specificity for human CCL20, or one or more constructs comprising such nucleic acid(s), can be introduced into a suitable host cell by a method appropriate to the host cell selected. In some embodiments, the method of introduction is, *e.g.*, transformation, transfection, electroporation, or infection. In some embodiments, the nucleic acid(s) are operably linked to one or more expression control elements. In certain embodiments, the nucleic acid(s) are in a vector, in a construct created by processes in the cell, or integrated into the host cell genome. Host cells can be maintained under conditions suitable for expression. In some embodiments, these conditions comprise the presence of an inducer, or of suitable media (supplemented with, *e.g.*, appropriate salts, growth factors, antibiotic, nutritional supplements, etc.), whereby the encoded polypeptide(s) are produced. These processes encompasses expression in a host cell (*e.g.*, a mammary gland cell) of a transgenic animal or plant (*e.g.*, tobacco) (see *e.g.*, WO 92/03918). In some embodiments, the antibodies or portions are isolated from the host cells, culture medium, or milk.

[0202] The invention also relates to fusion proteins in which an antibody or portion of the invention (*e.g.*, a humanized antibody or portion) is linked to another moiety (*e.g.*, a moiety which does not occur in antibodies as found in nature) in an N-terminal location, C-terminal location or internal to the fusion protein. In some embodiments, the fusion protein can be produced by the insertion of a nucleic acid encoding an antibody sequence(s) into a suitable expression vector, such as a pET vector (*e.g.*, pET-15b, Novagen), a phage vector (*e.g.*, pCANTAB 5 E, Pharmacia), or other vector (*e.g.*, pRIT2T Protein A fusion vector, Pharmacia). The resulting construct can be introduced into a suitable host cell for expression. In some embodiments, the expressed fusion proteins are isolated or purified from a cell lysate by means of a suitable affinity matrix (see, *e.g.*, *Current Protocols in Molecular Biology* (Ausubel *et al.*, Eds., Vol. 2, Suppl. 26, pp. 16.4.1-16.7.8 (1991))).

[0203] The invention relates to a host cell that comprises recombinant nucleic acid(s) encoding an antibody or portion, or heavy and/or light chains thereof, provided herein (*e.g.*, a humanized antibody, a humanized light chain or a humanized heavy chain, a mouse antibody, a mouse light chain or a

mouse heavy chain, a chimeric antibody, or a chimeric heavy chain or a chimeric light chain, of the invention). The invention also relates to a host cell that comprises recombinant nucleic acid(s) encoding an antigen-binding portion of the antibody or its chains. In some embodiments, the host cell comprises a recombinant vector of the invention as referred to herein. In some embodiments, said recombinant vector is an expression vector. In certain embodiments, said recombinant vector is a mammalian cell expression vector.

[0204] The invention also relates to a method of preparing an antibody or portion, or a heavy or light chain thereof, of this invention. In one embodiment, the method comprises maintaining a host cell of the invention as described herein under conditions appropriate for expression of the antibody or portion or heavy and/or light chain thereof. In some embodiments, the host cell contains one or more isolated nucleic acids that encode the antibody or portion, or heavy and/or light chain thereof, of the invention. In some embodiments, the host cell is cultured on a substrate or in suspension. In some embodiments, the method further comprises the step of purifying or isolating the antibody or antibody chain.

[0205] The invention further relates to a method of preparing antibodies or antigen-binding portions thereof through phage display. In some embodiments, a naïve antibody phage display library on CCL20 antigen is panned. In some embodiments, a method of preparing antibodies through guided selection is used (see, *e.g.*, U.S. Patent Publication No. US 2006-0251658 A1). In certain embodiments, a custom library built around, for example, a fixed heavy chain (and/or light chain) CDR3 region of a known anti-CCL20 antibody is created. The CDR1 and CDR2 regions of the heavy and light chains can be derived from a naïve repertoire (Osburn *et al., Methods* 36:61-68 (2005)). In one embodiment, anti-CCL20 scFvs are generated from scFv naïve antibody libraries which are used to obtain mouse-human chimeric antibodies with the desired binding properties. These libraries may be screened for antibodies with the desired binding properties. In some embodiments, scFv phage libraries are used. In certain embodiments, scFvs which recognize human CCL20 are isolated from scFv guided selection libraries following a series of repeated selection cycles on recombinant human CCL20 essentially as described in Vaughan *et al. Nature Biotech.* 14:309-314 (1996). In brief, following incubation with the library, the immobilized antigen, which is pre-coupled to paramagnetic beads, and bound phage can be recovered by magnetic separation while unbound phage is washed away. Bound phage can then be rescued as described by Vaughan *et al.* (1996; *supra*) and the selection process repeated.

[0206] In a particular embodiment, a library is constructed consisting of the entire variable domain of the heavy chain of a mouse anti-CCL20 antibody fused in a single chain format to a repertoire of naïve human light chain variable regions. After selection, the human light chain variable regions that complement the mouse heavy chain variable regions are identified. A library is then constructed

consisting of the repertoire of human light chain variable regions selected as described above fused in a single chain format to a chimeric heavy chain variable region consisting of naïve human CDR1 and CDR2 regions and a fixed CDR3 region from the mouse anti-CCL20 antibody heavy chain variable domain. After selection for CCL20 binders, the best binding clones are selected. Five of the six CDR regions can be human in origin while the CDR3 of the heavy chain variable region can be identical to the original CDR3 of the mouse heavy chain variable domain.

[0207] In some embodiments, selections are performed using CCL20 coupled to DYNABEADS M-270 amine (Dynal) according to the manufacturer's recommendations. In some embodiments, selections using biotinylated CCL20 can be prepared using the primary amine specific reagent succinimidyl-6-(biotinamido) hexanoate following the manufacturer's instructions (EZ link NHS LC Biotin, Pierce).

[0208] In some embodiments outputs from selections are tested as periplasmic preparations in high throughput screens based on competition assays which measure the ability of the scFvs present in the periplasmic preparation to compete for binding to CCL20.

[0209] Samples that are able to compete in the high throughput screens may be subjected to DNA sequencing as described in Vaughan *et al.* (1996; *supra*) and Osburn *et al.* (2005; *supra*). Clones may then be expressed and purified as scFvs or IgGs and assessed for their ability to bind CCL20, neutralize CCL20 or a combination thereof, *e.g.*, using assays such as antibody-dependent cell mediated cytotoxicity (ADCC) assay and complement dependent cytotoxicity (CDC) assay. Purified scFv preparations can then be prepared as described in Example 3 of WO 01/66754. Protein concentrations of purified scFv preparations were determined using the BCA method (Pierce). Similar approaches can be used to screen for an optimal partner (the opposite chain) of a fixed full-length antibody heavy or light chain or heavy or light chain variable domain.

[0210] In another embodiment, an anti-human CCL20 antibody or an antigen-binding portion thereof, as described herein, is first used to select heavy and light chain sequences having similar binding activity toward CCL20, using the epitope imprinting methods described in PCT Publication No. WO 93/06213, incorporated herein by reference. In certain embodiments, the antibody libraries used in this method are scFv libraries prepared and screened as described in PCT Publication No. WO 92/01047, McCafferty *et al.*, *Nature* 348:552-554 (1990); and Griffiths *et al.*, *EMBO J.* 12:725-734 (1993); all incorporated herein by reference. In certain embodiments, the scFv antibody libraries are screened using human CCL20 as the antigen.

[0211] Once initial V_L and V_H domains are selected, "mix and match" experiments can be performed, in which different pairs of the initially selected V_L and V_H segments are screened for CCL20 binding to select preferred V_L/V_H pair combinations. Additionally, to further improve the quality of the antibody, the V_L and V_H segments of the preferred V_L/V_H pair(s) can be randomly

mutated in a process analogous to the *in vivo* somatic mutation process responsible for affinity maturation of antibodies during a natural immune response. In some embodiments, the random mutations occur within the CDR3 region of V_H and/or V_L. In certain embodiments, this *in vitro* affinity maturation is accomplished by, *e.g.*, amplifying V_H and V_L domains using PCR primers complimentary to the V_H CDR3 or V_L CDR3, respectively, wherein the primers have been "spiked" with a random mixture of the four nucleotide bases at certain positions such that the resultant PCR products encode V_H and V_L segments into which random mutations have been introduced into the V_H and/or V_L CDR3 regions. These randomly mutated V_H and V_L segments can be re-screened for binding to CCL20.

[0212] Following screening and isolation of an anti-CCL20 antibody of the invention from a recombinant antibody display library, nucleic acids encoding the selected antibody can be recovered from the display package (*e.g.*, from the phage genome) and subcloned into other expression vectors by standard recombinant DNA techniques. In some embodiments, the nucleic acid is further manipulated to create other antibody forms of the invention, as described herein. In certain embodiments, to express a recombinant human antibody isolated by screening of a combinatorial library, the DNA encoding the antibody is cloned into a recombinant expression vector and introduced into mammalian host cells, as described herein.

[0213] In a particular embodiment, the invention provides a method of producing a hybridoma that secretes a monoclonal antibody that has binding specificity for human CCL20, comprising administering lymphocytes of a CCL20 transgenic mouse to a non-transgenic mouse having the same strain (*e.g.*, CD1) as the human CCL20 transgenic mouse, thereby producing an immunized, non-transgenic mouse. Splenocytes of the immunized, non-transgenic mouse are contacted with immortalized cells, thereby producing fused cells, and the fused cells are maintained under conditions in which hybridomas that secrete a monoclonal antibody having binding specificity for human CCL20 are produced, thereby producing a hybridoma that secretes a monoclonal antibody that has binding specificity for human CCL20.

[0214] The present invention also provides methods of preparing mutated forms of the anti-CCL20 antibodies and antigen-binding portions thereof of the invention. In some embodiments, the antibodies or portions are mutated in the variable domains of the heavy and/or light chains. In certain embodiments, said mutation alters one or more binding properties of the antibody or portion. In particular embodiments, a mutation is made in one or more of the CDR regions to increase or decrease the K_D of the anti-CCL20 antibody or portion, to increase or decrease k_{off}, or to alter the binding specificity of the antibody or portion. Techniques in site-directed mutagenesis are well-known in the art. See *e.g.* Sambrook *et al.* and Ausubel *et al.*, *supra*. In another embodiment, one or more mutations are made at an amino acid residue that is known to be changed compared to the

germline in a monoclonal antibody of the invention. In certain embodiments, the mutations are made in a CDR region or framework region of a variable domain, or in a constant domain. In a particular embodiment, the mutations are made in a variable domain. In some embodiments, one or more mutations are made at an amino acid residue that is known to be changed compared to the germline in a CDR region or framework region of a variable domain of an antibody or portion of the invention.

[0215] In another embodiment, the framework region is mutated so that the resulting framework region(s) have the amino acid sequence of the corresponding germline gene. In certain embodiments, one or more mutations are made in a framework region or constant domain to increase the half-life of the anti-CCL20 antibody or portion. See *e.g.* PCT Publication WO 00/09560. In certain embodiments, a mutation in a framework region or constant domain is made to alter the immunogenicity of the antibody, or to provide a site for covalent or non-covalent binding to another molecule. According to the invention, a single antibody or portion may have mutations in any one or more of the CDRs or framework regions of the variable domain or in the constant domain.

[0216] Anti-CCL20 antibodies or antigen-binding portions thereof of the invention also can be produced transgenically through the generation of a mammal or plant that is transgenic for the antibody heavy and light chain sequences of interest and production of the antibody in a recoverable form therefrom. In some embodiments, the anti-CCL20 antibodies or portions are produced in, and recovered from, the milk of goats, cows, or other mammals. See, *e.g.*, U.S. Patent Nos. 5,827,690, 5,756,687, 5,750,172, and 5,741,957. In some embodiments, non-human transgenic animals that comprise human antibody loci are immunized with human CCL20 or an immunogenic portion thereof, as described above. Methods for making antibodies in plants are described, *e.g.*, in U.S. Patent Nos. 6,046,037 and 5,959,177.

[0217] In some embodiments, non-human transgenic animals or plants are produced by introducing one or more nucleic acid molecules encoding an anti-CCL20 antibody or portion of the invention into the animal or plant by standard transgenic techniques. See Hogan and U.S. Patent No. 6,417,429, *supra*. In certain embodiments, transgenic cells used for making the transgenic animal can be embryonic stem cells or somatic cells or a fertilized egg. In some embodiments, the non-human transgenic animals or plants are chimeric, nonchimeric heterozygotes, or nonchimeric homozygotes. See, *e.g.*, Hogan *et al.*, *Manipulating the Mouse Embryo: A Laboratory Manual* 2nd ed., Cold Spring Harbor Press (1999); Jackson *et al.*, *Mouse Genetics and Transgenics: A Practical Approach*, Oxford University Press (2000); and Pinkert, *Transgenic Animal Technology: A Laboratory Handbook*, Academic Press (1999). In some embodiments, the non-human transgenic animals or plants have a targeted disruption and replacement by a targeting construct that encodes a heavy chain and/or a light chain of interest. In certain embodiments, the transgenic animals or plants comprise and express nucleic acid molecules encoding heavy and light chains that specifically bind to human CCL20. The

anti-CCL20 antibodies or portions may be made in any non-human transgenic animal or plant. In particular embodiments, the non-human animals are mice, rats, sheep, pigs, goats, cattle or horses. The non-human transgenic animal may express the encoded polypeptides in blood, milk, urine, saliva, tears, mucus and other bodily fluids.

[0218] The antibodies and antigen-binding portions thereof of the invention are useful in neutralizing CCL20-induced chemoattraction of, *e.g.*, CCR6+ cells such as immature dendritic cells (DC), effector/memory T-cells, and B-cells. The antibodies and portions may thus be useful in treating a variety of diseases and conditions such as inflammation, autoimmune diseases, and cancer. Examples of diseases and conditions that can be treated with the antibodies or antigen-binding portions of this invention include, without limitation, Grave's disease, vitiligo, hyperthyroidism, rheumatoid arthritis, psoriasis, atopic dermatitis, contact dermatitis, Crohn's disease, inflammatory bowel disease, B-cell malignancies, breast adenocarcinoma, chronic hepatitis, contact dermatitis, glioblastoma, hepatocellular carcinoma, human papillomavirus infection of the cervix, mycosis fungoides, pancreatic adenocarcinoma, periodontal disease, thyroid papillary carcinoma, pustulosis palmaris et plantaris, conditions associated with maculopapular exanthema, epidermolysis bullosa, alopecia areata, multiple sclerosis, polymyositis, dermatomyositis, Behcet's disease, acute generalized exanthematous pustulosis, vasculitides, juvenile idiopathic arthritis, sarcoidosis, bronchial asthma, allergic rhinitis, renal allograft rejection, graft-versus-host disease, liver allograft rejection, chronic obstructive pulmonary disease, cystic fibrosis, glomerulonephritis, respiratory syncytial virus infection, multiple myeloma, and Langerhans cell histiocytosis. In some embodiments, the invention provides methods for treating any CCR6-associated condition.

[0219] Accordingly, the invention provides methods for treating inflammation, an autoimmune disease, or cancer by administering an effective amount of an antibody or portion of the invention to a subject in need thereof. In some embodiments, the subject is a human patient having or at risk for having an autoimmune disease, a cancer, and/or inflammation. In some embodiments, the antibody or portion is administered prophylactically to prevent onset or relapse of inflammation, autoimmune disease, or cancer.

[0220] Antibodies and antigen-binding portions thereof of this invention can be administered to an individual (*e.g.*, a human) alone or in conjunction with another agent in a combination therapy. The antibodies or portions can be administered before, as an admixture with, separately but concurrently with, or subsequent to administration of the additional agent. In some embodiments, the additional agent is selected from the group including, but not limited to: inhibitors of immuno-stimulatory cytokines (*e.g.*, anti-TNF-α MAbs), immune cell eliminators (*e.g.*, anti-CD20 MAbs), blockers of accessory molecules (*e.g.* Abatacept), disease-modifying agents for rheumatic diseases (*e.g.*, non-steroidal anti-inflammatory drugs (NSAIDs), methotrexate, retinoic acids, and vitamin D3 analogs), and immunosuppressants

(*e.g.*, calcineurin inhibitors). In some embodiments, the additional agent is selected from the group including, but not limited to: steroidal agents, immunomodulatory agents, somatostatic agents, conventional immunotherapeutic agents, cytokines or cytokine antagonists; and/or growth factors or growth factor antagonists. In some embodiments, anti-CCL20 antibody therapy can be used in conjunction with standard-of-care cancer treatment such as chemotherapy, surgery, or radiation, or with another targeted therapy such as anti-VEGF antibody therapy. In some embodiments, anti-CCL20 antibody therapy can be used in conjunction with a prophylactic regimen. In one embodiment, a synthetic peptide mimetic can be administered in conjunction with an antibody of the present invention. In another embodiment, hormone therapy can be administered in conjunction with an antibody or portion of the present invention.

[0221] In one embodiment, the antibodies or antigen-binding portions of the invention are administered alone or in combination with an anti-inflammatory agent. Anti-inflammatory agents that may be administered with the antibodies of the invention include, but are not limited to, corticosteroids (*e.g.* betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, and triamcinolone), nonsteroidal anti-inflammatory drugs (*e.g.*, balsalazide, celecoxib, diclofenac, diflunisal, etodolac, fenoprofen, floctafenine, flurbiprofen, ibuprofen, indomethacin, ketoprofen, meclofenamate, mefenamic acid, meloxicam, nabumetone, naproxen, olsalazine, oxaprozin, phenylbutazone, piroxicam, salsalate, sulindac, tenoxicam, tiaprofenic acid, and tolmetin), as well as acetaminophen, antihistamines; aminoarylcarboxylic acid derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives (*e.g.* sulfasalazone and mesalamine), thiazinecarboxamides, e-acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide, ditazol, emorfazone, guaiazulene, nabumetone, nimesulide, orgotein, oxaceprol, paranyline, perisoxal, pifoxime, proquazone, proxazole, and tenidap.

[0222] Conventional nonspecific immunosuppressive agents that may be administered in combination with the antibodies of the invention include, but are not limited to, steroids, cyclosporine, cyclosporine analogs, cyclophosphamide methylprednisone, prednisone, azathioprine, FK-506, 15-deoxyspergualin, natalizumab, and other immunosuppressive agents that act by suppressing the function of responding T cells.

[0223] In some embodiments, the antibodies or antigen-binding portions of the invention are administered in combination with immunosuppressants. Immunosuppressant preparations that may be administered with the antibodies of the invention include, but are not limited to, ORTHOCLONE™ (OKT3), SANDIMMUNE™/NEORAL™/SANGDYA™ (cyclosporin), PROGRAF™ (tacrolimus), CELLCEPT™ (mycophenolate), Azathioprine, glucorticosteroids, AVONEX™ (interferon-beta 1A),

and RAPAMUNE™ (sirolimus). In one embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

[0224] In some embodiments, the antibodies or antigen-binding portions of the invention are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be administered with the antibodies of the invention include, but are not limited to, antibiotic derivatives (*e.g.*, doxorubicin, bleomycin, daunorubicin, and dactinomycin); antiestrogens (*e.g.*, tamoxifen); antimetabolites (*e.g.*, fluorouracil, 5-FU, floxuridine, interferon alpha-2b, glutamic acid, plicamycin, mercaptopurine, and 6-thioguanine); cytotoxic agents (*e.g.*, carmustine, BCNU, lomustine, CCNU, cytosine arabinoside, cyclophosphamide, estramustine, hydroxyurea, procarbazine, mitomycin, busulfan, cis-platin, and vincristine sulfate); hormones (*e.g.*, medroxyprogesterone, estramustine phosphate sodium, ethinyl estradiol, estradiol, epinephrine, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, and testolactone); nitrogen mustard derivatives (*e.g.*, mephalen, chorambucil, mechlorethamine (nitrogen mustard) and thiotepa); steroids and combinations (*e.g.*, bethamethasone sodium phosphate); and others (*e.g.*, dicarbazine, asparaginase, mitotane, vincristine sulfate, vinblastine sulfate, rituximab, and etoposide).

[0225] In some embodiments, the antibodies or antigen-binding portions of the invention are administered in combination with a TNF antagonist. TNF antagonists that may be administered with the antibodies or antigen-binding portions of the invention include, but are not limited to, infliximab (REMICADE™), adalimumab (HUMIRA™), certolizumab pegol (CIMZIA™), golimumab (SIMPONI™), etanercept (ENBREL™), xanthine derivatives (*e.g.* pentoxifylline) and bupropion (WELLBURTIN™, ZYBAN™).

[0226] In some embodiments, the antibodies or antigen-binding portions of the invention are administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with the antibodies or portions of the invention include, but not limited to, GAMMAR™, IVEEGAM™, SANDOGLOBULIN™, GAMMAGARD S/D™, and GAMIMUNE™. In some embodiments, the antibodies or antigen-binding portions of the invention are administered in combination with intravenous immune globulin preparations in transplantation therapy (*e.g.*, bone marrow transplant).

[0227] In some embodiments, the antibodies or antigen-binding portions of the invention are administered in combination with cytokines or cytokine antagonists. In some embodiments, the antibodies or antigen-binding portions of the invention may be administered with any cytokine or cytokine antagonist, including, but not limited to, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-γ and TNF-α or any antagonist thereof. In some embodiments, the antibodies or antigen-binding portions of the invention may be administered with any interleukin or interleukin antagonist, including, but not limited to, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6,

IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, and IL-21 or any antagonist thereof.

[0228] In some embodiments, the antibodies or antigen-binding portions of the invention are administered in combination with one or more chemokines or chemokine antagonists. In specific embodiments, the antibodies or antigen-binding portions of the invention are administered in combination with an α(CxC) chemokine selected from the group consisting of gamma-interferon inducible protein-10 (γIP-10), interleukin-8 (IL-8), platelet factor-4 (PF4), neutrophil activating protein (NAP-2), GRO-α, GRO-β, GRO-γ, neutrophil-activating peptide (ENA-78), granulocyte chemoattractant protein-2 (GCP-2), and stromal cell-derived factor-1 (SDF-1, or pre-B cell stimulatory factor (PBSF)); and/or a β(CC) chemokine selected from the group consisting of: RANTES (regulated on activation, normal T expressed and secreted), macrophage inflammatory protein-1 alpha (MIP-1α), macrophage inflammatory protein-1 beta (MIP-1β), monocyte chemotactic protein-1 (MCP-1), monocyte chemotactic protein-2 (MCP-2), monocyte chemotactic protein-3 (MCP-3), monocyte chemotactic protein-4 (MCP-4) macrophage inflammatory protein-1 gamma (MIP-1γ), macrophage inflammatory protein-3 alpha (MIP-3α), macrophage inflammatory protein-3 beta (MIP-3β), macrophage inflammatory protein-4 (MIP-4/DC-CK-1/PARC), eotaxin, Exodus, and I-309; and/or the γ(C) chemokine, lymphotactin; or any antagonist thereof.

[0229] In some embodiments, the antibodies or antigen-binding portions of the invention are administered with chemokine beta-8, chemokine beta-1, and/or macrophage inflammatory protein-4, or any antagonist thereof. In a preferred embodiment, the antibodies or antigen-binding portions of the invention are administered with chemokine beta-8 or an antagonist thereof.

[0230] In some embodiments, the antibodies or antigen-binding portions of the invention are administered in combination with an IL-4 antagonist. IL-4 antagonists that may be administered with the antibody and antibody compositions of the invention include, but are not limited to: soluble IL-4 receptor polypeptides, multimeric forms of soluble IL-4 receptor polypeptides, anti-IL-4 receptor antibodies that bind the IL-4 receptor without transducing the biological signal elicited by IL-4, anti-IL4 antibodies that block binding of IL-4 to one or more IL-4 receptors; and muteins of IL-4 that bind IL-4 receptors but do not transduce the biological signal elicited by IL-4. Preferably, the anti-IL4 antibodies employed according to this method are monoclonal antibodies; antigen-binding portions thereof may also be employed.

[0231] In some embodiments, the antibodies or antigen-binding portions of the invention are administered in combination with members of the TNF family, or antagonists thereof. In some embodiments, the antibodies or antigen-binding portions of the invention are administered in combination with agents that include, but are not limited to, soluble forms of TNF-α, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta),

OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), endokine-alpha (International Publication No. WO 98/07880), OPG, neutrokine-alpha (International Publication No. WO 98/18921), OX40, nerve growth factor (NGF), soluble forms of Fas, CD30, CD27, CD40 4-IBB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TR6 (International Publication No. WO 98/30694), TR7 (International Publication No. WO 98/41629), TRANK, TR9 (International Publication No. WO 98/56892), TR10 (International Publication No. WO 98/54202), 312C2 (International Publication No. WO 98/06842), TR12, and soluble forms CD154, CD70, and CD153, or any antagonist thereof.

[0232] As used herein, the term "therapeutically effective amount" refers to an amount of the administered therapeutic agent that will relieve or prevent to some extent one or more of the symptoms of the disorder being treated. An effective amount of anti-CCL20 antibody or antigen-binding portion thereof for treating a disease is an amount that helps the treated subject to reach one or more desired clinical end points.

[0233] In some embodiments, to minimize immunogenicity, a humanized antibody or portion is used to treat a human patient in therapeutic methods and compositions of this invention. In cases where repeated administration is not necessary, in some embodiments, it may also be appropriate to administer a mouse or chimeric antibody or portion of the invention to a human patient.

[0234] In some embodiments, the antibodies or antigen-binding portions thereof of the invention may be used to treat an individual who has previously been treated with, *e.g.*, inhibitors of immuno-stimulatory cytokines (*e.g.*, anti-TNF-α MAbs), immune cell eliminators (*e.g.*, anti-CD20 MAbs), and/or blockers of accessory molecules (*e.g.* Abatacept). In some embodiments, the antibodies or portions are used to treat a patient who has developed primary non-responsiveness or a gradual decline in response rate to these other treatments.

[0235] The antibody or antigen-binding portion thereof of this invention can be administered in a single unit dose or multiple doses at any time point deemed appropriate by a health care provider. The dosage can be determined by methods known in the art and can be dependent, for example, upon the individual's age, sensitivity, tolerance and overall well-being. Any administration method accepted in the art may be employed suitably for the antibodies and portions of the invention, including, but not necessarily limited to, parenteral (*e.g.*, intravenous, intraarterial, intramuscular, intrathecal, intraperitoneal, subcutaneous injection), oral (*e.g.*, dietary), locally, topical, inhalation (*e.g.*, intrabronchial, intranasal or oral inhalation, intranasal drops), or rectal, depending on the disease or condition to be treated. In one embodiment, the antibody or portion is administered parenterally.

[0236] Formulation will vary according to the route of administration selected (*e.g.*, solution, emulsion). An appropriate composition comprising the antibody or portion to be administered can be prepared in a physiologically acceptable vehicle or carrier. The composition can comprise multiple doses or be a single unit dose composition. For solutions or emulsions, suitable carriers include, for example, aqueous or alcoholic/aqueous solutions, emulsions, or suspensions, including saline and buffered media. Parenteral vehicles can include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles can include various additives, preservatives, or fluid, nutrient or electrolyte replenishers (See, generally, *Remington's Pharmaceutical Sciences*, 17th Edition, Mack Publishing Co., PA, 1985). For inhalation, the compound can be solubilized and loaded into a suitable dispenser for administration (*e.g.*, an atomizer, nebulizer or pressurized aerosol dispenser).

[0237] Dosage regimens may be adjusted to provide the optimum desired response. In certain embodiments, a single bolus may be administered, several divided doses may be administered over time, or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form, as used herein, refers to physically discrete units suited as unitary dosages for the patients/subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are generally dictated by and directly dependent on (a) the unique characteristics of the therapeutic agent and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

[0238] Thus, the skilled artisan would appreciate, based upon the disclosure provided herein, that the dose and dosing regimen is adjusted in accordance with methods well-known in the therapeutic arts. That is, the maximum tolerable dose can be readily established, and the effective amount providing a detectable therapeutic benefit to a patient may also be determined, as can the temporal requirements for administering each agent to provide a detectable therapeutic benefit to the patient. Accordingly, while certain dose and administration regimens are exemplified herein, these examples in no way limit the dose and administration regimen that may be provided to a patient in practicing the present invention.

[0239] It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated, and may include single or multiple doses. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of

the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. Further, the dosage regimen with the compositions of this invention may be based on a variety of factors, including the type of disease, the age, weight, sex, medical condition of the patient, the severity of the condition, the route of administration, and the particular antibody employed. Thus, the dosage regimen can vary widely, but can be determined routinely using standard methods. For example, doses may be adjusted based on pharmacokinetic or pharmacodynamic parameters, which may include clinical effects such as toxic effects and/or laboratory values. The present invention thus encompasses intra-patient dose-escalation as determined by the skilled artisan. Methods of determining appropriate dosages and regimens are well-known in the relevant art and would be understood to be encompassed by the skilled artisan once provided with the teachings disclosed herein.

[0240] In some embodiments, for administration to human subjects, the total monthly dose of the antibodies or antibody portion of the invention is in the range 0.5-1200 mg per patient, depending, of course, on the mode of administration. In certain embodiments, an intravenous monthly dose requires about 1-1000 mg/patient. The total monthly dose may be administered in single or divided doses and may, at the physician's discretion, fall outside of the typical range given herein.

[0241] In certain embodiments, a range for a therapeutically or prophylactically effective amount of an antibody or antibody portion of the invention is 1-1000 mg/kg/patient/month. In one embodiment, the antibody or portion thereof of the invention may be administered at about 1-200 or 1-150 mg/patient/month. In certain embodiments, the antibody or portion is administered in a 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mg/kg/patient shot 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 times a month. In a particular embodiment, the antibody or portion is administered in a 1 to 5 mg/kg/patient shot 1 or 2 times a month.

[0242] The antibodies and antigen-binding portions thereof of the present invention also are useful in a variety of processes with applications in research and diagnosis. In some embodiments, the antibodies and portions are used to detect, isolate, and/or purify human CCL20 or variants thereof (*e.g.*, by affinity purification or other suitable methods such as flow cytometry, *e.g.*, for cells, such as lymphocytes, in suspension), and to study human CCL20 structure (*e.g.*, conformation) and function. For *in vitro* applications, wherein immunogenicity of the antibody is not a concern, the mouse and chimeric antibodies and antigen-binding protions thereof of this invention will be useful in addition to humanized antibodies.

[0243] The antibodies or antigen-binding portions thereof of the present invention can be used in diagnostic applications (*e.g.*, *in vitro*, *ex vivo*). In some embodiments, the humanized antibodies or portions of the present invention are used to detect and/or measure the level of human CCL20 in a sample. In certain embodiments, the sample comprises, *e.g.*, cells or tissues expressing human CCL20, and/or

body fluids such as an inflammatory exudate, blood, serum, and/or bowel fluid bearing human CCL20. A sample can be obtained from an individual and an antibody or portion described herein can be used in a suitable immunological method to detect and/or measure human CCL20 expression, including methods such as flow cytometry (*e.g.*, for cells in suspension such as lymphocytes), enzyme-linked immunosorbent assays (ELISA), including chemiluminescence assays, radioimmunoassay, and immunohistology.

[0244] In one embodiment, a method of detecting human CCL20 in a sample comprises contacting the sample with an antibody or portion of the present invention under conditions suitable for specific binding of the antibody or portion to human CCL20 and detecting antibody-CCL20 complexes which are formed. In one embodiment, the antibodies or portions described herein can be used to analyze normal versus inflamed tissues (*e.g.*, from a human) for human CCL20 reactivity and/or expression (*e.g.*, immunohistologically) to detect associations between increased expression of human CCL20 (*e.g.*, in affected tissues) and one or more disorders selected from, but not limited to, Grave's disease, vitiligo, hyperthyroidism, rheumatoid arthritis, psoriasis, atopic dermatitis, contact dermatitis, Crohn's disease, inflammatory bowel disease, B-cell malignancies, breast adenocarcinoma, chronic hepatitis, contact dermatitis, glioblastoma, hepatocellular carcinoma, human papillomavirus infection of the cervix, mycosis fungoides, pancreatic adenocarcinoma, periodontal disease, thyroid papillary carcinoma, pustulosis palmaris et plantaris, conditions associated with maculopapular exanthema, epidermolysis bullosa, alopecia areata, multiple sclerosis, polymyositis, dermatomyositis, Behcet's disease, acute generalized exanthematous pustulosis, vasculitides, juvenile idiopathic arthritis, sarcoidosis, bronchial asthma, allergic rhinitis, renal allograft rejection, graft-versus-host disease, liver allograft rejection, chronic obstructive pulmonary disease, cystic fibrosis, glomerulonephritis, respiratory syncytial virus infection, multiple mycloma, Langerhans cell histiocytosis, or other conditions. Thus, the antibodies of the present invention permit immunological methods of assessment of the presence of human CCL20 in normal and inflamed tissues, through which the presence of disease, disease progress and/or the efficacy of anti-human CCL20 therapy in the treatment of disease, *e.g.*, inflammatory and/or immune disease, can be assessed.

[0245] The methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, *e.g.*, Sambrook J. & Russell D. *Molecular Cloning: A Laboratory Manual*, 3rd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (2000); Ausubel *et al.*, *Short Protocols in Molecular Biology: A Compendium of Methods from Current Protocols in Molecular Biology*, Wiley, John & Sons, Inc. (2002); Harlow and Lane *Using Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1998); and Coligan *et al.*, *Short Protocols in*

Protein Science, Wiley, John & Sons, Inc. (2003). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclature used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art.

[0246] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Exemplary methods and materials are described below, although methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention. All publications and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. Although a number of documents are cited herein, this citation does not constitute an admission that any of these documents forms part of the common general knowledge in the art. Throughout this specification and claims, the word "comprise," or variations such as "comprises" or "comprising" will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers. The materials, methods, and examples are illustrative only and not intended to be limiting.

[0247] In order that this invention may be better understood, the following examples are set forth. These examples are for purposes of illustration only and are not to be construed as limiting the scope of the invention in any manner.

Example 1: Involvement of CCL20 in autoimmune/inflammatory disorders

[0248] To obtain evidence for the involvement of CCL20 in autoimmune and inflammatory conditions, we studied the effects of knocking out the CCL20 receptor CCR6 in a type II collagen-induced arthritis (CIA) mouse rheumatoid arthritis (RA) model. CCR6 wild type mice, $CCR6^{+/-}$ (heterozygous) mice, and $CCR6^{-/-}$ (homozygous) knock-out mice (each n=10) were immunized at the base of the tail with bovine type II collagen (150 μg/mouse) emulsified in complete Freund's adjuvant (Chondrex, #7001). Three weeks later, a booster injection of the same amount of bovine type II collagen emulsion in incomplete adjuvant was administered at the base of the tail. We graded the severity of arthritic symptoms in the paws of each mouse as described in Griswold *et al.*, *Arthritis & Rheumatism* **31(11)**:1406-1412 (1988). Briefly, we graded the articular lesions of the extremities distal to the elbow or knee on a scale of 0 to 4 based on the number of joints involved and the degree of erythema and swelling. Arthritis scores were calculated as the sum of the scores of all four paws for each animal. CCR6 wild type animals developed arthritis, while $CCR6^{-/-}$ animals showed strong resistance to the disease (FIG. 1). Interestingly, $CCR6^{+/-}$ animals also exhibited some levels of

resistance, indicating that total abrogation of CCL20 function is not required to counteract the arthritic phenotype.

[0249] In addition, we immunohistochemically analyzed the hind paws of mice for the presence of CD3-positive T-cells or F4/80-positive macrophages. On day 43, mice were harvested and their hind paws were fixed in formalin. The paws were then sectioned and placed on slides. After decalcification of the paw samples, the slides were stained immunohistochemically with anti-CD3 (#N1580; DAKO) and F4/80 antibodies (Clone CI:A3-1, AbD Serotec). The intensity of staining was scored by two independent observers by scanning the samples with an Aperio instrument (Aperio Technologies). The scoring scale was as follows: 0, normal; 1, light staining throughout the paw or intense staining in one digit; 2, moderate staining in multiple digits; 3, intense staining in multiple digits or moderate throughout; 4; intense staining throughout all digits and paw. The number of T-cells (FIG. 2) and macrophages (FIG. 3) infiltrating the CIA-induced lesions were strongly reduced in both $CCR6^{+/-}$ and $CCR6^{-/-}$ mice.

[0250] We also studied the effects of knocking out CCR6 in a dinitrofluorobenzene (DNFB)-induced allergic contact dermatitis mouse model. Two groups of six mice each (one group of mice wild-type for CCR6 and one group CCR6 deficient) were sensitized by brushing 25 µl of 0.4% DNFB solution (in 4:1 acetone:olive oil) on the shaved abdomen for two successive days (days 0 and 1). On day 5, mice were re-challenged by applying 20 µl of 0. 1 % DNFB (in 4:1 acetone:olive oil) to one side of one ear. As an indicator of edema, ear thickness was measured before DNFB challenge and at 24 hr after the last challenge on Day 5 by using a thickness gauge. CCR6 deficient mice demonstrated almost no increased ear thickness after treatment with DNFB, indicating resistance against DNFB-induced contact hypersensitivity (FIG. 4).

[0251] These data support a role for CCR6 and CCL20 in autoimmune/inflammatory disorders.

Example 2: Inhibition of CCL20-induced chemotaxis by hamster anti-mouse CCL20 2F5-5 MAb

[0252] To further explore the role of CCL20 as a potential therapeutic target, we performed experiments using a hamster anti-mouse CCL20 antibody (2F5-5). We first characterized the ability of the 2F5-5 MAb to bind to mouse, human, and rhesus CCL20. Soluble CCL20-secreted alkaline phosphatase (SEAP) antigens of each species were prepared as follows. The cDNAs encoding the CCL20s of human, cynomolgus monkey, rhesus monkey, rat, and mouse were amplified and subcloned into a pcDNA3.1 (+) dSall SEAP vector, which contains SEAP cDNA and has its SalI site deleted (pcDNA 3.1 (+) purchased from Invitrogen; SEAP cDNA derived from a pSEAP-Enhancer vector, Clontech). The expression vectors were transfected into the human embryo kidney cell line HEK293EBNA (HEK293E, Invitrogen). The HEK293E cells were inoculated with DMEM

(Invitrogen) supplemented with 10% fetal bovine serum on the day before transfection. On the day of transfection, the culture medium was replaced with OPTI-MEM II serum free media (Invitrogen). The expression vectors were transfected using TransIT LT1 (TAKARA Bio Inc., Shiga, Japan) according to the manufacturer's protocol. After 3 days of incubation at 5% CO_2 and 37°C, culture supernatants were harvested. The concentration of CCL20-SEAP in each culture was measured by using the Great EscAPe SEAP Chemiluminescence Kit 2.0 (Clontech).

[0253] We then performed surface plasmon resonance (Biacore™) binding studies to measure the binding of the 2F5-5 MAb to mouse, human, rhesus, and cynomolgus CCL20-SEAP antigens. Anti-SEAP (monoclonal anti-mouse placental alkaline phosphatase, Thermo Scientific, Cat # MAI-19354, Lot # KL12748M) was immobilized on a CM5 sensor chip (GE Healthcare) using a standard NHS/EDC amine coupling procedure. SEAP-tagged mouse CCL20 (100 nM in supernatant, ID735, Lot # 091130) was diluted to 5 nM with TBS-P containing 0.005 % Tween-20 and captured on the CM5 sensor chip. 0.2 nM to 80 nM dilutions of hamster anti-mouse CCL20 (2F5-5, Lot # 060215, 2 mg/ml) in TBS-P were injected over the sensor chip at a flow rate of 30 µl/min. Association and dissociation of hamster anti-mouse CCL20 with mouse CCL20 were monitored for 4 min and 16 min, respectively. The chip surface was regenerated between injections using 10 mM glycine, pH 2.25. Sensograms were double referenced by subtracting hamster anti-mouse CCL20 injections into a reference cell without captured mouse CCL20 and a TBS-P injection over captured mouse CCL20. The sensograms were analyzed using a 1:1 Langmuir binding model in BIAevaluation software (GE Healthcare, version 3.2). In this assay, 2F5-5 MAb binds to mouse CCL20 with an affinity of 32 pM, but does not bind to human, rhesus, or cynomolgus CCL20 (Table 1).

Table 1: 2F5-5 MAb binding affinity to CCL20 orthologs

CCL20 Ortholog	k_a ($x10^5 M^{-1}sec^{-1}$)	k_d ($x10^{-5} sec^{-1}$)	K_D ($x10^{-11} M$)
Mouse	9.63	0.474	0.492
Human	Not detectable	Not detectable	Not detectable
Rhesus	Not detectable	Not detectable	Not detectable
Cynomolgus	Not detectable	Not detectable	Not detectable

[0254] To test the effect of 2F5-5 MAb on CCL20 function, we evaluated its neutralizing activity against mouse CCL20 using an *in vitro* chemotaxis assay. CCR6-transduced B300.19 cells and recombinant mouse CCL20 (1 nM) were added to transwell culture plates. Four hours later, the cells that migrated into the lower chamber were counted by fluorescence-activated cell sorting (FACS).

2F5-5 MAb completely inhibited chemotaxis at 1 μg/ml with an estimated IC_{50} value of 0.04 μg/ml (0.27 nM) (FIG. 5). By contrast, a control hamster antibody (10 μg/ml) did not inhibit chemotaxis (100% control; data not shown).

Example 3: Humanization of mouse anti-CCL20 MAbs

[0255] To obtain monoclonal antibodies against human CCL20, we generated a panel of mouse anti-human CCL20 antibodies. Recombinant human CCL20 (R&D, #360-MP-025/CF, 17.5 μg/head) emulsified with Freund's complete adjuvant (Mitsubishi-Kagaku Yatron, RM606-1) was injected subcutaneously into the footpads of the mice. Two consecutive injections were then administered every three days. Three days after the final immunization, the animals were sacrificed and the inguinal lymph-node cells were fused with P3U1 myeloma cells at a 2:1 to 10:1 ratio in the presence of 50% polyethylene glycol. The cells were then cultured in 96-well plastic plates.

[0256] Sandwich ELISA was used for primary screening. A 96-well plate was coated with a polyclonal anti-human IgG antibody (Jackson, #709-005-149, 2 μg/ml in PBS (-)). After overnight incubation at 4°C, the wells were blocked with 1 x Block-Ace (Dainippon Sumitomo Pharma, UK-B80) for 1 hour at room temperature. The wells were then washed with 0.02% Tween 20/PBS (-), after which 1 nM human CCL20-hIgG Fc chimera protein in 0.02% Tween 20/PBS (-) was added to the wells (50 μl/well). After a one hour incubation at room temperature, three additional washes were performed as described above. Culture supernatants from each hybridoma were then diluted twofold with 20% FBS and 200 μg/ml human IgG (Mitsubishi Welpharma) in 0.02% Tween 20/PBS (-), added to the wells, and incubated for one hour at room temperature. After three further washes, the wells were incubated with horseradish peroxidase-conjugated anti-mouse IgG antibody (Jackson, #715-035-150, 5000 fold dilution with 0.02% Tween 20/PBS (-)) for one hour at room temperature. The wells were then washed three times and incubated in a TMBZ (3,3',5,5'-tetramethylbenzidine) solution for 15-30 minutes. An equal volume of 2 M H_2SO_4 was then added to stop the reaction, and the optical density was read at 450 nm by ARVO (PerkinElmer). The sandwich ELISA identified 24 positive wells.

[0257] We then performed a chemotaxis assay as a secondary screen. The chemotaxis assay was performed in transwell culture plates (MultiScreen pore 5 μm; Millipore, #MAMIC 5S10). First, 50 μl/well of 300 ng/ml recombinant human CCL20 (R&D, #360-MP-025/CF) in chemotaxis buffer (0.5% BSA, 0.5% FBS, 20 mM HEPES (pH 7.4), 50 μM 2-mercaptoethanol in RPMI1640 (Invitrogen)) was pre-incubated with 100 μl/well of culture supernatants from the hybridomas at room temperature for 30 minutes (for a final CCL20 concentration of 100 ng/ml) in the lower wells of the plates. After 30 minutes, B300.19 cells transfected with human CCR6 (SEQ ID NO: 104) ($2x10^5$ cells/75 μl) were applied to the upper wells and incubated in a 5% CO_2 incubator at 37°C for 4 hours.

Following the incubation, 150 µl from the lower wells were harvested and fixed with 50 µl of 4% PFA/PBS (-). 30 µl of each sample were applied to the FACSCantoII cell analyzer (BD Biosciences) to count migrated cells. Neutralizing activity was found in four wells.

[0258] We then used standard limiting dilution to obtain hybridoma clones from the four positive wells. We confirmed the neutralizing activity of supernatant from each clone using the *in vitro* chemotaxis assay. Three of the mouse anti-human CCL20 monoclonal antibodies produced by these clones (antibodies 36F7C10 (Table 2), 42G5B10 (Table 3), and 40-1C10B9 (Table 4)) demonstrated neutralizing activity against human CCL20.

Table 2: Amino acid and nucleotide sequences of 36F7C10

DESCRIPTION	SEQUENCE
Heavy Chain Signal Sequence (Amino Acid) (SEQ ID NO: 33)	MRWSCIILFLVATATGVNS
Light Chain Signal Sequence (Amino Acid) (SEQ ID NO: 34)	MGVPTQLLLLWLTVVVVRC
Heavy Chain Variable Domain Amino Acid Sequence (SEQ ID NO: 39)	QVQLQQPGAELVKPGASVKMSCKASGYT FTNYWMHWVKQRPGQGLEWIGVIDPSDS YTTYNQKFKGKATLTVDTSSSTAYMQLS SLTSEDSAVYYCTRGNYGVDYAMDYWG QGTSVTVSS
Light Chain Variable Domain Amino Acid Sequence (SEQ ID NO: 40)	DIQMTQSPASLSASVGETVTITCGASENIY GALNWYQRKQGKSPQLLIYGATNLADG MSSRFSGSGSGRQYSLKISSLHPDDVATY YCQNVLITPYTFGGGTKLEIK
Heavy Chain Signal Sequence (Nucleotide) (SEQ ID NO: 45)	ATGAGATGGAGCTGTATCATCCTCTTCT TGGTAGCAACAGCTACAGGTGTCAACT CC
Light Chain Signal Sequence (Nucleotide) (SEQ ID NO: 46)	ATGGGTGTACCCACTCAGCTCCTGTTGC TGTGGCTTACAGTCGTAGTTGTCAGATG T
Heavy Chain Variable Domain Nucleotide Sequence (SEQ ID NO: 51)	CAGGTCCAACTGCAGCAGCCTGGGGCT GAGCTGGTGAAGCCTGGGGCTTCAGTG AAGATGTCCTGCAAGGCTTCTGGCTACA CCTTCACCAACTACTGGATGCACTGGGT GAAGCAGAGGCCTGGACAAGGCCTTGA GTGGATCGGAGTGATTGATCCTTCTGAT AGTTATACTACCTACAATCAAAAGTTCA AGGGCAAGGCCACATTGACTGTAGACA CATCCTCCAGCACAGCCTACATGCAGCT CAGCAGCCTGACATCTGAGGACTCTGC GGTCTATTACTGTACAAGAGGTAACTAC GGAGTAGACTATGCTATGGACTACTGG GGTCAAGGAACCTCAGTCACCGTCTCCT CG
Light Chain Variable Domain Nucleotide Sequence	GACATCCAGATGACTCAGTCTCCAGCTT CACTGTCTGCATCTGTGGGAGAAACTGT

(SEQ ID NO: 52)	CACCATCACATGTGGAGCAAGTGAGAA TATTTACGGTGCTTTAAATTGGTATCAG CGGAAACAGGGAAAATCTCCTCAGCTC CTGATCTATGGTGCAACCAACTTGGCAG ATGGCATGTCATCGAGGTTCAGTGGCA GTGGATCTGGTAGACAGTATTCTCTCAA GATCAGTAGCCTGCATCCTGACGATGTT GCAACGTATTACTGTCAAAATGTGTTAA TTACTCCGTACACGTTCGGAGGGGGGA CCAAGCTGGAAATAAAA

Table 3: Amino acid and nucleotide sequences of 42G5B10

DESCRIPTION	SEQUENCE
Heavy Chain Signal Sequence (Amino Acid) (SEQ ID NO: 37)	MRWSCIILFLVATATGVNS
Light Chain Signal Sequence (Amino Acid) (SEQ ID NO: 38)	MGVPTQLLLLWLTVVVVRC
Heavy Chain Variable Domain Amino Acid Sequence (SEQ ID NO: 43)	QVQLQQPGAELVKPGASVKMSCKASGYT FTSYWMHWVKQRPGQGLEWIGLIDPSDK YTNYNQKFKGKATLTVDTSSSTAYMQLS SLTSEDSAVYYCTRGNYGVDYGMDYWG QGTSVTVSS
Light Chain Variable Domain Amino Acid Sequence (SEQ ID NO: 44)	DIQMTQSPASLSASVGETVTITCGASENIY GALNWYQRKQGKSPQLLIYGATNLADG MSSRFSGSGSGRQYSLKISSLHPDDVATY YCQNVLSTPYTFGGGTKLEIK
Heavy Chain Signal Sequence (Nucleotide) (SEQ ID NO: 49)	ATGAGATGGAGCTGTATCATCCTCTTCT TGGTAGCAACAGCTACAGGTGTCAACT CC
Light Chain Signal Sequence (Nucleotide) (SEQ ID NO: 50)	ATGGGTGTACCCACTCAGCTCCTGTTGC TGTGGCTTACAGTCGTAGTTGTCAGATG T
Heavy Chain Variable Domain Nucleotide Sequence (SEQ ID NO: 55)	CAGGTCCAACTGCAGCAGCCTGGGGCT GAGCTGGTGAAGCCTGGGGCTTCAGTG AAGATGTCCTGCAAGGCTTCTGGCTACA CCTTCACCAGCTACTGGATGCACTGGGT GAAGCAGAGGCCTGGACAAGGCCTTGA GTGGATCGGACTGATTGATCCTTCTGAT AAGTATACTAACTACAATCAAAAGTTC AAGGGCAAGGCCACATTGACTGTAGAC ACATCCTCCAGCACAGCCTACATGCAGC TCAGCAGCCTGACATCTGAGGACTCTGC GGTCTATTACTGTACAAGAGGTAACTAC GGAGTAGACTATGGTATGGACTACTGG GGTCAAGGAACCTCAGTCACCGTCTCCT CA
Light Chain Variable Domain Nucleotide Sequence (SEQ ID NO: 56)	GACATCCAGATGACTCAGTCTCCAGCTT CACTGTCTGCATCTGTGGGAGAAACTGT CACCATCACATGTGGAGCAAGTGAGAA TATTTACGGTGCTTTAAATTGGTATCAG

	CGGAAACAGGGAAAATCTCCTCAGCTC CTGATCTATGGTGCAACCAACTTGGCAG ATGGCATGTCATCGAGGTTCAGTGGCA GTGGATCTGGTAGACAGTATTCTCTCAA GATCAGTAGCCTGCATCCTGACGATGTT GCAACGTATTACTGTCAAAATGTGTTAA GTACTCCGTACACGTTCGGAGGGGGGA CCAAGCTGGAAATAAAA

Table 4: Amino acid and nucleotide sequences of 40.1C10B9

DESCRIPTION	SEQUENCE
Heavy Chain Signal Sequence (Amino Acid) (SEQ ID NO: 35)	MEWSWVFLFLLSVIAGVQS
Light Chain Signal Sequence (Amino Acid) (SEQ ID NO: 36)	MGVPTQLLLLWLTVVVVRC
Heavy Chain Variable Domain Amino Acid Sequence (SEQ ID NO: 41)	QVQLQQSGAELVRPGASVTLSCKASGYT FTDYEMHWVKQTPVHGLEWIGAIDPETT STAYNQKFKGKATLTADKSSSTAYMELR SLTSEDSAVYYCTKCYYGSADYAMDYW GQGTSVTVSS
Light Chain Variable Domain Amino Acid Sequence (SEQ ID NO: 42)	DIQMTQSPASLSASVGETVTITCGASENIY GALNWYQRKQGKSPQLLIYGATNLADG MSSRFSGSGSGRQYSLKISSLHPDDVATY YCQNVLSTPWTFGGGTKLEIK
Heavy Chain Signal Sequence (Nucleotide) (SEQ ID NO: 47)	ATGGAATGGAGCTGGGTCTTTCTCTTCC TCCTGTCAGTAATTGCAGGTGTCCAATC C
Light Chain Signal Sequence (Nucleotide) (SEQ ID NO: 48)	ATGGGTGTACCCACTCAGCTCCTGTTGC TGTGGCTTACAGTCGTAGTTGTCAGATG T
Heavy Chain Variable Domain Nucleotide Sequence (SEQ ID NO: 53)	CAGGTTCAACTGCAGCAGTCTGGGGCT GAGCTGGTGAGGCCTGGGGCTTCAGTG ACGCTGTCCTGCAAGGCTTCGGGCTACA CATTTACTGACTATGAAATGCACTGGGT GAAGCAGACACCTGTGCATGGCCTGGA ATGGATTGGAGCTATTGATCCTGAAACT ACTAGTACTGCCTACAATCAGAAGTTCA AGGGCAAGGCCACACTGACTGCAGACA AATCCTCCAGCACAGCCTACATGGAGCT CCGCAGCCTGACATCTGAGGACTCTGCC GTCTATTACTGTACCAAATGTTACTACG GTAGCGCGGACTATGCTATGGACTACTG GGGTCAAGGAACCTCAGTCACCGTCTCC TCA
Light Chain Variable Domain Nucleotide Sequence (SEQ ID NO: 54)	GACATCCAGATGACTCAGTCTCCAGCTT CACTGTCTGCATCTGTGGGAGAAACTGT CACCATCACATGTGGAGCAAGTGAGAA TATTTACGGTGCTTTAAATTGGTATCAG CGGAAACAGGGAAAATCTCCTCAGCTC CTGATCTATGGTGCAACCAACTTGGCAG ATGGCATGTCATCGAGGTTCAGTGGCA

	GTGGATCTGGTAGACAGTATTCTCTCAA GATCAGTAGCCTGCATCCTGACGATGTT GCAACGTATTACTGTCAAAATGTGTTAA GTACTCCGTGGACGTTCGGTGGAGGCA CCAAGCTGGAAATCAAA

[0259] The 36F7C10, 42G5B10, and 40-1C10B9 mouse antibodies were then used to produce humanized antibody heavy and light chains. The humanization method involved grafting mouse complementarity determining regions (CDRs), as identified by Kabat and/or Chothia definition methods according to Kabat numbering, into the human heavy and light chain germline sequences representing the best framework matches with the original mouse sequences from which the CDRs were derived (FIGS. 6A-C; bolding denotes residues that differ between the mouse antibody and the human germline sequence; underlining and bolding denotes grafted mouse CDRs; italicizing and bolding denotes framework residues substituted with corresponding mouse antibody residues; and underlining, italicizing and bolding denotes CDR residues substituted with corresponding human germline residues). Framework matches were identified using the IgBlast database, according to the methods described in Altschul et al., Nucl. Acids Res. 25:3389-3402 (1997). To derive alternative humanized chain versions, we used two methods: 1) 3D modeling techniques to predict critical murine residues within the framework interacting with the CDR residues, and 2) sequence alignments to identify murine residues immediately adjacent to the canonical CDR sequences.

[0260] The humanized sequences were constructed into expression vectors and transfected into mammalian cell lines (*e.g.*, HEK293E cells (Invitrogen)) for antibody production. Antibodies obtained by this process were then characterized in functional and physiochemical assays.

Example 4: *In vitro* chemotaxis assays demonstrating neutralization activity of humanized anti-human CCL20 Abs

[0261] We identified humanized antibodies that actively neutralize the human CCL20 ligand by performing *in vitro* chemotaxis assays using CCR6-transduced B300.19 cells. Upon determination of IC_{50}, IC_{90}, and IC_{95} values (FIGS. 7A-C), antibodies demonstrating a reduced value in the IC_{95} table were deemed to possess significant neutralizing activity (FIG. 7C). Of the 14 humanized heavy chains and 26 humanized light chains produced, we tested 41 combinations. Eight of these combinations were shown to significantly neutralize chemotaxis. These eight humanized antibodies are listed below:

- 36LK3/36HKK3
- 36LK3/42HKK1
- 36LK3/42HKK2

- 36LK3/42HKK3
- 36LK3/36HC2
- 36LK3/36HC3
- 36LC3/36HKK3
- 36LC3/36HC2

[0262] The amino acid and nucleotide sequences of humanized heavy chains HC2, HC3, 36HKK3, 42HKK1, 42HKK2, and 42HKK3, and of humanized light chains LC3 and LK3, are shown in Tables 5-12. The amino acid sequences encoded by the human germline genes utilized by these heavy and light chains are shown in Table 13.

Table 5: Amino acid and nucleotide sequences of humanized anti-human CCL20 antibody heavy chain 36HC2 ("HC2")

DESCRIPTION	SEQUENCE (signal sequence underlined, variable domain in bold)
Heavy Chain Amino Acid Sequence with the signal sequence (SEQ ID NO: 1)	MGWSCIILFLVATATGVHS**QVQLVQSGA EVKKPGASVKVSCKASGYTFTNYWMH WVRQAPGQGLEWMGVIDPSDSYTTYA QKFQGRVTMTVDTSTSTVYMELSSLRS EDTAVYYCARGNYGVDYAMDYWGQG TLVTVSS**ASTKGPSVFPLAPSSKSTSGGTA ALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTY ICNVNHKPSNTKVDKRVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
Heavy Chain Amino Acid Sequence without the signal sequence (SEQ ID NO: 108)	**QVQLVQSGAEVKKPGASVKVSCKASG YTFTNYWMHWVRQAPGQGLEWMGVI DPSDSYTTYAQKFQGRVTMTVDTSTST VYMELSSLRSEDTAVYYCARGNYGVDY AMDYWGQGTLVTVSS**ASTKGPSVFPLA PSSKSTSGGTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVT VPSSSLGTQTYICNVNHKPSNTKVDKRVE PKSCDKTHTCPPCPAPELLGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSREEMT

	KNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSP GK
Heavy Chain Variable Domain Amino Acid Sequence (SEQ ID NO: 9)	QVQLVQSGAEVKKPGASVKVSCKASG YTFTNYWMHWVRQAPGQGLEWMGVI DPSDSYTTYAQKFQGRVTMTVDTSTST VYMELSSLRSEDTAVYYCARGNYGVDY AMDYWGQGTLVTVSS
Heavy Chain Nucleotide Sequence with the signal sequence (SEQ ID NO: 17)	ATGGGCTGGTCCTGCATCATTCTGTTCC TGGTGGCCACTGCTACCGGAGTGCACA GCCAGGTGCAGCTGGTGCAGTCTGGG GCTGAGGTGAAGAAACCCGGTGCAA GTGTGAAGGTGTCATGTAAAGCATCC GGCTATACATTCACTAACTACTGGAT GCATTGGGTGAGGCAGGCTCCAGGA CAGGGACTGGAATGGATGGGCGTGA TCGACCCTTCAGATTCCTACACCACA TATGCCCAGAAGTTTCAGGGCAGGGT GACCATGACAGTGGACACTAGCACCT CTACAGTGTACATGGAGCTGTCCAGC CTGAGAAGTGAAGATACAGCAGTGTA CTATTGCGCCCCGCGGCAATTACGGAG TGGACTATGCCATGGATTACTGGGGG CAGGGTACTCTGGTGACCGTGTCTAG TGCTTCTACCAAGGGCCCATCGGTCTTC CCCCTGGCACCCTCCTCCAAGAGCACCT CTGGGGGCACAGCGGCCCTGGGCTGCC TGGTCAAGGACTACTTCCCCGAACCGGT GACGGTGTCGTGGAACTCAGGCGCCCT GACCAGCGGCGTGCACACCTTCCCGGCT GTCCTACAGTCCTCAGGACTCTACTCCC TCAGCAGCGTGGTGACCGTGCCCTCCAG CAGCTTGGGCACCCAGACCTACATCTGC AACGTGAATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGCCCAAA TCTTGTGACAAAACTCACACATGCCCAC CGTGCCCAGCACCTGAACTCCTGGGGG GACCGTCAGTCTTCCTCTTCCCCCCAAA ACCCAAGGACACCCTCATGATCTCCCGG ACCCCTGAGGTCACATGCGTGGTGGTG GACGTGAGCCACGAAGACCCTGAGGTC AAGTTCAACTGGTACGTGGACGGCGTG GAGGTGCATAATGCCAAGACAAAGCCG CGGGAGGAGCAGTACAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGC ACCAGGACTGGCTGAATGGCAAGGAGT ACAAGTGCAAGGTCTCCAACAAAGCCC TCCCAGCCCCCATCGAGAAAACCATCTC CAAAGCCAAAGGGCAGCCCCGAGAACC ACAGGTGTACACCCTGCCCCCATCCCGG GAGGAGATGACCAAGAACCAGGTCAGC CTGACCTGCCTGGTCAAAGGCTTCTATC

	CCAGCGACATCGCCGTGGAGTGGGAGA GCAATGGGCAGCCGGAGAACAACTACA AGACCACGCCTCCCGTGCTGGACTCCGA CGGCTCCTTCTTCCTCTATAGCAAGCTC ACCGTGGACAAGAGCAGGTGGCAGCAG GGGAACGTCTTCTCATGCTCCGTGATGC ATGAGGCTCTGCACAACCACTACACGC AGAAGAGCCTCTCCCTGTCTCCCGGGAA ATGA
Heavy Chain Nucleotide Sequence without the signal sequence (SEQ ID NO: 109)	CAGGTGCAGCTGGTGCAGTCTGGGG CTGAGGTGAAGAAACCCGGTGCAAGT GTGAAGGTGTCATGTAAAGCATCCGG CTATACATTCACTAACTACTGGATGC ATTGGGTGAGGCAGGCTCCAGGACA GGGACTGGAATGGATGGGCGTGATC GACCCTTCAGATTCCTACACCACATA TGCCCAGAAGTTTCAGGGCAGGGTGA CCATGACAGTGGACACTAGCACCTCT ACAGTGTACATGGAGCTGTCCAGCCT GAGAAGTGAAGATACAGCAGTGTACT ATTGCGCCCCGCGGCAATTACGGAGTG GACTATGCCATGGATTACTGGGGGCA GGGTACTCTGGTGACCGTGTCTAGTG CTTCTACCAAGGGCCCATCGGTCTTCCC CCTGGCACCCTCCTCCAAGAGCACCTCT GGGGGCACAGCGGCCCTGGGCTGCCTG GTCAAGGACTACTTCCCCGAACCGGTG ACGGTGTCGTGGAACTCAGGCGCCCTG ACCAGCGGCGTGCACACCTTCCCGGCTG TCCTACAGTCCTCAGGACTCTACTCCCT CAGCAGCGTGGTGACCGTGCCCTCCAG CAGCTTGGGCACCCAGACCTACATCTGC AACGTGAATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGCCCAAA TCTTGTGACAAAACTCACACATGCCCAC CGTGCCCAGCACCTGAACTCCTGGGGG GACCGTCAGTCTTCCTCTTCCCCCCAAA ACCCAAGGACACCCTCATGATCTCCCGG ACCCCTGAGGTCACATGCGTGGTGGTG GACGTGAGCCACGAAGACCCTGAGGTC AAGTTCAACTGGTACGTGGACGGCGTG GAGGTGCATAATGCCAAGACAAAGCCG CGGGAGGAGCAGTACAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGC ACCAGGACTGGCTGAATGGCAAGGAGT ACAAGTGCAAGGTCTCCAACAAAGCCC TCCCAGCCCCCATCGAGAAAACCATCTC CAAAGCCAAAGGGCAGCCCCGAGAACC ACAGGTGTACACCCTGCCCCCATCCCGG GAGGAGATGACCAAGAACCAGGTCAGC CTGACCTGCCTGGTCAAAGGCTTCTATC CCAGCGACATCGCCGTGGAGTGGGAGA GCAATGGGCAGCCGGAGAACAACTACA

	AGACCACGCCTCCCGTGCTGGACTCCGA CGGCTCCTTCTTCCTCTATAGCAAGCTC ACCGTGGACAAGAGCAGGTGGCAGCAG GGGAACGTCTTCTCATGCTCCGTGATGC ATGAGGCTCTGCACAACCACTACACGC AGAAGAGCCTCTCCCTGTCTCCCGGGAA ATGA
Heavy Chain Variable Domain Nucleotide Sequence (SEQ ID NO: 25)	**CAGGTGCAGCTGGTGCAGTCTGGGG CTGAGGTGAAGAAACCCGGTGCAAGT GTGAAGGTGTCATGTAAAGCATCCGG CTATACATTCACTAACTACTGGATGC ATTGGGTGAGGCAGGCTCCAGGACA GGGACTGGAATGGATGGGCGTGATC GACCCTTCAGATTCCTACACCACATA TGCCCAGAAGTTTCAGGGCAGGGTGA CCATGACAGTGGACACTAGCACCTCT ACAGTGTACATGGAGCTGTCCAGCCT GAGAAGTGAAGATACAGCAGTGTACT ATTGCGCCCCGCGGCAATTACGGAGTG GACTATGCCATGGATTACTGGGGGCA GGGTACTCTGGTGACCGTGTCTAGT**

Table 6: Amino acid and nucleotide sequences of humanized anti-human CCL20 antibody heavy chain 36HC3 ("HC3")

DESCRIPTION	SEQUENCE (signal sequence underlined, variable domain in bold)
Heavy Chain Amino Acid Sequence (SEQ ID NO: 2)	MGWSCIILFLVATATGVHSQVQLVQSGA **EVKKPGASVKVSCKASGYTFTNYWMH WVKQAPGQGLEWIGVIDPSDSYTTYNQ KFKGKATMTRDTSTSTVYMELSSLRSE DTAVYYCTRGNYGVDYAMDYWGQGT SVTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKRVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK**
Heavy Chain Variable Domain Amino Acid Sequence (SEQ ID NO: 10)	**QVQLVQSGAEVKKPGASVKVSCKASG YTFTNYWMHWVKQAPGQGLEWIGVID PSDSYTTYNQKFKGKATMTRDTSTSTV YMELSSLRSEDTAVYYCTRGNYGVDYA MDYWGQGTSVTVSS**
Heavy Chain Nucleotide Sequence	ATGGGCTGGTCCTGCATCATTCTGTTCC TGGTGGCAACTGCCACCGGAGTGCACA

(SEQ ID NO: 18)	GCCAGGTGCAGCTGGTGCAGTCTGGG GCTGAGGTGAAGAAACCCGGTGCAA GTGTGAAAGTGTCATGCAAGGCATCC GGCTATACATTCACTAACTACTGGAT GCATTGGGTGAAGCAGGCACCAGGA CAGGGACTGGAATGGATCGGCGTGAT CGACCCTTCAGATTCCTACACCACAT ATAATCAGAAGTTTAAAGGCAAGGCT ACCATGACAAGGGACACTAGCACCTC TACAGTGTACATGGAGCTGTCCAGCC TGAGGTCCGAAGATACAGCCGTGTAC TATTGCACTCGGGGCAACTACGGAGT GGACTATGCTATGGATTACTGGGGGC AGGGTACTAGTGTGACCGTGTCTAGT GCATCTACCAAGGGCCCATCGGTCTTCC CCCTGGCACCCTCCTCCAAGAGCACCTC TGGGGGCACAGCGGCCCTGGGCTGCCT GGTCAAGGACTACTTCCCCGAACCGGT GACGGTGTCGTGGAACTCAGGCGCCCT GACCAGCGGCGTGCACACCTTCCCGGCT GTCCTACAGTCCTCAGGACTCTACTCCC TCAGCAGCGTGGTGACCGTGCCCTCCAG CAGCTTGGGCACCCAGACCTACATCTGC AACGTGAATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGCCCAAA TCTTGTGACAAAACTCACACATGCCCAC CGTGCCCAGCACCTGAACTCCTGGGGG GACCGTCAGTCTTCCTCTTCCCCCCAAA ACCCAAGGACACCCTCATGATCTCCCGG ACCCCTGAGGTCACATGCGTGGTGGTG GACGTGAGCCACGAAGACCCTGAGGTC AAGTTCAACTGGTACGTGGACGGCGTG GAGGTGCATAATGCCAAGACAAAGCCG CGGGAGGAGCAGTACAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGC ACCAGGACTGGCTGAATGGCAAGGAGT ACAAGTGCAAGGTCTCCAACAAAGCCC TCCCAGCCCCCATCGAGAAAACCATCTC CAAAGCCAAAGGGCAGCCCCGAGAACC ACAGGTGTACACCCTGCCCCCATCCCGG GAGGAGATGACCAAGAACCAGGTCAGC CTGACCTGCCTGGTCAAAGGCTTCTATC CCAGCGACATCGCCGTGGAGTGGGAGA GCAATGGGCAGCCGGAGAACAACTACA AGACCACGCCTCCCGTGCTGGACTCCGA CGGCTCCTTCTTCCTCTATAGCAAGCTC ACCGTGGACAAGAGCAGGTGGCAGCAG GGGAACGTCTTCTCATGCTCCGTGATGC ATGAGGCTCTGCACAACCACTACACGC AGAAGAGCCTCTCCCTGTCTCCCGGGAA ATGA
Heavy Chain Variable Domain Nucleotide Sequence	CAGGTGCAGCTGGTGCAGTCTGGGG CTGAGGTGAAGAAACCCGGTGCAAGT

(SEQ ID NO: 26)	GTGAAAGTGTCATGCAAGGCATCCGG CTATACATTCACTAACTACTGGATGC ATTGGGTGAAGCAGGCACCAGGACA GGGACTGGAATGGATCGGCGTGATC GACCCTTCAGATTCCTACACCACATA TAATCAGAAGTTTAAAGGCAAGGCTA CCATGACAAGGGACACTAGCACCTCT ACAGTGTACATGGAGCTGTCCAGCCT GAGGTCCGAAGATACAGCCGTGTACT ATTGCACTCGGGGCAACTACGGAGTG GACTATGCTATGGATTACTGGGGGCA GGGTACTAGTGTGACCGTGTCTAGT

Table 7: Amino acid and nucleotide sequences of humanized anti-human CCL20 antibody heavy chain 36HKK3

DESCRIPTION	SEQUENCE (signal sequence underlined, variable domain in bold)
Heavy Chain Amino Acid Sequence (SEQ ID NO: 3)	MDWTWRILFLVAAATGAHSQVQLVQSG AEVKKPGASVKVSCKASGYTFTNYWM HWVRQAPGQGLEWMGVIDPSDSYTTY NQKFKGKATLTVDTSTSTAYMELSSLR SEDTAVYYCTRGNYGVDYAMDYWGQ GTLVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKRVEPKSCDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
Heavy Chain Variable Domain Amino Acid Sequence (SEQ ID NO: 11)	QVQLVQSGAEVKKPGASVKVSCKASG YTFTNYWMHWVRQAPGQGLEWMGVI DPSDSYTTYNQKFKGKATLTVDTSTST AYMELSSLRSEDTAVYYCTRGNYGVDY AMDYWGQGTLVTVSS
Heavy Chain Nucleotide Sequence (SEQ ID NO: 19)	ATGGACTGGACATGGAGAATCCTGTTCC TGGTGGCCGCTGCAACCGGAGCACACA GCCAGGTGCAGCTGGTGCAGTCTGGA GCAGAGGTGAAGAAACCCGGTGCTA GTGTGAAAGTGTCATGCAAGGCCTCC GGGTATACTTTCACCAACTACTGGAT GCATTGGGTGAGGCAGGCTCCAGGA CAGGGACTGGAATGGATGGGCGTGA TTGACCCTTCAGATTCCTACACCACA TATAATCAGAAGTTTAAAGGAAAGGC AACACTGACTGTGGACACCAGCACAT

	CTACTGCCTACATGGAGCTGTCCAGC CTGAGGTCCGAAGATACTGCCGTGTA CTATTGTACCCGGGGCAACTACGGAG TGGACTATGCAATGGATTACTGGGGG CAGGGTACCCTGGTCACAGTGTCTAG TGCTAGCACCAAGGGCCCATCGGTCTTC CCCCTGGCACCCTCCTCCAAGAGCACCT CTGGGGGCACAGCGGCCCTGGGCTGCC TGGTCAAGGACTACTTCCCCGAACCGGT GACGGTGTCGTGGAACTCAGGCGCCCT GACCAGCGGCGTGCACACCTTCCCGGCT GTCCTACAGTCCTCAGGACTCTACTCCC TCAGCAGCGTGGTGACCGTGCCCTCCAG CAGCTTGGGCACCCAGACCTACATCTGC AACGTGAATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGCCCAAA TCTTGTGACAAAACTCACACATGCCCAC CGTGCCCAGCACCTGAACTCCTGGGGG GACCGTCAGTCTTCCTCTTCCCCCCAAA ACCCAAGGACACCCTCATGATCTCCCGG ACCCCTGAGGTCACATGCGTGGTGGTG GACGTGAGCCACGAAGACCCTGAGGTC AAGTTCAACTGGTACGTGGACGGCGTG GAGGTGCATAATGCCAAGACAAAGCCG CGGGAGGAGCAGTACAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGC ACCAGGACTGGCTGAATGGCAAGGAGT ACAAGTGCAAGGTCTCCAACAAAGCCC TCCCAGCCCCCATCGAGAAAACCATCTC CAAAGCCAAAGGGCAGCCCCGAGAACC ACAGGTGTACACCCTGCCCCCATCCCGG GAGGAGATGACCAAGAACCAGGTCAGC CTGACCTGCCTGGTCAAAGGCTTCTATC CCAGCGACATCGCCGTGGAGTGGGAGA GCAATGGGCAGCCGGAGAACAACTACA AGACCACGCCTCCCGTGCTGGACTCCGA CGGCTCCTTCTTCCTCTATAGCAAGCTC ACCGTGGACAAGAGCAGGTGGCAGCAG GGGAACGTCTTCTCATGCTCCGTGATGC ATGAGGCTCTGCACAACCACTACACGC AGAAGAGCCTCTCCCTGTCTCCGGGTAA ATGA
Heavy Chain Variable Domain Nucleotide Sequence (SEQ ID NO: 27)	CAGGTGCAGCTGGTGCAGTCTGGAGC AGAGGTGAAGAAACCCGGTGCTAGTG TGAAAGTGTCATGCAAGGCCTCCGGG TATACTTTCACCAACTACTGGATGCA TTGGGTGAGGCAGGCTCCAGGACAG GGACTGGAATGGATGGGCGTGATTGA CCCTTCAGATTCCTACACCACATATA ATCAGAAGTTTAAAGGAAAGGCAACA CTGACTGTGGACACCAGCACATCTAC TGCCTACATGGAGCTGTCCAGCCTGA GGTCCGAAGATACTGCCGTGTACTAT

	TGTACCCGGGGCAACTACGGAGTGGA CTATGCAATGGATTACTGGGGGCAGG GTACCCTGGTGACAGTGTCTAGT

Table 8: Amino acid and nucleotide sequences of humanized anti-human CCL20 antibody heavy chain 42HKK1

DESCRIPTION	SEQUENCE (signal sequence underlined, variable domain in bold)
Heavy Chain Amino Acid Sequence (SEQ ID NO: 4)	MDWTWRILFLVAAATGAHSQVQLVQSG **AEVKKPGASVKVSCKASGYTFTSYWM HWVRQAPGQGLEWMGLIDPSDKYTNY NQKFKGRVTMTRDTSTSTVYMELSSLR SEDTAVYYCARGNYGVDYGMDYWGQ GTLVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKRVEPKSCDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK**
Heavy Chain Variable Domain Amino Acid Sequence (SEQ ID NO: 12)	**QVQLVQSGAEVKKPGASVKVSCKASG YTFTSYWMHWVRQAPGQGLEWMGLI DPSDKYTNYNQKFKGRVTMTRDTSTST VYMELSSLRSEDTAVYYCARGNYGVDY GMDYWGQGTLVTVSS**
Heavy Chain Nucleotide Sequence (SEQ ID NO: 20)	ATGGACTGGACCTGGCGAATCCTGTTCC TGGTGGCCGCTGCAACAGGAGCACACT CACAGGTGCAGCTGGTGCAGTCCGGG GCAGAGGTGAAGAAACCCGGTGCCA GCGTGAAGGTGTCTTGCAAAGCTAGT GGCTATACCTTCACAAGCTACTGGAT GCATTGGGTGCGGCAGGCACCAGGA CAGGGACTGGAATGGATGGGCCTGAT TGACCCTTCTGATAAGTACACTAACT ACAACCAGAAGTTTAAAGGAAGGGTG ACTATGACCCGGGACACATCAACTTC CACCGTGTACATGGAGCTGTCCAGCC TGAGATCCGAAGATACCGCCGTGTAC TATTGTGCTCGCGGCAACTACGGAGT GGACTATGGCATGGATTACTGGGGCC AGGGTACACTGGTGACCGTGTCCAGT GCTAGCACCAAGGGCCCATCGGTCTTCC CCCTGGCACCCTCCTCCAAGAGCACCTC TGGGGGCACAGCGGCCCTGGGCTGCCT GGTCAAGGACTACTTCCCCGAACCGGT

	GACGGTGTCGTGGAACTCAGGCGCCCT GACCAGCGGCGTGCACACCTTCCCGGCT GTCCTACAGTCCTCAGGACTCTACTCCC TCAGCAGCGTGGTGACCGTGCCCTCCAG CAGCTTGGGCACCCAGACCTACATCTGC AACGTGAATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGCCCAAA TCTTGTGACAAAACTCACACATGCCCAC CGTGCCCAGCACCTGAACTCCTGGGGG GACCGTCAGTCTTCCTCTTCCCCCCAAA ACCCAAGGACACCCTCATGATCTCCCGG ACCCCTGAGGTCACATGCGTGGTGGTG GACGTGAGCCACGAAGACCCTGAGGTC AAGTTCAACTGGTACGTGGACGGCGTG GAGGTGCATAATGCCAAGACAAAGCCG CGGGAGGAGCAGTACAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGC ACCAGGACTGGCTGAATGGCAAGGAGT ACAAGTGCAAGGTCTCCAACAAAGCCC TCCCAGCCCCCATCGAGAAAACCATCTC CAAAGCCAAAGGGCAGCCCCGAGAACC ACAGGTGTACACCCTGCCCCCATCCCGG GAGGAGATGACCAAGAACCAGGTCAGC CTGACCTGCCTGGTCAAAGGCTTCTATC CCAGCGACATCGCCGTGGAGTGGGAGA GCAATGGGCAGCCGGAGAACAACTACA AGACCACGCCTCCCGTGCTGGACTCCGA CGGCTCCTTCTTCCTCTATAGCAAGCTC ACCGTGGACAAGAGCAGGTGGCAGCAG GGGAACGTCTTCTCATGCTCCGTGATGC ATGAGGCTCTGCACAACCACTACACGC AGAAGAGCCTCTCCCTGTCTCCGGGTAA ATGA
Heavy Chain Variable Domain Nucleotide Sequence (SEQ ID NO: 28)	CAGGTGCAGCTGGTGCAGTCCGGGG CAGAGGTGAAGAAACCCGGTGCCAG CGTGAAGGTGTCTTGCAAAGCTAGTG GCTATACCTTCACAAGCTACTGGATG CATTGGGTGCGGCAGGCACCAGGAC AGGGACTGGAATGGATGGGCCTGATT GACCCTTCTGATAAGTACACTAACTA CAACCAGAAGTTTAAAGGAAGGGTGA CTATGACCCGGGACACATCAACTTCC ACCGTGTACATGGAGCTGTCCAGCCT GAGATCCGAAGATACCGCCGTGTACT ATTGTGCTCGCGGCAACTACGGAGTG GACTATGGCATGGATTACTGGGGGCA GGGTACACTGGTGACCGTGTCCAGT

Table 9: Amino acid and nucleotide sequences of humanized anti-human CCL20 antibody heavy chain 42HKK2

DESCRIPTION	SEQUENCE

	(signal sequence underlined, variable domain in bold)
Heavy Chain Amino Acid Sequence (SEQ ID NO: 5)	MDWTWRILFLVAAATGAHSQVQLVQSG AEVKKPGASVKVSCKASGYTFTSYWM HWVRQAPGQGLEWMGLIDPSDKYTNY NQKFKGRVTLTVDTSTSTVYMELSSLR SEDTAVYYCTRGNYGVDYGMDYWGQ GTLVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKRVEPKSCDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
Heavy Chain Variable Domain Amino Acid Sequence (SEQ ID NO: 13)	QVQLVQSGAEVKKPGASVKVSCKASG YTFTSYWMHWVRQAPGQGLEWMGLI DPSDKYTNYNQKFKGRVTLTVDTSTST VYMELSSLRSEDTAVYYCTRGNYGVDY GMDYWGQGTLVTVSS
Heavy Chain Nucleotide Sequence (SEQ ID NO: 21)	ATGGACTGGACTTGGAGGATCCTGTTCC TGGTGGCCGCTGCAACCGGAGCTCACTC ACAGGTGCAGCTGGTGCAGTCCGGA GCAGAGGTGAAGAAACCCGGTGCCTC CGTGAAGGTGTCTTGCAAAGCAAGTG GCTATACCTTCACAAGCTACTGGATG CATTGGGTGAGACAGGCACCAGGACA GGGACTGGAATGGATGGGCCTGATTG ACCCTTCTGATAAGTACACCAACTAC AACCAGAAGTTTAAAGGACGCGTGAC TCTGACCGTGGACACATCAACTTCCA CCGTGTACATGGAGCTGTCCAGCCTG AGGTCCGAAGATACCGCAGTGTACTA TTGTACACGGGGCAACTACGGAGTGG ACTATGGCATGGATTACTGGGGGCAG GGTACACTGGTGACCGTGTCCAGTGC TAGCACCAAGGGCCCATCGGTCTTCCCC CTGGCACCCTCCTCCAAGAGCACCTCTG GGGGCACAGCGGCCCTGGGCTGCCTGG TCAAGGACTACTTCCCCGAACCGGTGAC GGTGTCGTGGAACTCAGGCGCCCTGAC CAGCGGCGTGCACACCTTCCCGGCTGTC CTACAGTCCTCAGGACTCTACTCCCTCA GCAGCGTGGTGACCGTGCCCTCCAGCA GCTTGGGCACCCAGACCTACATCTGCAA CGTGAATCACAAGCCCAGCAACACCAA GGTGGACAAGAGAGTTGAGCCCAAATC TTGTGACAAAACTCACACATGCCCACCG TGCCCAGCACCTGAACTCCTGGGGGGA

	CCGTCAGTCTTCCTCTTCCCCCCAAAAC CCAAGGACACCCTCATGATCTCCCGGAC CCCTGAGGTCACATGCGTGGTGGTGGA CGTGAGCCACGAAGACCCTGAGGTCAA GTTCAACTGGTACGTGGACGGCGTGGA GGTGCATAATGCCAAGACAAAGCCGCG GGAGGAGCAGTACAACAGCACGTACCG TGTGGTCAGCGTCCTCACCGTCCTGCAC CAGGACTGGCTGAATGGCAAGGAGTAC AAGTGCAAGGTCTCCAACAAAGCCCTC CCAGCCCCCATCGAGAAAACCATCTCC AAAGCCAAAGGGCAGCCCCGAGAACCA CAGGTGTACACCCTGCCCCCATCCCGGG AGGAGATGACCAAGAACCAGGTCAGCC TGACCTGCCTGGTCAAAGGCTTCTATCC CAGCGACATCGCCGTGGAGTGGGAGAG CAATGGGCAGCCGGAGAACAACTACAA GACCACGCCTCCCGTGCTGGACTCCGAC GGCTCCTTCTTCCTCTATAGCAAGCTCA CCGTGGACAAGAGCAGGTGGCAGCAGG GGAACGTCTTCTCATGCTCCGTGATGCA TGAGGCTCTGCACAACCACTACACGCA GAAGAGCCTCTCCCTGTCTCCGGGTAAA TGA
Heavy Chain Variable Domain Nucleotide Sequence (SEQ ID NO: 29)	**CAGGTGCAGCTGGTGCAGTCCGGAG CAGAGGTGAAGAAACCCGGTGCCTCC GTGAAGGTGTCTTGCAAAGCAAGTGG CTATACCTTCACAAGCTACTGGATGC ATTGGGTGAGACAGGCACCAGGACA GGGACTGGAATGGATGGGCCTGATTG ACCCTTCTGATAAGTACACCAACTAC AACCAGAAGTTTAAAGGACGCGTGAC TCTGACCGTGGACACATCAACTTCCA CCGTGTACATGGAGCTGTCCAGCCTG AGGTCCGAAGATACCGCAGTGTACTA TTGTACACGGGGCAACTACGGAGTGG ACTATGGCATGGATTACTGGGGGCAG GGTACACTGGTGACCGTGTCCAGT**

Table 10: Amino acid and nucleotide sequences of humanized anti-human CCL20 antibody heavy chain 42HKK3

DESCRIPTION	SEQUENCE (signal sequence underlined, variable domain in bold)
Heavy Chain Amino Acid Sequence (SEQ ID NO: 6)	<u>MDWTWRILFLVAAATGAHS</u>**QVQLVQSG AEVKKPGASVKVSCKASGYTFTSYWM HWVRQAPGQGLEWMGLIDPSDKYTNY NQKFKGKATLTVDTSTSTAYMELSSLR SEDTAVYYCTRGNYGVDYGMDYWGQ GTLVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGV**

	HTFPAVLQSSGLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKRVEPKSCDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISR TPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
Heavy Chain Variable Domain Amino Acid Sequence (SEQ ID NO: 14)	**QVQLVQSGAEVKKPGASVKVSCKASG YTFTSYWMHWVRQAPGQGLEWMGLI DPSDKYTNYNQKFKGKATLTVDTSTST AYMELSSLRSEDTAVYYCTRGNYGVDY GMDYWGQGTLVTVSS**
Heavy Chain Nucleotide Sequence (SEQ ID NO: 22)	ATGGACTGGACTTGGAGAATCCTGTTCC TGGTGGCCGCTGCAACCGGAGCTCACTC ACAGGTGCAGCTGGTGCAGTCCGGA GCAGAGGTGAAGAAACCCGGTGCCTC CGTGAAAGTGTCTTGCAAGGCTAGTG GCTATACCTTCACAAGCTACTGGATG CATTGGGTGAGGCAGGCACCAGGAC AGGGACTGGAATGGATGGGCCTGATT GACCCTTCTGATAAGTACACCAACTA CAACCAGAAGTTTAAAGGAAAGGCAA CTCTGACCGTGGACACATCAACTTCC ACCGCCTACATGGAGCTGTCCAGCCT GAGGTCCGAAGATACCGCCGTGTACT ATTGTACACGGGGCAACTACGGAGTG GACTATGGCATGGATTACTGGGGGCA GGGTACACTGGTGACCGTGTCCAGTG CTAGCACCAAGGGCCCATCGGTCTTCCC CCTGGCACCCTCCTCCAAGAGCACCTCT GGGGGCACAGCGGCCCTGGGCTGCCTG GTCAAGGACTACTTCCCCGAACCGGTG ACGGTGTCGTGGAACTCAGGCGCCCTG ACCAGCGGCGTGCACACCTTCCCGGCTG TCCTACAGTCCTCAGGACTCTACTCCCT CAGCAGCGTGGTGACCGTGCCCTCCAG CAGCTTGGGCACCCAGACCTACATCTGC AACGTGAATCACAAGCCCAGCAACACC AAGGTGGACAAGAGAGTTGAGCCCAAA TCTTGTGACAAAACTCACACATGCCCAC CGTGCCCAGCACCTGAACTCCTGGGGG GACCGTCAGTCTTCCTCTTCCCCCCAAA ACCCAAGGACACCCTCATGATCTCCCGG ACCCCTGAGGTCACATGCGTGGTGGTG GACGTGAGCCACGAAGACCCTGAGGTC AAGTTCAACTGGTACGTGGACGGCGTG GAGGTGCATAATGCCAAGACAAAGCCG CGGGAGGAGCAGTACAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGC ACCAGGACTGGCTGAATGGCAAGGAGT

	ACAAGTGCAAGGTCTCCAACAAAGCCC TCCCAGCCCCCATCGAGAAAACCATCTC CAAAGCCAAAGGGCAGCCCCGAGAACC ACAGGTGTACACCCTGCCCCCATCCCGG GAGGAGATGACCAAGAACCAGGTCAGC CTGACCTGCCTGGTCAAAGGCTTCTATC CCAGCGACATCGCCGTGGAGTGGGAGA GCAATGGGCAGCCGGAGAACAACTACA AGACCACGCCTCCCGTGCTGGACTCCGA CGGCTCCTTCTTCCTCTATAGCAAGCTC ACCGTGGACAAGAGCAGGTGGCAGCAG GGGAACGTCTTCTCATGCTCCGTGATGC ATGAGGCTCTGCACAACCACTACACGC AGAAGAGCCTCTCCCTGTCTCCGGGTAA ATGA
Heavy Chain Variable Domain Nucleotide Sequence (SEQ ID NO: 30)	**CAGGTGCAGCTGGTGCAGTCCGGAG CAGAGGTGAAGAAACCCGGTGCCTCC GTGAAAGTGTCTTGCAAGGCTAGTGG CTATACCTTCACAAGCTACTGGATGC ATTGGGTGAGGCAGGCACCAGGACA GGGACTGGAATGGATGGGCCTGATTG ACCCTTCTGATAAGTACACCAACTAC AACCAGAAGTTTAAAGGAAAGGCAAC TCTGACCGTGGACACATCAACTTCCA CCGCCTACATGGAGCTGTCCAGCCTG AGGTCCGAAGATACCGCCGTGTACTA TTGTACACGGGGCAACTACGGAGTGG ACTATGGCATGGATTACTGGGGGCAG GGTACACTGGTGACCGTGTCCAGT**

Table 11: Amino acid and nucleotide sequences of humanized anti-human CCL20 antibody light chain LC3

DESCRIPTION	SEQUENCE (signal sequence underlined; variable domain in bold)
Light Chain Amino Acid Sequence with the signal sequence (SEQ ID NO: 7)	MGWSCIILFLVATATGVHS**DIQMTQSPSS LSASVGDRVTITCRASENIYGALNWYQ QKPGKAPKLLIYGATNLADGVPSRFSG SGSGRQYSLTISSLQPEDFATYYCQNVL ITPYTFGGGTKLEIKRTVAAPSVFIFPPSD EQLKSGTASVVCLLNNFYPREAKVQWKV DNALQSGNSQESVTEQDSKDSTYSLSSTL TLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC**
Light Chain Amino Acid Sequence without the signal sequence (SEQ ID NO: 110)	**DIQMTQSPSSLSASVGDRVTITCRASENI YGALNWYQQKPGKAPKLLIYGATNLA DGVPSRFSGSGSGRQYSLTISSLQPEDFA TYYCQNVLITPYTFGGGTKLEIKRTVAA PSVFIFPPSDEQLKSGTASVVCLLNNFYPR EAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSTLTLSKADYEKHKVYACEVTH**

	QGLSSPVTKSFNRGEC
Light Chain Variable Domain Amino Acid Sequence (SEQ ID NO: 15)	DIQMTQSPSSLSASVGDRVTITCRASENI YGALNWYQQKPGKAPKLLIYGATNLA DGVPSRFSGSGSGRQYSLTISSLQPEDFA TYYCQNVLITPYTFGGGTKLEIK
Light Chain Nucleotide Sequence with the signal sequence (SEQ ID NO: 23)	ATGGGCTGGTCCTGCATCATTCTGTTCC TGGTGGCAACCGCCACAGGAGTGCACA GCGACATCCAGATGACCCAGTCTCCA TCCAGCCTGAGTGCCTCAGTGGGCGA TAGGGTGACTATCACCTGTCGGGCCA GCGAGAACATCTACGGCGCTCTGAAT TGGTATCAGCAGAAGCCAGGAAAAGC TCCCAAGCTGCTGATCTACGGGGCTA CAAACCTGGCAGACGGTGTGCCCAGT CGATTCTCCGGTAGCGGCTCTGGACG ACAGTATTCACTGACTATCTCTAGTCT GCAGCCTGAAGATTTCGCCACTTACT ATTGCCAGAATGTGCTGATTACTCCA TATACCTTTGGCGGAGGGACAAAACT GGAGATCAAGAGAACTGTGGCCGCTCC CAGTGTGTTCATTTTTCCCCCTTCAGAC GAACAGCTGAAATCAGGGACCGCTTCC GTGGTGTGTCTGCTGAACAATTTCTACC CTCGCGAGGCAAAAGTGCAGTGGAAGG TGGATAACGCCCTGCAGAGTGGCAATT CACAGGAGTCCGTGACCGAACAGGACA GCAAAGATTCTACATATAGTCTGTCATC CACCCTGACACTGAGCAAGGCTGATTA CGAGAAGCACAAAGTGTATGCATGCGA AGTGACTCATCAGGGGCTGAGCTCTCCC GTGACCAAGTCTTTTAACCGGGGTGAAT GTTGA
Light Chain Nucleotide Sequence without the signal sequence (SEQ ID NO: 111)	GACATCCAGATGACCCAGTCTCCATC CAGCCTGAGTGCCTCAGTGGGCGATA GGGTGACTATCACCTGTCGGGCCAGC GAGAACATCTACGGCGCTCTGAATTG GTATCAGCAGAAGCCAGGAAAAGCTC CCAAGCTGCTGATCTACGGGGCTACA AACCTGGCAGACGGTGTGCCCAGTCG ATTCTCCGGTAGCGGCTCTGGACGAC AGTATTCACTGACTATCTCTAGTCTG CAGCCTGAAGATTTCGCCACTTACTA TTGCCAGAATGTGCTGATTACTCCAT ATACCTTTGGCGGAGGGACAAAACTG GAGATCAAGAGAACTGTGGCCGCTCCC AGTGTGTTCATTTTTCCCCCTTCAGACG AACAGCTGAAATCAGGGACCGCTTCCG TGGTGTGTCTGCTGAACAATTTCTACCC TCGCGAGGCAAAAGTGCAGTGGAAGGT GGATAACGCCCTGCAGAGTGGCAATTC ACAGGAGTCCGTGACCGAACAGGACAG CAAAGATTCTACATATAGTCTGTCATCC ACCCTGACACTGAGCAAGGCTGATTAC

	GAGAAGCACAAAGTGTATGCATGCGAA GTGACTCATCAGGGGCTGAGCTCTCCCG TGACCAAGTCTTTTAACCGGGGTGAATG TTGA
Light Chain Variable Domain Nucleotide Sequence (SEQ ID NO: 31)	**GACATCCAGATGACCCAGTCTCCATC CAGCCTGAGTGCCTCAGTGGGCGATA GGGTGACTATCACCTGTCGGGCCAGC GAGAACATCTACGGCGCTCTGAATTG GTATCAGCAGAAGCCAGGAAAAGCTC CCAAGCTGCTGATCTACGGGGCTACA AACCTGGCAGACGGTGTGCCCAGTCG ATTCTCCGGTAGCGGCTCTGGACGAC AGTATTCACTGACTATCTCTAGTCTG CAGCCTGAAGATTTCGCCACTTACTA TTGCCAGAATGTGCTGATTACTCCAT ATACCTTTGGCGGAGGGACAAAACTG GAGATCAAG**

Table 12: Amino acid and nucleotide sequences of humanized anti-human CCL20 antibody light chain LK3

DESCRIPTION	SEQUENCE (signal sequence underlined, variable domain in bold)
Light Chain Amino Acid Sequence with the signal sequence (SEQ ID NO: 8)	MDMRVPAQLLGLLLLWLRGARC**DIQMT QSPSSLSASVGDRVTITCGASENIYGALN WYQRKPGKAPKLLIYGATNLADGVPSR FSGSGSGRDYTLTISSLQPEDFATYYCQ NVLITPYTFGQGTKLEIKRTVAAPSVFIF PPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSP** VTKSFNRGEC
Light Chain Amino Acid Sequence without the signal sequence (SEQ ID NO: 112)	**DIQMTQSPSSLSASVGDRVTITCGASENI YGALNWYQRKPGKAPKLLIYGATNLA DGVPSRFSGSGSGRDYTLTISSLQPEDF ATYYCQNVLITPYTFGQGTKLEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVT HQGLSSPVTKSFNRGEC**
Light Chain Variable Domain Amino Acid Sequence (SEQ ID NO: 16)	**DIQMTQSPSSLSASVGDRVTITCGASENI YGALNWYQRKPGKAPKLLIYGATNLA DGVPSRFSGSGSGRDYTLTISSLQPEDF ATYYCQNVLITPYTFGQGTKLEIK**
Light Chain Nucleotide Sequence with the signal sequence (SEQ ID NO: 24)	ATGGACATGAGGGTGCCTGCTCAGCTG CTGGGACTGCTGCTGCTGTGGCTGAGGG GAGCACGATGC**GACATCCAGATGACT CAGAGCCCATCCAGCCTGTCAGCCTC CGTGGGCGACAGGGTGACCATCACAT GTGGAGCATCCGAGAACATCTACGGG GCCCTGAATTGGTATCAGAGGAAGCC**

	CGGCAAAGCTCCTAAGCTGCTGATCT ACGGTGCCACAAACCTGGCTGATGGC GTGCCCTCCAGATTCAGCGGCTCTGG AAGTGGGCGCGACTATACTCTGACCA TTTCTAGTCTGCAGCCAGAGGATTTC GCCACCTACTATTGCCAGAATGTGCT GATCACACCCTACACTTTTGGTCAGG GCACAAAACTGGAAATTAAGCGTACG GTGGCTGCACCATCTGTCTTCATCTTCC CGCCATCTGATGAGCAGTTGAAATCTGG AACTGCCTCTGTTGTGTGCCTGCTGAAT AACTTCTATCCCAGAGAGGCCAAAGTA CAGTGGAAGGTGGATAACGCCCTCCAA TCGGGTAACTCCCAGGAGAGTGTCACA GAGCAGGACAGCAAGGACAGCACCTAC AGCCTCAGCAGCACCCTGACGCTGAGC AAAGCAGACTACGAGAAACACAAAGTC TACGCCTGCGAAGTCACCCATCAGGGC CTGAGCTCGCCCGTCACAAAGAGCTTCA ACAGGGGAGAGTGTTGA
Light Chain Nucleotide Sequence without the signal sequence (SEQ ID NO: 113)	GACATCCAGATGACTCAGAGCCCATC CAGCCTGTCAGCCTCCGTGGGCGACA GGGTGACCATCACATGTGGAGCATCC GAGAACATCTACGGGGCCCTGAATTG GTATCAGAGGAAGCCCGGCAAAGCTC CTAAGCTGCTGATCTACGGTGCCACA AACCTGGCTGATGGCGTGCCCTCCAG ATTCAGCGGCTCTGGAAGTGGGCGCG ACTATACTCTGACCATTTCTAGTCTGC AGCCAGAGGATTTCGCCACCTACTAT TGCCAGAATGTGCTGATCACACCCTA CACTTTTGGTCAGGGCACAAAACTGG AAATTAAGCGTACGGTGGCTGCACCAT CTGTCTTCATCTTCCCGCCATCTGATGA GCAGTTGAAATCTGGAACTGCCTCTGTT GTGTGCCTGCTGAATAACTTCTATCCCA GAGAGGCCAAAGTACAGTGGAAGGTGG ATAACGCCCTCCAATCGGGTAACTCCCA GGAGAGTGTCACAGAGCAGGACAGCAA GGACAGCACCTACAGCCTCAGCAGCAC CCTGACGCTGAGCAAAGCAGACTACGA GAAACACAAAGTCTACGCCTGCGAAGT CACCCATCAGGGCCTGAGCTCGCCCGTC ACAAAGAGCTTCAACAGGGGAGAGTGT TGA
Light Chain Variable Domain Nucleotide Sequence, (SEQ ID NO: 32)	GACATCCAGATGACTCAGAGCCCATC CAGCCTGTCAGCCTCCGTGGGCGACA GGGTGACCATCACATGTGGAGCATCC GAGAACATCTACGGGGCCCTGAATTG GTATCAGAGGAAGCCCGGCAAAGCTC CTAAGCTGCTGATCTACGGTGCCACA AACCTGGCTGATGGCGTGCCCTCCAG ATTCAGCGGCTCTGGAAGTGGGCGCG

	ACTATACTCTGACCATTTCTAGTCTGC AGCCAGAGGATTTCGCCACCTACTAT TGCCAGAATGTGCTGATCACACCCTA CACTTTTGGTCAGGGCACAAAACTGG AAATTAAG

Table 13: Amino acid sequences encoded by human germline genes

DESCRIPTION	SEQUENCE
IGHV1-46*03 (SEQ ID NO: 57)	QVQLVQSGAEVKKPGASVKVSCKASGYT FTSYYMHWVRQAPGQGLEWMGIINPSGG STSYAQKFQGRVTMTRDTSTSTVYMELSS LRSEDTAVYYCAR
JH4 (SEQ ID NO: 58)	WGQGTLVTVSS
IGKV1D-39*01 (SEQ ID NO: 59)	DIQMTQSPSSLSASVGDRVTITCRASQSISS YLNWYQQKPGKAPKLLIYAASSLQSGVP SRFSGSGSGTDFTLTISSLQPEDFATYYCQ QSYSTPP

[0263] Based on the evaluation results, we selected 36LK3/36HC2 ("HC2/LK3") and 36LC3/36HC2 ("HC2/LC3") for further studies. For these two antibodies, *in vitro* chemotaxis assays were performed in parallel with parental mouse clone 36F7C10 and its chimeric form (comprising a human Fc portion). In this assay, we employed transwell culture plates with B300.19 CCR6+ cells seeded in the upper layer and recombinant human CCL20 ligand in the lower layer. Recombinant human CCL20 (10 nM final, R&D Systems) was pre-incubated with the humanized anti-CCL20 antibodies at room temperature. After 30 min, human CCR6-transduced murine pre-B cells (B300.19, provided by Dr. H. Kawasaki at Tokyo University) were applied to the upper layer and chemotaxis was developed at 37°C for 4 hrs. At the end of incubation, FACS was used to measure migrated cells. The 50%, 90%, and 95% inhibitory concentrations (IC_{50}, IC_{90}, and IC_{95}, respectively) for HC2/LK3 and HC2/LC3 were then calculated (Table 14). Neither of the humanized antibodies lost neutralization activity in comparison with the parental mouse MAb, and IC_{50} values were calculated at around 1 nM.

Table 14: Neutralizing activity of HC2/LK3 and HC2/LC3 against human CCL20 in a chemotaxis assay (in nM)

	Mouse MAb	Chimeric MAb
IC_{50}	1.697 ± 0.464	1.438 ± 0.216
IC_{90}	3.944 ± 0.593	3.510 ± 0.601
IC_{95}	7.680 ± 2.966	5.480 ± 1.179

HuMAb HC2/LK3				
	Assay-1	-2	-3	Average
IC_{50}	0.65	1.09	1.30	1.014 ± 0.330
IC_{90}	3.55	3.35	6.79	4.564 ± 1.928
IC_{95}	7.59	5.41	13.91	8.972 ± 4.417

HuMAb HC2/LC3				
	Assay-1	-2	-3	Average
IC_{50}	0.90	1.26	1.20	1.117 ± 0.193
IC_{90}	4.64	5.82	5.30	5.251 ± 0.591
IC_{95}	11.77	>66.77	10.33	--

The dose responses for a representative trial with the HC2/LK3 antibody are shown in FIGS. 8A-C. Three independent experiments are depicted.

[0264] We further confirmed the neutralizing activity of HC2/LK3 and HC2/LC3 using a transendothelial migration (TEM) assay in which freshly isolated human peripheral blood mononuclear cells were used instead of CCR6-transduced artificial cells. Because CD3+CD4+CD45RO+ memory T cells and CD19+ B cells are well known to be enriched with CCR6-positive cells, we measured the migrated cell numbers of these populations in the presence or absence of the HC2/LK3 and HC2/LC3 antibodies, as well as the parental mouse antibody 36F7C10. Both humanized antibodies (FIGS. 9B and C) demonstrated dose dependent inhibition of cell migration comparable to that of 36F7C10 (FIG. 9A), providing further evidence of their neutralizing activity.

Example 5: Binding of humanized anti-human CCL20 MAbs to human CCL20

[0265] To measure the binding affinity of humanized antibodies HC2/LC3 and HC2/LK3 by surface plasmon resonance (Biacore™), we expressed and purified the antibodies from conditioned media of transiently-transfected HEK293F cells, and captured the antibodies on a CM5 chip coated with anti-human Fc monoclonal antibodies using HBS-EP as the running buffer. We then injected several dilutions (100, 20, 4, 0.8, 0.16, and 0 nM) of human CCL20 protein (R&D Systems) over the coated chip surface, and observed dissociation of bound CCL20 for up to 20 minutes (FIGS. 10A and B). We fitted binding data globally to a 1:1 Langmuir model. The results are shown in Table 15 and represent the average of two independent experiments. Affinity between CCL20 and its receptor,

CCR6, has been reported as 500 pM in primary human cells (Liao *et al.*, *J. Immunol.* 168(10):4871-4880 (2002)); these data demonstrate that the affinities of the HC2/LC3 and HC2/LK3 antibodies for CCL20 are approximately 10 times higher than the affinity of CCR6 for CCL20.

Table 15: SPR results of humanized anti-CCL20 antibodies of the invention

Heavy chain/ Light chain	k_a ($x10^5$ M^{-1} sec^{-1})	k_d ($x10^{-5}$ sec^{-1})	K_D (pM)	Standard deviation
HC2/LC3	145	61	44	15
HC2/LK3	166	117	70	7

Example 6: Cross-reactivity of humanized anti-human CCL20 MAbs to chemokine paralogs

[0266] We examined HC2/LK3 and HC2/LC3 for their specificity for human CCL20 by analyzing their cross-reactivity against a chemokine panel (Table 16) using an enzyme-linked immunosorbent assay (ELISA).

Table 16: Recombinant human chemokines used in ELISA assay

Chemokine		Maker	Cat. No.
CCL20	MIP-3a/LARC	R&D	#360-MP-025/CF
XCL1	Ltn	R&D	#695-LT-025/CF
CCL28		R&D	#717-VC-025/CF
CCL27	CTACK	R&D	#376-CT-025/CF
CCL25	TECK	GT	#2234X
CCL24	Eotaxin-2/MPIF-2	GT	#2343X
CCL22	MDC	R&D	#336-MD-025/CF
CCL21	6Ckine/SLC	R&D	#366-6C-025/CF
CCL19	MIP-3b	R&D	#361-MI-025/CF
CCL17	TARC	GT	#2364
CCL16	HCC-4	R&D	#802-HC-025/CF
CCL13	MCP-4	GT	#2327X
CCL11	Eotaxin	R&D	#320-EO-020/CF
CCL7	MARC	R&D	#282-P3-010/CF
CCL5	Rantes	CHEMICON	#GF020
CCL4	MIP-1β	R&D	#271-BME-010/CF
CCL3	MIP-1a	GT	#2270X
CCL2	MCP-1/MCAF/JE	PEPRO TECH	#300-04
CCL1	TCA3/I-309	GT	#2272
CXCL16	CXCL16	GT	#2976X
CXCL13	BCA-1/BLC	R&D	#801-CX-025/CF
CXCL12	SDF-1/PBSF	R&D	#350-NS-010/CF

CXCL10	IP-10	PEPRO TECH	#300-12
CXCL9	MIG	GT	#2392X
CXCL8	IL-8	PEPRO TECH	#200-08M
CXCL4	PF4	R&D	#795-P4-025/CF
CXCL2	GROβ	R&D	#276-GB-010/CF
CXCL1	GROα	R&D	#275-GR-010/CF
CX3CL1 (Chemokine Domain)	FKN	R&D	#362-CX-025/CF
CX3CL1 (Extracellular Domain)	FKN	R&D	#365-FR-025/CF

The wells of a 96-well plate were coated with 1 μg/ml of the recombinant human chemokines in PBS (-). After overnight incubation at 4°C, the wells were blocked with 1 x Block-Ace (Dainippon Sumitomo Pharma, UK-B80) for 1 hour at room temperature. After washing twice with 0.02% Tween 20/PBS (-), we added 50 μl of 10 μg/ml purified 36F7C10, chimeric 36F710, HC2/LK3, or HC2/LC3 in 0.02% Tween 20/PBS (-) to each well. The wells were incubated for 1 hour at room temperature and washed three times as described in Example 3. Horseradish peroxidase (HRP)-conjugated anti-mouse IgG antibody (Jackson, #715-035-150, for 36F7C10) or HRP-conjugated anti-human IgG Fcγ fragment (Jackson, #109-035-098, for chimeric and humanized mAbs) was then added (in a 5000 fold dilution with 0.02% Tween 20/PBS (-)) and the wells were incubated for 1 hour at room temperature. After washing five times, a TMBZ (3,3',5,5'-tetramethylbenzidine) solution (1%, in N,N-Dimethylformamide) was added to the wells and incubated for 15-30 minutes. The reaction was stopped by adding an equal volume of a 2 M H_2SO_4 stopping solution, and the optical density was read at 450 nm by ARVO (PerkinElmer). As demonstrated in FIG. 11B, HC2/LK3 and HC2/LC3 bind specifically to human CCL20 over the other chemokines in the panel.

[0267] In these assays, although HC2/LK3 was reactive against plate-bound human CCL20, it appeared less potent than the mouse 36F7C10 or chimeric 36F7C10-hFc antibodies (FIG. 11A). However, the HC2/LK3 showed clear and strong binding against CCL20 anchored via a His-tag in ELISA assays (FIG. 11C), which allowed exposure of all CCL20 portions to the solvent. This may indicate that the CCL20 epitope(s) for HC2/LK3 are buried or occluded in the procedure used to bind CCL20 to the plate surface in the first assay format. To avoid this possible artifact, we employed Biacore™ analysis in which free CCL20 was applied over MAbs captured on a sensor chip. In the Biacore™ assays, HC2/LK3 and HC2/LC3 showed strong CCL20 binding comparable to that of the 36F7C10-hFc chimeric antibody (FIG. 11D). Further, the small reaction against CXCL4 observed in FIG. 11B was found to be negligible in the Biacore™ assays (FIG. 11D).

[0268] Phylogenetic analysis by Derceper *et al.*, (*Nucleic Acids Res.* 1(36):W465-469 (2008)) indicates that CCL16 is the chemokine closest in sequence to CCL20, although the percent identity between CCL20 and CCL16 is less than 37.5% (homology in only 56 out of 70 amino acids comprising the mature CCL20 peptide (SIM – Alignment Tool for protein sequences, Swiss Institute of Bioinformatics)) (FIG. 12). We used Biacore™ analysis to test whether HC2/LK3 and HC2/LC3 cross-react with CCL16. Monoclonal mouse anti-human Fc was immobilized on all four flow cells of a CM5 chip at a flow rate of 25 µl/min with HBS-EP buffer containing 0.2 mg/mL BSA. Subsequently, 50 µl of a 1 µg/ml solution of chimeric, HC2/LC3, or HC2/LK3 antibody in HBS-EP buffer with 0.2 mg/ml BSA was injected over flow cells 2, 3, and 4, respectively. 150 µl of a 100 nM solution of CCL20 or CCL16 in HBS-EP buffer (or buffer alone) with 0.2 mg/ml BSA was then injected over all four flow cells at a flow rate of 40 µl/min. Dissociation followed for 20 min. Flow cell 1 (anti-human Fc antibody alone) was used as a reference for all flow cells.

[0269] Under these conditions, human CCL20 bound to the chimeric (FIG. 13A), HC2/LC3 (FIG. 13B), and HC2/LK3 (FIG. 13B) antibodies captured on the chip with similar strength. By contrast, no significant binding was detected when human CCL16 was run through the chip, indicating that although CCL16 is the chemokine closest in sequence to CCL20, CCL20 and CCL16 do not possess sufficient homology (<37%, as discussed above) to allow the humanized anti-CCL20 antibodies to cross-react with CCL16.

[0270] These data indicate that HC2/LK3 and HC2/LC3 are specific for human CCL20 over other chemokines.

Example 7: Cross-reactivity of humanized anti-human CCL20 MAbs to species orthologs

[0271] We then determined the cross-reactivity of the HC2/LC3 and HC2/LK3 MAbs to CCL20 from other species. Amino acid sequence alignments among CCL20 orthologs (obtained using SIM – alignment tool for protein sequences (Swiss Institute of Bioinformatics)) indicate that the identity between cynomolgus/rhesus and human CCL20 is 86%, while the homology between mouse and human CCL20 is 64% (FIG. 14; human CCL20 – SEQ ID NO: 85; rhesus CCL20 – SEQ ID NO: 86; cynomolgus CCL20 – SEQ ID NO: 87; partial mouse CCL20 – SEQ ID NO: 88 (the entire mouse CCL20 amino acid sequence may be found in SEQ ID NO: 102; residues 1-27 constitute the signal sequence; see Table 18)).

[0272] To test whether the chimeric and humanized antibodies can bind to mouse, cynomolgus, or rhesus macaque CCL20 orthologs, we used ELISA assays as illustrated in FIG. 15. Recombinant secreted alkaline phosphatase (SEAP)-chemokine fusion proteins were engineered as described in Example 2, expressed and purified for all of the CCL20 orthologs tested. Nunc MaxiSorb flat-bottom black plates were coated with 1 µg/ml mouse anti-placental alkaline phosphatase antibody (Pierce,

cat# MA1-19354) in 50 mM sodium bicarbonate, pH 9.4, overnight at 4°C. Plates were then blocked using 1X phosphate buffered saline with Tween-20 (PBST) with 5% bovine serum albumin (BSA) for 2 hours at room temperature. 20 nM SEAP-CCL20 fusion proteins were serially diluted 5-fold in Opti-MEM medium (Invitrogen). Chimeric and humanized (HC2/LC3 and HC2/LK3) antibodies were diluted to 1 µg/ml in 1X PBST with 5% BSA, added to the plates, and incubated for 1 hr at room temperature. The plates were then washed with 1X PBST with 5% BSA three times. Goat anti-human IgG+IgM (H+L) HRP conjugate (Jackson ImmunoResearch cat# 109-035-127) was diluted to 80 ng/ml, added to the plate, and incubated for 1 hour at room temperature. QuantaBlu fluorescent HRP substrate (Pierce cat# 15169) was added for detection of bound antibody by measuring fluorescence in a Molecular Devices M5 (Excitation 325 nm/Emission 420 nm) plate reader.

[0273] While the hamster anti-mouse CCL20 antibody 2F5-5 MAb bound mouse CCL20 with a 50% effective dose (EC_{50}) of 64 pM (data not shown), neither the chimeric nor humanized anti-human CCL20 antibodies bound detectably to mouse CCL20 or rat CCL20 under the conditions described above. By contrast, both the chimeric and humanized antibodies effectively bound to human, rhesus, and cynomolgus CCL20 (FIGS. 16A-C, 17A and B). While the EC_{50} for human and rhesus CCL20 appeared similar (for example, 62 and 101 pM for HC2/LC3), the EC_{50} for cynomolgus CCL20 was 340 pM for HC2/LC3, a 5.4 fold difference versus human CCL20. Similar results were seen for HC2/LK3. This suggests that a much greater concentration of antibody was required to achieve the same amount of binding to cynomolgus CCL20 as to human or rhesus CCL20.

Example 8: Surface plasmon resonance assays for cross-reactivity of humanized anti-CCL20 MAbs to CCL20 species orthologs

[0274] We also used surface plasmon resonance (Biacore™) analysis to test whether HC2/LC3 and HC2/LK3 can bind to mouse, cynomolgus, or rhesus macaque CCL20 orthologs. Monoclonal mouse anti-human placental alkaline phosphatase was immobilized on all four flow cells of a CM5 chip at a flow rate of 25 µl/min. 25 µl of a 2 nM solution of human, rhesus, cynomolgus, or mouse CCL20-SEAP in HBS-EP buffer was injected over flow cell 2, 3, or 4. For antibody binding, 240 µl of 0-80 nM dilutions of HC2/LC3, HC2/LK3, or 2F5-5 MAb in HBS-EP buffer (or buffer alone) was injected over all four flow cells at a flow rate of 50 µl/min. Dissociation followed for 45 min. Flow cell 1 (anti-SEAP alone) was used as a reference for all flow cells. Regeneration of the flow cells was performed with 10 mM glycine at pH 2.25. Due to regeneration effects on the capturing capacity of the chips, antibody injections were performed sequentially from low to high concentration. Data fitting was performed using a 1:1 Langmuir model.

[0275] Under the above conditions, hamster anti-mouse CCL20 antibody 2F5-5 bound mouse CCL20 with a (bivalent) K_D of 4.9 pM, but did not significantly bind human, rhesus, or cynomolgus

CCL20. In contrast, chimeric, HC2/LC3, and HC2/LK3 antibodies effectively bound to human, rhesus, and cynomolgus CCL20 (Table 17).

[0276] The apparent affinity values measured were higher in this assay than previously detected in Example 5 (for example 4.7 pM for HC2/LC3 in the present assay vs. 44 pM in Table 3) due to the bivalent (higher affinity) nature of the assay format used (versus the monovalent format used for the data shown in Table 15). The K_D values for rhesus and cynomolgus CCL20 were generally higher (3-fold) than for human CCL20, indicating that the antibodies bound more specifically to human CCL20. In contrast to the ELISA data from Example 7, however, we observed no significant difference between binding affinity for rhesus and cynomolgus CCL20.

Table 17: Assessment of anti-CCL20 humanized monoclonal antibody binding to CCL20 orthologs by Biacore™

Antibody	Ligand	k_a ($x10^5 M^{-1}scc^{-1}$)	k_d ($x10^{-5} scc^{-1}$)	K_D (pM)
Chimeric clone 36F7C10[a]	hCCL20	29	2.08	7.16
	rCCL20	29.4	1.78	6.05
	cCCL20	38.3	5.04	13.2
HC2/LC3	hCCL20	56.9	2.68	4.71
	rCCL20	41.3	7.51	18.2
	cCCL20	86.4	13.4	15.5
HC2/LK3	hCCL20	28.8	3.39	11.8
	rCCL20	26.3	8.21	31.2
	cCCL20	52.8	14.9	28.3
Hamster anti-mouse 2F5-5[b]	hCCL20	No binding	No binding	No binding
	rCCL20	No binding	No binding	No binding
	cCCL20	No binding	No binding	No binding
	mCCL20	9.63	0.474	4.92

[a]: Previously shown not to bind mCCL20

[b]: Hamster anti-mouse data from earlier chip

Example 9: Epitope Mapping for Mouse Anti-Human CCL20 Clone 36F7C10

[0277] To determine the human CCL20 epitope(s) to which mouse anti-human CCL20 monoclonal antibody 36F7C10 binds, we used a hydrogen/deuterium exchange method in which antibody binding protects and thus preserves deuteration of the epitope. As shown in FIG. 18A, we observed very

strong perturbations near the N-terminus of human CCL20 at residues 7-9, 10-19, and 20-22, possibly representing the epitope(s). Marginal protection was also observed near the C-terminus at residues 39-55, 56-67, and 61-70. These segments may be peripheral to the epitope, or their breathing motions may be conjugated to the epitope. The deuteration level of each indicated amino acid is shown at four timepoints: from top to bottom, 150, 500, 1,500, and 5,000 s.

[0278] The regions identified as the epitope for 36F7C10 fall within the N-terminal and loop regions of CCL20, which are known to be critical for CCR6 binding and signaling (Malik *et al.*, *Acta Cryst* F62:631-634 (2006))(FIG. 18B). Chimeric and humanized antibodies derived from 36F7C10, especially antibodies with the same heavy and light chain CDR1, CDR2 and CDR3 amino acid sequences as 36F7C10, or similar heavy and light chain CDR1, CDR2 and CDR3 amino acid sequences as 36F7C10 (*e.g.*, with less than 3, 2 or 1 amino acid substitutions as compared to the CDRs of 36F7C10) are expected to bind to the same epitope. The identification of this epitope may thus explain the ability of the 36F7C10 MAb and chimeric and humanized antibodies derived therefrom to neutralize CCL20 activity.

Example 10: *In vivo* chemotaxis assay for humanized anti-human CCL20 antibodies

[0279] To test whether HC2/LC3 and HC2/LK3 can inhibit CCL20-induced chemotaxis *in vivo*, we took advantage of the fact that human CCL20 can interact with mouse CCR6 (SEQ ID NO: 106) to induce chemotaxis of mouse T cells (FIG. 19). We used a hybrid *in vivo* system measuring the migration of mouse T cells towards intradermally injected human CCL20 (FIG. 20).

[0280] Recombinant human CCL20 (10 ng/head) and vehicle were intradermally injected into the shaved skin on the right and left sides, respectively, of the backs of the test mice (groups of n=3). Calcein-AM-labeled mouse splenic T cells ($5x10^6$ cells/mouse) were then transferred intravenously into the tail vein, with simultaneous injection of the indicated antibodies into the tail vein at the dosages described in FIG. 20. After one hour, the fluorescent positive cells were counted using a stereoscopic microscope at the site of the intradermal injections.

[0281] Both HC2/LK3 and HC2/LC3 significantly inhibited cell migration to the CCL20-injected sites at a level comparable to that of parental mouse antibody 36F7C10 and its chimeric form. Because the chemotaxis of T cells is a key step in the inflammatory cascade, prevention of this migration has clinical implications for the treatment of autoimmune and inflammatory conditions.

Example 11: Effect of 2F5-5 MAb on type II collagen-induced arthritis

[0282] To further evaluate the use of anti-CCL20 antibodies for therapeutic indications, we performed further *in vivo* studies in mice using the hamster anti-mouse 2F5-5 MAb. First, we evaluated the ability of 2F5-5 to neutralize CCL20-mediated chemotaxis in CIA mice (an animal

model of rheumatoid arthritis). CIA was induced essentially as described in Example 1. After the development of arthritis (arthritis score 1-3), mice were randomized and treated with 500 μg/mouse of either 2F5-5 MAb or a control IgG antibody. In both cases, the antibody was administered intravenously every other day. Compared to the control IgG, 2F5-5 MAb inhibited the further development of arthritis symptoms (FIGS. 21, 22).

[0283] We also used X-ray scoring to determine the effects of 2F5-5 MAb on bone lesions, which are frequently seen in rheumatoid arthritis. Scoring was performed as described in Inoue *et al.*, *Agents Actions* 39:187-194 (1993). Briefly, each paw of a CIA mouse was graded on a scale of 0 to 3 based on the severity of osteoporosis (O), bone erosion (E), and new bone formation (N) according to X-ray images. The scale used was: 0, no change; 1, slight change; 2, moderate change; and 3, severe change. The scores for each factor were added to develop a cumulative X-ray score for bone lesions. Mice treated with 2F5-5 MAb demonstrated remarkably less severe X-ray scores on day 39, as compared to mice treated with a control IgG (FIGS. 23, 24).

[0284] We confirmed the effect of 2F5-5 MAb treatment on bone pathology by analyzing several biomarkers using ELISA. CIA mice were immunized and treated with 500 μg of 2F5-5 or control IgG antibody as described above. Plasma samples were prepared from the mice on day 11 or 12 after the second immunization. A marker of cartilage destruction, serum cartilage oligomeric matrix protein (COMP), was quantified using the Animal COMP ELISA Enzyme-immunoassay kit (AnaMar Medical) according to the manufacturer's instructions (incorporated herein by reference), except that animal plasma samples were diluted 1:20. Because COMP is pre-coated on the plate, this is a competition ELISA, whereby addition of the COMP standard or COMP-containing plasma results in a decrease in OD_{450} (FIG. 25A). Raw data from a sample assay is shown in FIGS. 25B and 25C. COMP serum levels in CIA mice were reduced almost to normal levels by treatment with 2F5-5 MAb (FIG. 26), demonstrating the ability of 2F5-5 to inhibit cartilage destruction.

[0285] Because the formation and differentiation of osteoclasts is responsible for RA-related osteoporosis and erosion, we measured the levels of osteoclast induction molecule receptor activator for nuclear factor κB ligand (RANKL), and osteoclast markers such as the receptor activator for nuclear factor κB (RANK), tartrate resistant acid phosphatase (TRAP), and cathepsin K, as indicators for arthritic therapy. We evaluated mRNA expression levels of these markers by quantitative PCR using total RNA isolated from homogenized mouse paws. The paws were first homogenized with a homogenizer after soaking in a tissue lysing buffer containing Trizol reagent (Invitrogen, CA, USA). After adding chloroform, samples were centrifuged at 14,000 rpm for 15 minutes at 4°C to separate the solution into aqueous and organic phases. The aqueous phase was removed and isopropanol added to it, followed by centrifugation at 14,000 rpm for 15 minutes at 4°C to obtain RNA pellets. RNA pellets dissolved with RNAse free water were used in an RNAeasy mini kit (QIAGEN,

Valencia, CA, USA) to isolate the RNA and treated with DNAse to remove any DNA. Complimentary DNA (cDNA) was generated from the RNA with an RT reaction kit (RNA PCR Kit, TAKARA Bio, Inc. Shiga, Japan) according to the manufacturer's protocol. Quantitative real time PCR for each cDNA species was performed and compared to the level of a housekeeping gene, hypoxanthine guanine phosphoribosyl transferase (HPRT). The following forward and reverse primer sets were used in the PCR reactions:

RANKL, 5'-CATTTGCACACCTCACCATC-3' (SEQ ID NO: 89) and
5'-TCCGTTGCTTAACGTCATGT-3' (SEQ ID NO: 90);
RANK, 5'-CGGCGTTTACTACAGGAAGG-3' (SEQ ID NO: 91) and
5'-TTCTTGCTGACTGGAGGTTG-3' (SEQ ID NO: 92);
TRAP, 5'-GCTGGAAACCATGATCACCT-3' (SEQ ID NO: 93) and
5'-GGTAGTAAGGGCTGGGGAAG-3' (SEQ ID NO: 94);
Cathepsin K, 5'-CAGTGTTGGTGGTGGGCTAT-3' (SEQ ID NO: 95) and
5'-CCGAGCCAAGAGAGCATATC-3' (SEQ ID NO: 96); and
HPRT, 5'-CAGGCCAGACTTTGTTGGAT-3' (SEQ ID NO: 97) and
5'-TTGCGCTCATCTTAGGCTTT-3' (SEQ ID NO: 98).

All primers were designed using web-based software Primer 3 (Whitehead Institute for Biomedical Research, Cambridge, MA, USA) to avoid non-specific amplification of RNA. Reaction mixtures with cDNA template, primers, uracil DNA glycosylase (Invitrogen, CA, USA), and QuantiTect SYBR Green PCR Master Mix (QIAGEN, Valencia, CA, USA) were used in the amplification reaction in an ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster, CA, USA). Expression levels were automatically quantified by ABI PRISM 7700 Sequence Detector Software.

[0286] Following 2F5-5 MAb treatment, mRNA levels were suppressed for all markers in the joint tissue (FIGS. 27A and B, show fold change in expression level compared to housekeeping gene, HPRT), providing further evidence that 2F5-5 inhibits bone pathology *in vivo*.

Example 12: Effects of 2F5-5 MAb on glucose-6-phosphate isomerase-induced arthritis

[0287] We tested the anti-arthritic effects of 2F5-5 MAb treatment in mice with glucose-6-phosphate isomerase (G6PI)-induced arthritis, another mouse model for RA. Arthritis was induced by intradermal sensitization with recombinant GST-G6PI (300 µg/mouse) emulsified in complete Freund's adjuvant and injected at the base of the tail of DBA/1 mice. Six days after the G6PI immunization and just prior to the onset of joint swelling, mice were randomized and treated with 500 µg/mouse of either isotype-matched antibody or 2F5-5 MAb. The severity of arthritic symptoms in the paws of each mouse was graded as described previously in Example 1. Mice treated with 2F5-5

MAb exhibited significantly lower arthritic scores than mice treated with isotype-matched antibodies, showing that 2F5-5 strongly suppresses arthritis development compared to the control (FIG. 28).

[0288] These data demonstrate the efficacy of anti-CCL20 antibody treatment in *in vivo* arthritis models, and suggest that the use of anti-CCL20 antibodies may be beneficial in the treatment of rheumatoid arthritis.

Example 13: Effect of 2F5-5 MAb on oxazolone-induced atopic dermatitis

[0289] We then evaluated the effect of 2F5-5 MAb treatment in mouse models of dermatitis. In one model, oxazolone was used to induce atopic dermatitis in mice prone to the disease (NC/Nga strain). The abdominal skin of the mice was shaved and exposed to oxazolone on days 0, 5, and 8. On day 13, mice showing signs of dermatitis (score 2) were selected and immunized again with oxazolone. Mice were thereafter randomized in groups of six for treatment with either 2F5-5 MAb or an isotype-matched control IgG antibody (500 µg/mouse, administered intravenously every other day). We assayed dermatitis scores in a blind study as follows: each dermatitis symptom such as dryness, scale; erythema, oozing/crusting, and excoriation was scored on a scale of 0 to 3 (0 = none, 1 = slight, 2 = moderate, 3 = severe); these scores were then added for a cumulative dermatitis score (as described in Leung *et al., J. Allergy Clin. Immunol.* 85(5):927-933 (1990)). We compared the magnitude of disease suppression between the two groups by quantifying the area under the curve ("AUC") from day 13 to day 18. In this dermatitis model, 2F5-5 MAb induced a statistically significant ($p<0.05$) suppression of disease progression compared to control IgG (FIG. 29).

Example 14: Effect of 2F5-5 MAb on DNFB-induced allergic contact dermatitis

[0290] We tested the effect of 2F5-5 MAb treatment in dinitrofluorobenzene (DNFB)-induced allergic contact dermatitis, a second mouse model of dermatitis. Mice were divided into groups of 6 and sensitized by brushing 25 µl of DNFB solution (0.4% DNFB in a 4:1 solution of acetone:olive oil) on the shaved abdomen for two successive days (days 0 and 1). One group was treated with 2F5-5 MAb, while the other group was treated with an isotype-matched control antibody (500 µg/mouse intravenously on days 0, 2, and 5). On day 5, the mice were re-challenged by applying 20 µl of 0.2% DNFB solution (in 4:1 acetone:olive oil) to one side of one ear. Ear thickness was measured as an indicator of edema on day 6 using a thickness gauge. Treatment with 2F5-5 MAb reduced ear thickness, indicating successful prevention of dermatitis development (FIG. 30).

[0291] All publications, patents, and patent applications cited in this specification are incorporated herein by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of

ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

Table 18: Amino acid and nucleotide sequences for human and mouse CCL20 and CCR6

Description	Sequence Type	Sequence (signal sequences underlined)	SEQ ID NO
Human CCL20 with signal sequence	Amino Acid	mcctkslllaalmsvlllhlcgeseaasnfdcclgytdri lhpkfivgftrqlanegcdinaiifhtkkklsvcanpkqt wvkyivrllskkvknm	85
	Nucleotide	atgtgctgtaccaagagtttgctcctggctgctttgatgt cagtgctgctactccacctctgcggcgaatcagaagcagc aagcaactttgactgctgtcttggatacacagaccgtatt cttcatcctaaatttattgtgggcttcacacggcagctgg ccaatgaaggctgtgacatcaatgctatcatctttcacac aaagaaaaagttgtctgtgtgcgcaaatccaaaacagact tgggtgaaatatattgtgcgtctcctcagtaaaaaagtca agaacatg	101
Human CCL20 without signal sequence	Amino Acid	asnfdcclgytdrilhpkfivgftrqlanegcdinaiifh tkkklsvcanpkqtwvkyivrllskkvknm	99
	Nucleotide	gcaagcaactttgactgctgtcttggatacacagaccgta ttcttcatcctaaatttattgtgggcttcacacggcagct ggccaatgaaggctgtgacatcaatgctatcatctttcac acaaagaaaaagttgtctgtgtgcgcaaatccaaaacaga cttgggtgaaatatattgtgcgtctcctcagtaaaaaagt caagaacatg	100
Mouse CCL20 with signal sequence	Amino Acid	macggkrllflalawvllahlcsqaeaasnydcclsyiqt plpsraivgftrqmadeacdinaiifhtkkrksvcadpkq nwvkravnllslrvkkm	102
	Nucleotide	atggcctgcggtggcaagcgtctgctcttccttgctttgg catgggtactgctggctcacctctgcagccaggcagaagc agcaagcaactacgactgttgcctctcgtacatacagacg cctcttccttccagagctattgtgggtttcacaagacaga tggccgatgaagcttgtgacattaatgctatcatctttca cacgaagaaaagaaaatctgtgtgcgctgatccaaagcag aactgggtgaaaagggctgtgaacctcctcagcctaagag tcaagaagatg	103
Human CCR6	Amino Acid	msgesmnfsdvfdssedyfvsvntsyysvdsemllcslqe vrqfsrlfvpiayslicvfgllgnilvvitfafykkarsm tdvyllnmaiadilfvltlpfwavshatgawvfsnatckl lkgiyainfncgmlllticismdryiaivqatksfrlrsrt lprskiiclvvwglsviissstfvfnqkyntqgsdvcepk yqtvsepirwkllmlglellfgffiplmfmifcytfivkt lvqaqnskrhkairviiavvlvflacqiphnmvllvtaan lgkmnrscqsekligytktvtevlaflhcclnpvlyafig qkfrnyflkilkdlwcvrrkykssgfscagrysenisrqt setadndnassftm	104
	Nucleotide	atgagcggggaatcaatgaatttcagcgatgttttcgact ccagtgaagattattttgtgtcagtcaatacttcatatta ctcagttgattctgagatgttactgtgctccttgcaggag gtcaggcagttctccaggctatttgtaccgattgcctact ccttgatctgtgtctttggcctcctggggaatattctggt ggtgatcacctttgctttttataagaaggccaggtctatg acagacgtctatctcttgaacatggccattgcagacatcc tctttgttcttactctcccattctgggcagtgagtcatgc caccggtgcgtgggttttcagcaatgccacgtgcaagttg ctaaaaggcatctatgccatcaactttaactgcgggatgc	105

		tgctcctgacttgcattagcatggaccggtacatcgccat tgtacaggcgactaagtcattccggctccgatccagaaca ctaccgcgcagcaaaatcatctgccttgttgtgtgggggc tgtcagtcatcatctccagctcaacttttgtcttcaacca aaaatacaacacccaaggcagcgatgtctgtgaacccaag taccagactgtctcggagcccatcaggtggaagctgctga tgttgggcttgagctactctttggtttctttatcccttt gatgttcatgatattttgttacacgttcattgtcaaaacc ttggtgcaagctcagaattctaaaaggcacaaagccatcc gtgtaatcatagctgtggtgcttgtgtttctggcttgtca gattcctcataacatggtcctgcttgtgacggctgcaaat ttgggtaaaatgaaccgatcctgccagagcgaaaagctaa ttggctatacgaaaactgtcacagaagtcctggctttcct gcactgctgcctgaaccctgtgctctacgcttttattggg cagaagttcagaaactactttctgaagatcttgaaggacc tgtggtgtgtgagaaggaagtacaagtcctcaggctttctc ctgtgccgggaggtactcagaaaacatttctcggcagacc agtgagaccgcagataacgacaatgcgtcgtccttcacta tg	
Mouse CCR6	Amino Acid	mnstesyfgtddydnteyysippdhgpcsleevrnftkvf vpiayslicvfgllgnimvvmtfafykkarsmtdvyllnm aitdilfvltlpfwavthatntwvfsdalcklmkgtyavn fncgmlllacismdryiaivqatksfrvrsrtlthskvic vavwfisiiissptfifnkkyelqdrdvcepryrsvsepi twkllgmglelffgfftpllfmvfcylfiiktlvqaqnsk rhrairvviavvlvflacqiphnmvllvtavntgkvgrsc stekvlaytrnvaevlaflhcclnpvlyafigqkfrnyfm kimkdvwcmrrknkmpgflcarvysesyisrqtsetvend nassftm	106
	Nucleotide	atgaattccacagagtcctactttggaacggatgattatg acaacacagagtattattctattcctccagaccatgggcc atgctccctagaagaggtcagaaacttcaccaaggtattt gtgccaattgcctactccttaatatgtgtctttggcctcc tgggcaacattatggtggtgatgacctttgccttctacaa gaaagccagatccatgactgacgtctacctgttgaacatg gccatcacagacatactctttgtcctcaccctaccgttct gggcagttactcatgccaccaacacttgggttttcagcga tgcactgtgtaaactgatgaaaggcacatatgcggtcaac tttaactgtgggatgctgctcctggcctgtatcagcatgg accggtacattgccatcgtccaggcaaccaaatctttccg ggtacgctccagaacactgacgcacagtaaggtcatctgt gtggcagtgtggttcatctccatcatcatctcaagcccta catttatcttcaacaagaaatacgagctgcaggatcgtga tgtctgtgagccacggtacaggtctgtctcagagcccatc acgtggaagctgctgggtatgggactggagctgttctttg ggttcttcaccccttttgctgtttatggtgttctgctatct gttcattatcaagaccttggtgcaggcccagaactccaag aggcacagagcaatccgagtcgtgatcgctgtggttctcg tgttcctggcttgtcagatccctcacaacatggtcctcct cgtgactgcggtcaacacgggcaaagtgggccggagctgc agcaccgagaaagtcctcgcctacaccaggaacgtggccg aggtcctggctttcctgcattgctgcctcaaccccgtgtt gtatgcgtttattggacagaaattcagaaactacttcatg aagatcatgaaggatgtgtggtgtatgagaaggaagaata agatgcctggcttcctctgtgcccgggtttactcggaaag ctacatctccaggcagaccagtgagaccgtcgaaaatgat aatgcatcgtcctttaccatg	107

Table 19: Sequence Identification Numbers

SEQ ID NO	Description		
1	HC2	Full-Length Heavy Chain (HC)	Amino Acid Sequence
2	HC3		
3	36HKK3		
4	42HKK1		
5	42HKK2		
6	42HKK3		
7	LC3	Full-Length Light Chain (LC)	
8	LK3		
9	HC2	Heavy Chain Variable Domain (VH)	
10	HC3		
11	36HKK3		
12	42HKK1		
13	42HKK2		
14	42HKK3		
15	LC3	Light Chain Variable Domain (VL)	
16	LK3		
17	HC2	Full-Length Heavy Chain (HC)	Nucleotide Sequence
18	HC3		
19	36HKK3		
20	42HKK1		
21	42HKK2		
22	42HKK3		
23	LC3	Full-Length Light Chain (LC)	
24	LK3		
25	HC2	Heavy Chain Variable Domain (VH)	
26	HC3		
27	36HKK3		
28	42HKK1		
29	42HKK2		
30	42HKK3		
31	LC3	Light Chain Variable Domain (VL)	
32	LK3		
33	36F7C10	Heavy Chain Signal Sequence	Amino Acid Sequence
34		Light Chain Signal Sequence	
35	40-1C10B9	Heavy Chain Signal Sequence	
36		Light Chain Signal Sequence	
37	42G5B10	Heavy Chain Signal Sequence	
38		Light Chain Signal Sequence	
39	36F7C10	VH	
40		VL	
41	40-1C10B9	VH	
42		VL	
43	42G5B10	VH	
44		VL	
45	36F7C10	Heavy Chain Signal	Nucleotide Sequence

		Sequence	
46		Light Chain Signal Sequence	
47	40-1C10B9	Heavy Chain Signal Sequence	
48		Light Chain Signal Sequence	
49	42G5B10	Heavy Chain Signal Sequence	
50		Light Chain Signal Sequence	
51	36F7C10	VH	
52		VL	
53	40-1C10B9	VH	
54		VL	
55	42G5B10	VH	
56		VL	
57	IGHV1-46*03 Germline Sequence		
58	JH4 Germline Sequence		
59	IGKV1D-39*01 Germline Sequence		
60	Mouse 36F7C10 HC2 HC3 36HKK3	H-CDR1 (Kabat)	NYWMH
61	Mouse 42G5B10 42HKK1 42HKK2 42HKK3		SYWMH
62	IGHV1-46*03/ ABM67212		SYYMH
63	Mouse 36F7C10 HC3 36HKK3	H-CDR2 (Kabat)	VIDPSDSYTTYNQKFKG
64	HC2		VIDPSDSYTTYAQKFQG
65	Mouse 42G5B10 42HKK1 42HKK2 42HKK3		LIDPSDKYTNYNQKFKG
66	IGHV1-46*03/ ABM67212		IINPSGGSTSYAQKFQG
67	Mouse 36F7C10 HC2 HC3 36HKK3	H-CDR3 (Kabat or Chothia)	GNYGVDYAMDY
68	Mouse 42G5B10 42HKK1 42HKK2 42HKK3		GNYGVDYGMDY
69	ABM67212		EGDGYIQAFDY
70	Mouse 36F7C10 LK3	L-CDR1 (Kabat or Chothia)	GASENIYGALN
71	LC3		RASENIYGALN
72	IGKV1D-39*01/ BAH04867.1		RASQSISSYLN
73	Mouse 36F7C10	L-CDR2	GATNLAD

	LC3 LK3	(Kabat or Chothia)	
74	IGKV1D-39*01/ BAH04867.1		AASSLQS
75	Mouse 36F7C10 LC3 LK3	L-CDR3 (Kabat or Chothia)	QNVLITPYT
76	BAH04867.1		QQSYSTPYT
77	Mouse 36F7C10 HC2 HC3 36HKK3	H-CDR1 (Chothia)	GYTFTNY
78	Mouse 42G5B10 42HKK1 42HKK2 42HKK3 IGHV1-46*03/ ABM67212		GYTFTSY
79	Mouse 36F7C10 HC2 HC3 36HKK3	H-CDR2 (Chothia)	VIDPSDSYTT
80	Mouse 42G5B10 42HKK1 42HKK2 42HKK3		LIDPSDKYTN
81	IGHV1-46*03/ ABM67212		IINPSGGSTS
82	ABM67212	VH	
83	BAH04867.1	VL	
84	Human CCL20 variant (without signal sequence) amino acid sequence		
85	Human CCL20 (with signal sequence) amino acid sequence		
86	Rhesus CCL20 amino acid sequence		
87	Cynomolgus CCL20 amino acid sequence		
88	Mouse CCL20 amino acid sequence (partial)		
89	5'-CATTTGCACACCTCACCATC-3'	RANKL	Primers
90	5'-TCCGTTGCTTAACGTCATGT-3'		
91	5'-CGGCGTTTACTACAGGAAGG-3'	RANK	
92	5'-TTCTTGCTGACTGGAGGTTG-3'		
93	5'-GCTGGAAACCATGATCACCT-3'	TRAP	
94	5'-GGTAGTAAGGGCTGGGGAAG-3'		
95	5'-CAGTGTTGGTGGTGGGCTAT-3'	Cathepsin K	
96	5'-CCGAGCCAAGAGAGCATATC-3'		
97	5'-CAGGCCAGACTTTGTTGGAT-3'	HPRT	
98	5'-TTGCGCTCATCTTAGGCTTT-3'		
99	Human CCL20 (without signal sequence) amino acid sequence		
100	Human CCL20 (without signal sequence) nucleotide sequence		
101	Human CCL20 (with signal sequence) nucleotide sequence		
102	Mouse CCL20 (with signal sequence) amino acid sequence		
103	Mouse CCL20 (with signal sequence) nucleotide sequence		
104	Human CCR6 amino acid sequence		

105	Human CCR6 nucleotide sequence
106	Mouse CCR6 amino acid sequence
107	Mouse CCR6 nucleotide sequence
108	HC2 full-length amino acid sequence (without signal sequence)
109	HC2 full-length nucleotide sequence (without signal sequence)
110	LC3 full-length amino acid sequence (without signal sequence)
111	LC3 full-length nucleotide sequence (without signal sequence)
112	LK3 full-length amino acid sequence (without signal sequence)
113	LK3 full-length nucleotide sequence (without signal sequence)
114	partial CCL20 amino acid sequence
115	partial CCL16 amino acid sequence

Table 20: Humanized and Mouse Antibody Sequences

		Amino Acid Sequences										Nucleotide Sequences			
						H-CDR			L-CDR						
		HC	LC	VH	VL	1	2	3	1	2	3	HC	LC	VH	VL
Humanized Anti-Human CCL20 Ab Chains	HC2	1		9		K: 60 C: 77	K: 64 C: 79	K: 67 C: 67				17		25	
	HC3	2		10		K: 60 C: 77	K: 63 C: 79	K: 67 C: 67				18		26	
	36HKK3	3		11		K: 60 C: 77	K: 63 C: 79	K: 67 C: 67				19		27	
	42HKK1	4		12		K: 61 C: 78	K: 65 C: 80	K: 68 C: 68				20		28	
	42HKK2	5		13		K: 61 C: 78	K: 65 C: 80	K: 68 C: 68				21		29	
	42HKK3	6		14		K: 61 C: 78	K: 65 C: 80	K: 68 C: 68				22		30	
	LC3		7		15				71	73	75		23		31
	LK3		8		16				70	73	75		24		32
Mouse Anti-Human CCL20 Abs	36F7C10			39	40	K: 60 C: 77	K: 63 C: 79	K: 67 C: 67	70	73	75			51	52
	40-1C10B9			41	42									53	54
	42G5B10			43	44	K: 61 C: 78	K: 65 C: 80	K: 68 C: 68						55	56

What is claimed is:

1. A monoclonal anti-human CCL20 antibody or an antigen-binding portion thereof, wherein said antibody comprises a heavy chain whose complementarity determining region 3 (CDR3) comprises SEQ ID NO: 67 or 68.

2. The monoclonal antibody or antigen-binding portion of claim 1, wherein the complementarity determining region 1 (CDR1), complementarity determining region 2 (CDR2), and complementarity determining region 3 (CDR3) of said heavy chain respectively comprise amino acid sequences selected from the group consisting of:
   a) SEQ ID NOS: 60, 64, and 67;
   b) SEQ ID NOS: 60, 63, and 67;
   c) SEQ ID NOS: 61, 65, and 68;
   d) SEQ ID NOS: 77, 79, and 67; and
   e) SEQ ID NOS: 78, 80, and 68.

3. A monoclonal anti-human CCL20 antibody or an antigen-binding portion thereof, wherein said antibody comprises a light chain whose complementarity determining region 3 (CDR3) comprises SEQ ID NO: 75.

4. The monoclonal antibody or antigen-binding portion of claim 3, wherein the complementarity determining region 1 (CDR1), complementarity determining region 2 (CDR2), and complementarity determining region 3 (CDR3) of said light chain respectively comprise amino acid sequences selected from the group consisting of:
   a) SEQ ID NOS: 70, 73, and 75; and
   b) SEQ ID NOS: 71, 73, and 75.

5. The antibody or antigen-binding portion of claim 1 or 3, wherein the heavy chain of said antibody comprises a CDR3 comprising SEQ ID NO: 67 or 68 and the light chain of said antibody comprises a CDR3 comprising SEQ ID NO: 75.

6. The antibody or antigen-binding portion according to claim 2 or 4, wherein said heavy chain CDR1, CDR2, and CDR3 and said light chain CDR1, CDR2, and CDR3 respectively comprise amino acid sequences selected from the group consisting of:
   a) SEQ ID NOS: 60, 64, 67, 70, 73, and 75;

b) SEQ ID NOS: 60, 64, 67, 71, 73, and 75;
c) SEQ ID NOS: 60, 63, 67, 70, 73, and 75;
d) SEQ ID NOS: 60, 63, 67, 71, 73, and 75;
e) SEQ ID NOS: 61, 65, 68, 70, 73, and 75;
f) SEQ ID NOS: 61, 65, 68, 71, 73, and 75;
g) SEQ ID NOS: 77, 79, 67, 70, 73, and 75;
h) SEQ ID NOS: 77, 79, 67, 71, 73, and 75;
i) SEQ ID NOS: 78, 80, 68, 70, 73, and 75; and
j) SEQ ID NOS: 78, 80, 68, 71, 73, and 75.

7. A monoclonal anti-human CCL20 antibody or an antigen-binding portion thereof, wherein said antibody comprises a heavy chain whose variable domain comprises an amino acid sequence selected from SEQ ID NOS: 9-14.

8. A monoclonal anti-human CCL20 antibody or an antigen-binding portion thereof, wherein said antibody comprises a light chain whose variable domain comprises SEQ ID NO: 15 or 16.

9. The antibody or antigen-binding portion according to claim 7 or 8, wherein said heavy chain variable domain and said light chain variable domain respectively comprise amino acid sequences selected from the group consisting of:
a) SEQ ID NOS: 9 and 15;
b) SEQ ID NOS: 9 and 16;
c) SEQ ID NOS: 10 and 16;
d) SEQ ID NOS: 11 and 15;
e) SEQ ID NOS: 11 and 16;
f) SEQ ID NOS: 12 and 16;
g) SEQ ID NOS: 13 and 16; and
h) SEQ ID NOS: 14 and 16.

10. A monoclonal anti-human CCL20 antibody whose heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-6 without the signal sequence and SEQ ID NO: 108.

11. A monoclonal anti-human CCL20 antibody whose heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-6 without the signal sequence and SEQ ID NO: 108, wherein said amino acid sequence lacks the C-terminal lysine.

12. A monoclonal anti-human CCL20 antibody whose light chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 7 and 8 without the signal sequence and SEQ ID NOS: 110 and 112.

13. The antibody according to any one of claims 10-12, wherein said heavy chain and said light chain respectively comprise amino acid sequences selected from the group consisting of:

a) SEQ ID NOS: 1 and 7;
b) SEQ ID NOS: 1 and 8;
c) SEQ ID NOS: 2 and 8;
d) SEQ ID NOS: 3 and 7;
e) SEQ ID NOS: 3 and 8;
f) SEQ ID NOS: 4 and 8;
g) SEQ ID NOS: 5 and 8;
h) SEQ ID NOS: 6 and 8;
i) SEQ ID NOS: 108 and 110; and
j) SEQ ID NOS: 108 and 112;

wherein said amino acid sequences lack signal sequences, if present, and wherein SEQ ID NOS: 1-6 optionally lack the C-terminal lysine.

14. A monoclonal antibody whose heavy chain comprises SEQ ID NO: 108 and whose light chain comprises SEQ ID NO: 110.

15. A monoclonal antibody whose heavy chain comprises SEQ ID NO: 108 without the C-terminal lysine and whose light chain comprises SEQ ID NO: 110.

16. A monoclonal antibody whose heavy chain comprises SEQ ID NO: 108 and whose light chain comprises SEQ ID NO: 112.

17. A monoclonal antibody whose heavy chain comprises SEQ ID NO: 108 without the C-terminal lysine and whose light chain comprises SEQ ID NO: 112.

18. A monoclonal anti-human CCL20 antibody, or an antigen-binding portion thereof, that binds to the same epitope of human CCL20 as the monoclonal antibody or antigen-binding portion of any one of claims 1-17.

19. A monoclonal anti-human CCL20 antibody, or an antigen-binding portion thereof, that competes for binding to human CCL20 with the monoclonal antibody or antigen-binding portion of any one of claims 1-17.

20. A monoclonal anti-human CCL20 antibody, or an antigen-binding portion thereof, that cross-competes for binding to human CCL20 with the monoclonal antibody or antigen-binding portion of any one of claims 1-17.

21. The monoclonal antibody or antigen-binding portion of any one of claims 1-12, wherein the antibody is a humanized antibody.

22. The monoclonal antibody or antigen-binding portion of any one of claims 1-6, wherein the framework regions of said heavy chain utilize a IGHV1-46*03 human germline sequence, and wherein the framework regions of said light chain utilize a IGKV1D-39*01 human germline sequence.

23. The monoclonal antibody of any one of claims 1-9 and 18-22, wherein said antibody comprises a human IgG1, IgG2, IgG3, or IgG4 constant domain.

24. A monoclonal anti-human CCL20 antibody, or an antigen-binding portion thereof, whose heavy chain comprises a variable domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 39, 41, and 43.

25. A monoclonal anti-human CCL20 antibody, or an antigen-binding portion thereof, whose light chain comprises a variable domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 40, 42, and 44.

26. The antibody or antigen-binding portion of claim 24 or 25, wherein said heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 39, 41, and 43 and wherein said light chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 40, 42, and 44.

27. The antibody of claim 26, wherein said heavy chain and said light chain respectively comprise amino acid sequences selected from the group consisting of:

a) SEQ ID NOS: 39 and 40;

b) SEQ ID NOS: 41 and 42; and

c) SEQ ID NOS: 43 and 44.

28. A monoclonal anti-human CCL20 antibody, or an antigen-binding portion thereof, wherein said antibody comprises a heavy chain comprising SEQ ID NO: 39 and a light chain comprising SEQ ID NO: 40.

29. The monoclonal antibody or antigen-binding portion of any one of claims 24-28, wherein said antibody is a chimeric antibody.

30. The antigen-binding portion of any one of claims 1-9, 18-22, and 24-28, wherein said portion is a single chain antibody, Fv, Fab, Fab', $F(ab')_2$, Fd, single chain Fv molecule (scFv), bispecific single chain Fv dimer, diabody, domain-deleted antibody or single domain antibody (dAb).

31. The monoclonal antibody or antigen-binding portion according to any one of claims 1-30, wherein said antibody or antigen-binding portion has one or more properties selected from the group consisting of:

a) does not bind to human CCL16;

b) binds to cynomolgus or rhesus CCL20, but not to mouse or rat CCL20;

c) has a binding affinity for human CCL20 of 70 pM or less using a monovalent surface plasmon resonance assay;

d) has a binding affinity for human CCL20 of 12 pM or less using a bivalent surface plasmon resonance assay;

e) has a binding affinity for human CCL20 greater than that of human CCR6;

f) has a selectivity for human CCL20 over human CX3CL1, CXCL1, CXCL2, CXCL4, CXCL8, CXCL9, CXCL10, CXCL12, CXCL13, CXCL16, CCL1, CCL2, CCL3, CCL4, CCL5, CCL7, CCL11, CCL13, CCL16, CCL17, CCL19, CCL21, CCL22, CCL24, CCL25, CCL27, CCL28, or XCL1;

g) reduces human CCL20-induced chemotaxis of CCR6+ cells with an IC_{50} of 1.7 nM or less;

h) reduces human CCL20-induced chemotaxis of CCR6+ cells *in vivo*;

i) reduces human CCL20-induced chemotaxis of CCR6 + cells *in vitro*;

j) reduces progression of arthritis symptoms in a subject;

k) reduces osteoporosis, bone erosion, or new bone formation in a subject;

l) reduces cartilage oligomeric matrix protein (COMP) serum levels in a subject;

m) reduces mRNA levels of RANKL, RANK, TRAP, or cathepsin K in a subject;

n) reduces progression of atopic dermatitis in a subject; and

o) reduces progression of allergic contact dermatitis in a subject.

32. A monoclonal anti-human CCL20 antibody, or an antigen-binding portion thereof, that binds to an epitope of human CCL20 comprising one or more amino acid sequences selected from the group consisting of:

a) residues 7-9 of SEQ ID NO: 84;

b) residues 10-19 of SEQ ID NO: 84; and

c) residues 20-22 of SEQ ID NO: 84.

33. The monoclonal antibody or antigen-binding portion of claim 32, wherein said epitope comprises residues 7-9, 10-19, and 20-22 of SEQ ID NO: 84.

34. The monoclonal antibody or antigen-binding portion of claim 32, wherein said epitope further comprises one or more amino acid sequences selected from the group consisting of:

a) residues 39-55 of SEQ ID NO: 84;

b) residues 56-67 of SEQ ID NO: 84; and

c) residues 61-70 of SEQ ID NO: 84.

35. The monoclonal antibody or antigen-binding portion of claim 34, wherein said epitope comprises residues 7-9, 10-19, 20-22, 39-55, 56-67, and 61-70 of SEQ ID NO: 84.

36. An isolated nucleic acid molecule encoding the heavy chain or an antigen-binding portion thereof of an antibody or portion according to any one of claims 1-35.

37. An isolated nucleic acid molecule encoding the light chain or an antigen-binding portion thereof of an antibody or portion according to any one of claims 1-35.

38. An isolated nucleic acid molecule encoding the heavy chain or an antigen-binding portion thereof, and the light chain or an antigen-binding portion thereof, of an antibody or portion according to any one of claims 1-35.

39. An isolated nucleic acid molecule encoding the heavy chain or an antigen-binding portion thereof of a monoclonal anti-human CCL20 antibody, wherein said nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS: 17-22, 25-30, and 109, or said nucleotide sequence without the sequence encoding a signal sequence, if present.

40. An isolated nucleic acid molecule encoding the light chain or an antigen-binding portion thereof of a monoclonal anti-human CCL20 antibody, wherein said nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS: 23, 24, 31, 32, 111, and 113, or said nucleotide sequence without the sequence encoding a signal sequence, if present.

41. The isolated nucleic acid molecule of claim 39 or 40, wherein said nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS: 17-22, 25-30, and 109, or said nucleotide sequence without the sequence encoding a signal sequence if present; and wherein said nucleic acid molecule further comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS: 23, 24, 31, 32, 111, and 113, or said nucleotide sequence without the sequence encoding a signal sequence if present.

42. Use of (1) a nucleic acid sequence encoding the heavy chain or an antigen-binding portion thereof, (2) a nucleic acid sequence encoding the light chain or an antigen-binding portion thereof, or (3) both, of an antibody or portion according to any one of claims 1-35, as a medicament.

43. A recombinant vector comprising (1) a nucleic acid sequence encoding the heavy chain or an antigen-binding portion thereof, (2) a nucleic acid sequence encoding the light chain or an antigen-binding portion thereof, or (3) both, of an antibody or portion according to any one of claims 1-35.

44. A host cell comprising a first nucleic acid sequence encoding the heavy chain or an antigen-binding portion thereof of an antibody or portion according to any one of claims 1-35, said

first nucleic acid sequence operably linked to an expression control element, and a second nucleic acid sequence encoding the light chain or an antigen-binding portion thereof of said antibody or portion, said second nucleic acid sequence operably linked to an expression control element.

45. A method of making an anti-human CCL20 antibody or an antigen-binding portion thereof, comprising maintaining the host cell of claim 44 under conditions appropriate for expression of the antibody or portion.

46. The method of claim 45, further comprising isolating the antibody or portion.

47. A composition comprising the monoclonal antibody or antigen-binding portion of any one of claims 1-35 and a pharmaceutically acceptable vehicle or carrier.

48. A method for treating a subject in need thereof, comprising administering to the subject an effective amount of the antibody or antigen-binding portion of any one of claims 1-35 or the composition of claim 47.

49. A method for treating a condition in a subject in need thereof, comprising administering to the subject an effective amount of the antibody or antigen-binding portion of any one of claims 1-35 or the composition of claim 47.

50. The method of claim 49, wherein said condition is a CCR6-associated condition.

51. The method of claim 49, wherein said condition is an autoimmune or inflammatory condition.

52. The method of claim 49, wherein said condition is rheumatoid arthritis, psoriasis, atopic dermatitis, contact dermatitis, Crohn's disease, inflammatory bowel disease, Grave's disease, vitiligo, hyperthyroidism, chronic hepatitis, human papillomavirus infection of the cervix, mycosis fungoides, osteoporosis, or periodontal disease.

53. A method for treating cancer, comprising administering to a subject an effective amount of the antibody or antigen-binding portion of any one of claims 1-35 or the composition of claim 47.

54. The method of claim 53, wherein said cancer is a B-cell malignancy, breast adenocarcinoma, glioblastoma, hepatocellular carcinoma, pancreatic adenocarcinoma, or thyroid papillary carcinoma.

55. A method for reducing CCL20-mediated chemotaxis of CCR6+ cells in a subject in need thereof, comprising administering to the subject the antibody or antigen-binding portion of any one of claims 1-35 or the composition of claim 47.

56. A method for reducing CCL20-mediated chemotaxis of CCR6+ cells *in vitro*, using the antibody or antigen-binding portion of any one of claims 1-35 or the composition of claim 47.

57. A method for treating a condition in a subject, comprising administering to the subject the antibody or antigen-binding portion of any one of claims 1-35 or the composition of claim 47, wherein said condition is selected from the group consisting of:

a) articular lesions of extremities distal to the elbow or knee;
b) erythema;
c) swelling;
d) increased cartilage oligomeric matrix protein (COMP) serum levels;
e) increased mRNA levels of receptor activator for nuclear factor κB ligand (RANKL), receptor activator for nuclear factor κB (RANK), tartrate resistant acid phosphatase (TRAP), or cathepsin K;
f) atopic dermatitis; and
g) allergic contact dermatitis.

58. Use of the antibody or antigen-binding portion of any one of claims 1-35 or the composition of claim 47 for the manufacture of a medicament.

59. Use of the antibody or antigen-binding portion of any one of claims 1-35 or the composition of claim 47 as a medicament.

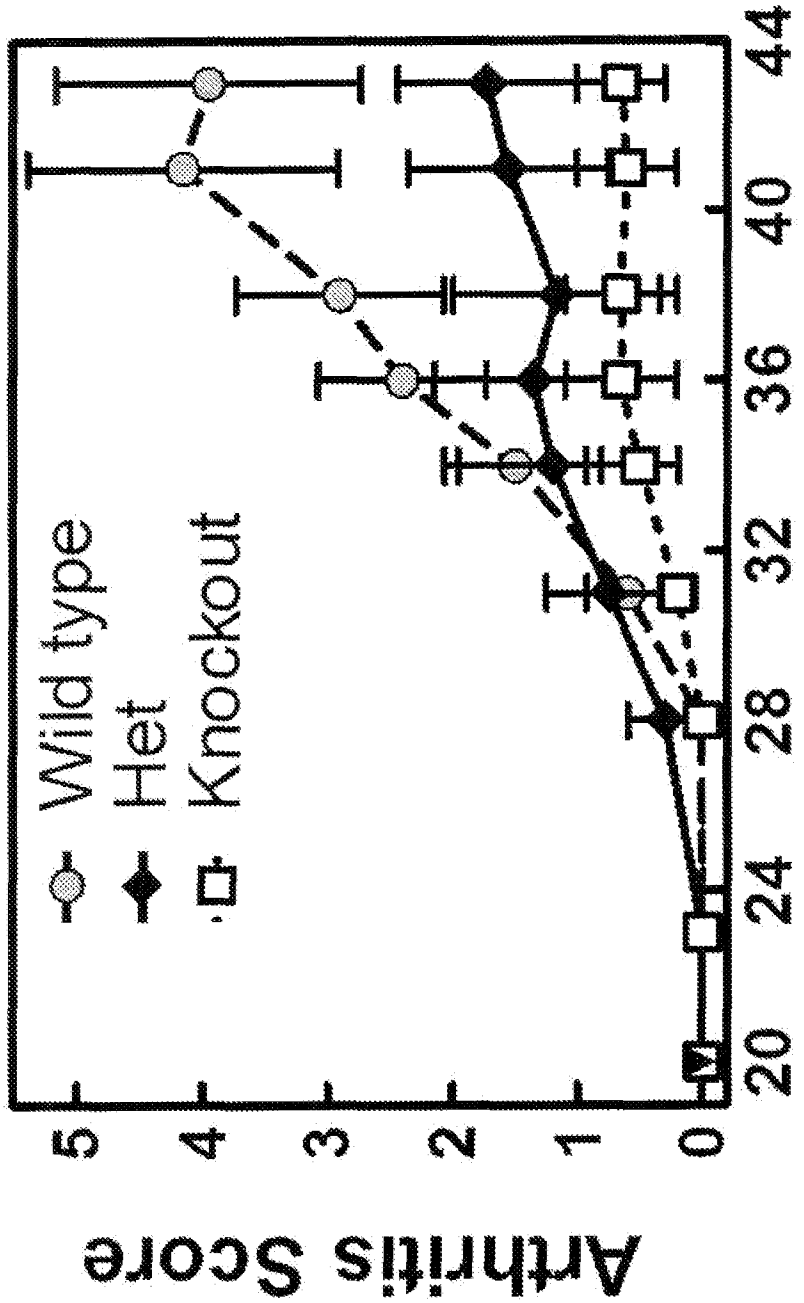

Arthritis Score
0
1
2
3
4
5
20
24
28
32
36
40
44
Days
Wild type
Het
Knockout

FIG. 1

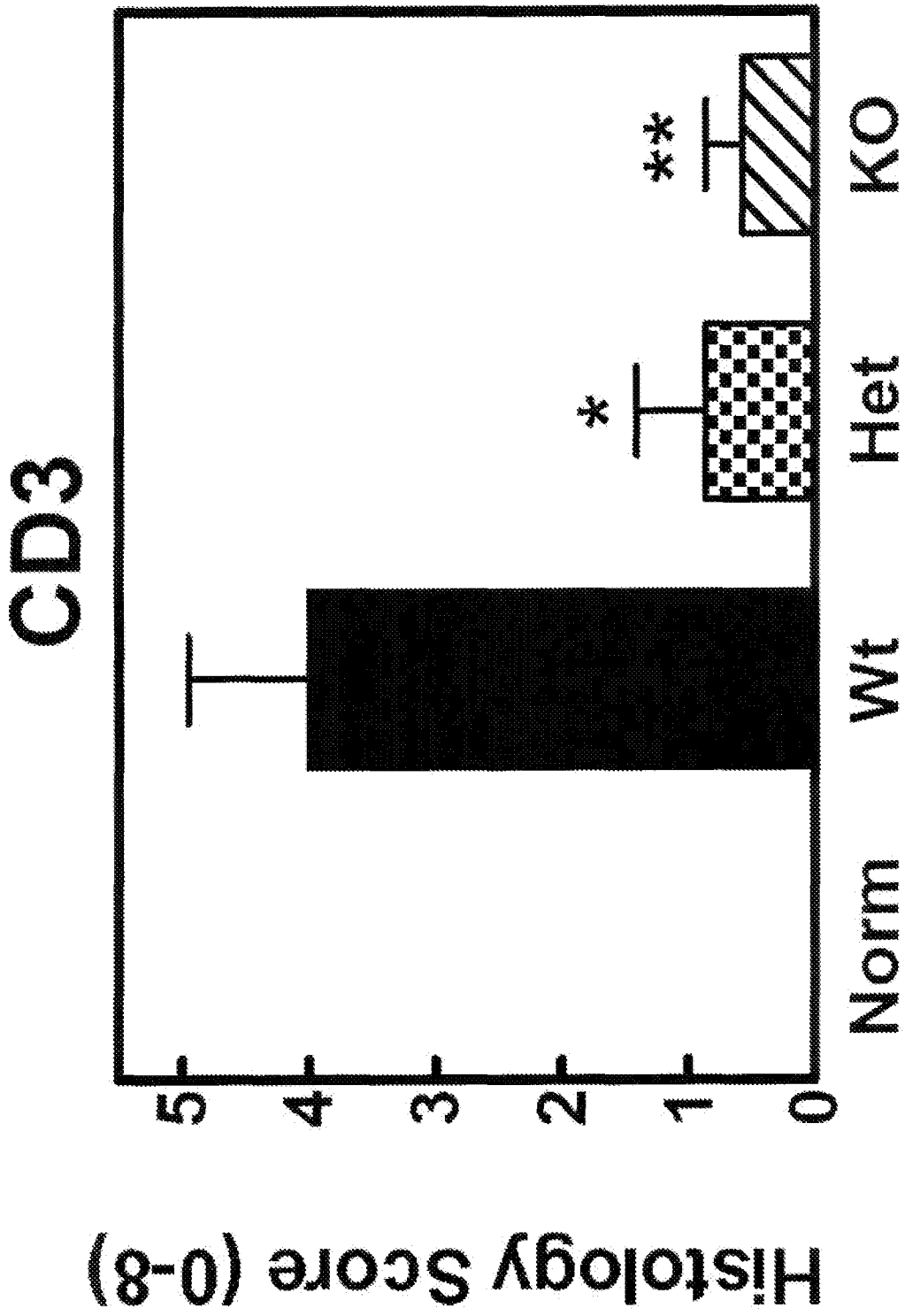


FIG. 2

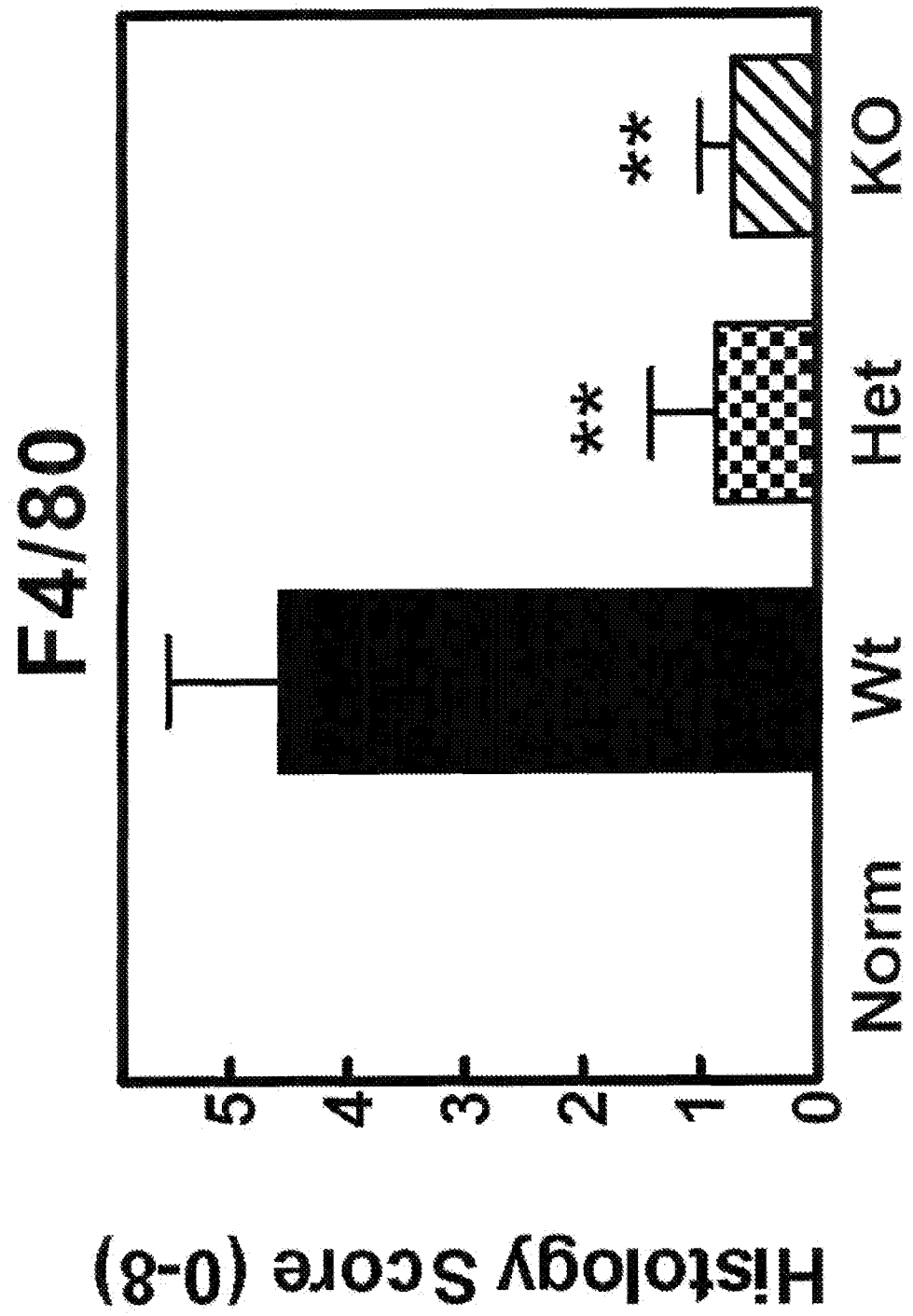


FIG. 3

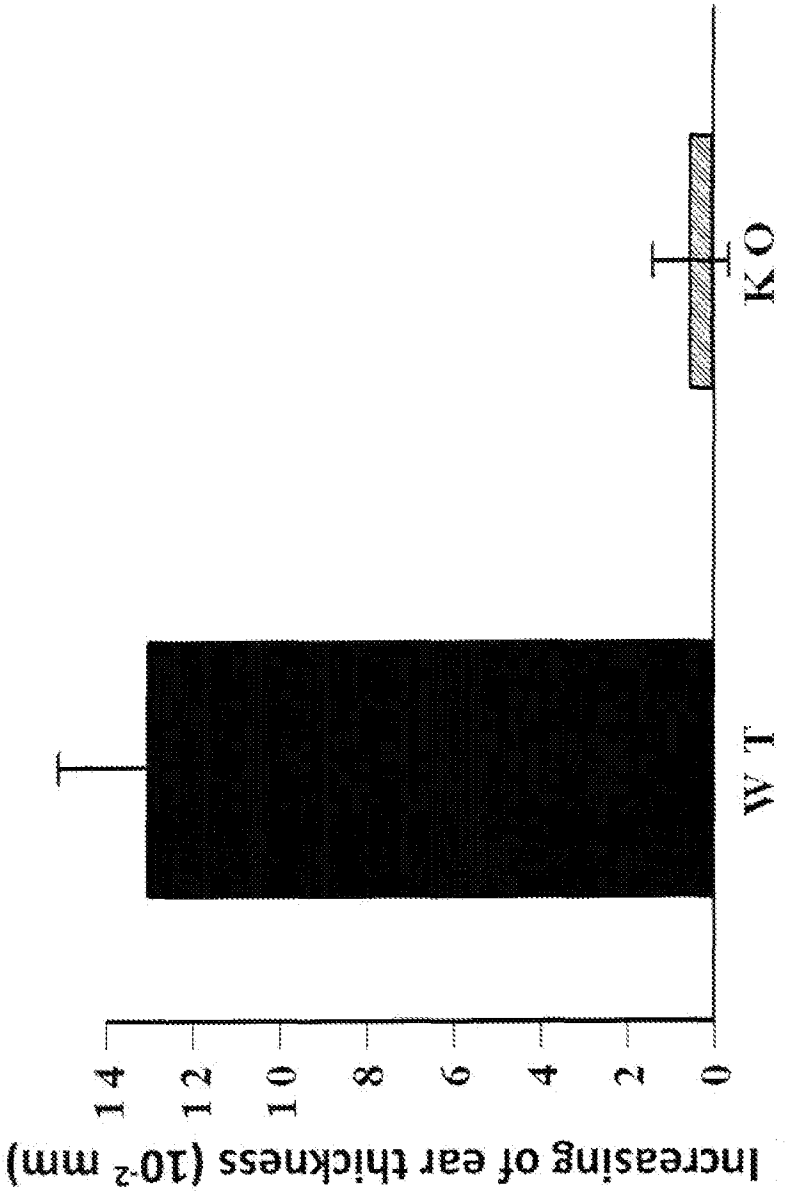


FIG. 4

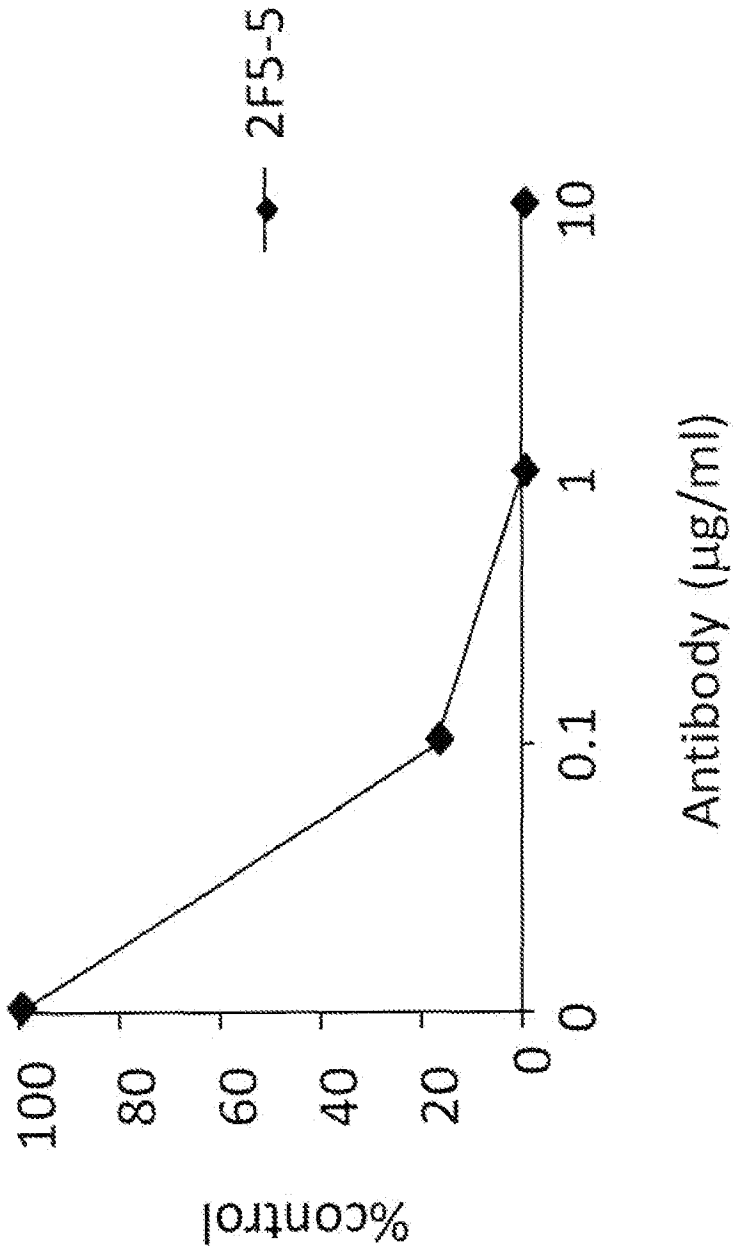


FIG. 5

Heavy chain_36										
Kabat definition		<----------FWR1---------->		<CDR1>	<----FWR2---->		<-------CDR2----->		<---	
Chothia definition			<-CDR1-->				<--CDR2-->			
mouse 36F7C10	1	QVQL**Q**QPGAE**L**VKPGASVK**M**SCKASGYTFT		**N**Y**W**MH	W**V**K**Q**R**P**GQGLE**W**IG		**V**IDP**S**D**S**YTT**Y**N**Q**KFK**G**		**K**ATL	70
HC2	1	QVQLVQSGAEVKKPGASVKVSCKASGYTFT		NYWMH	WVRQAPGQGLEWMG		VIDPSDSYTTYAQKFQG		RVTM	70
HC3	1	QVQLVQSGAEVKKPGASVKVSCKASGYTFT		NYWMH	WV***K***QAPGQGLEW***I***G		VIDPSDSYTTYNQKFKG		***KA***TM	70
36HKK3	1	QVQLVQSGAEVKKPGASVKVSCKASGYTFT		NYWMH	WVRQAPGQGLEWMG		VIDPSDSYTTYNQKFKG		**KATL**	70
ABM67212	1	QVQLVQSGAEVKKPGASVKVSCKASGYTFT		SYYMH	WVRQAPGQGLEWMG		IINPSGGSTSYAQKFQG		RVTM	70
IGHV1-46*03	1	QVQLVQSGAEVKKPGASVKVSCKASGYTFT		SYYMH	WVRQAPGQGLEWMG		IINPSGGSTSYAQKFQG		RVTM	70

Kabat definition		----------FWR3---------->	<--CDR3--->	<---FWR4-->	
Chothia definition			<--CDR3--->		
mouse 36F7C10	71	T**V**DTS**S**STA**Y**M**Q**LSSLT**S**EDSAVYYCT**R**	**GNYGVDYAM**DY	WGQGT**S**VTVSS	120
HC2	71	T**V**DTSTSTVYMELSSLRSEDTAVYYCAR	GNYGVDYAMDY	WGQGTLVTVSS	120
HC3	71	TRDTSTSTVYMELSSLRSEDTAVYYCTR	GNYGVDYAMDY	WGQGT***S***VTVSS	120
36HKK3	71	T**V**DTSTST**A**YMELSSLRSEDTAVYYCTR	GNYGVDYAMDY	WGQGTLVTVSS	120
ABM67212	71	TRDTSTSTVYMELSSLRSEDTAVYYCAR	EGDGYIQAFDY	WGQGTLVTVSS	120
IGHV1-46*03	71	TRDTSTSTVYMELSSLRSEDTAVYYCAR			98
JH4	1		YFDY	WGQGTLVTVSS	15

Bold; different residues between mouse and human

Underline; grafted CDRs

Bold Italic; substituted residues with mouse residues

FIG. 6A

Heavy chain_42							
Kabat definition	<----------FWR1---------->	<CDR1>	<----FWR2---->	<-------CDR2------>	<---		
Chothia definition	<-CDR1-->			<--CDR2-->			
mouse 42G5B10	1 QVQLQQPGAELVKPGASVKMSCKASGYTFT	SYWMH	WVKQRPGQGLEWIG	LIDPSDKYTNYNQKFKG	KATL	70	
42HKK1	1 QVQLVQSGAEVKKPGASVKVSCKASGYTFT	SYWMH	WVRQAPGQGLEWMG	LIDPSDKYTNYNQKFKG	RVTM	70	
42HKK2	1 QVQLVQSGAEVKKPGASVKVSCKASGYTFT	SYWMH	WVRQAPGQGLEWMG	LIDPSDKYTNYNQKFKG	RVTL	70	
42HKK3	1 QVQLVQSGAEVKKPGASVKVSCKASGYTFT	SYWMH	WVRQAPGQGLEWMG	LIDPSDKYTNYNQKFKG	KATL	70	
ABM67212	1 QVQLVQSGAEVKKPGASVKVSCKASGYTFT	SYYMH	WVRQAPGQGLEWMG	IINPSGGSTSYAQKFQG	RVTM	70	
IGHV1-46*03	1 QVQLVQSGAEVKKPGASVKVSCKASGYTFT	SYYMH	WVRQAPGQGLEWMG	IINPSGGSTSYAQKFQG	RVTM	70	

Kabat definition	----------FWR3---------->	<--CDR3--->	<---FWR4-->		
Chothia definition		<--CDR3--->			
mouse 42G5B10	71 TVDTSSSTAYMQLSSLTSEDSAVYYCTR	GNYGVDYGMDY	WGQGTSVTVSS	120	
42HKK1	71 TRDTSTSTVYMELSSLRSEDTAVYYCAR	GNYGVDYGMDY	WGQGTLVTVSS	120	
42HKK2	71 TVDTSTSTVYMELSSLRSEDTAVYYCTR	GNYGVDYGMDY	WGQGTLVTVSS	120	
42HKK3	71 TVDTSTSTAYMELSSLRSEDTAVYYCTR	GNYGVDYGMDY	WGQGTLVTVSS	120	
ABM67212	71 TRDTSTSTVYMELSSLRSEDTAVYYCAR	EGDGYIQAFDY	WGQGTLVTVSS	120	
IGHV1-46*03	71 TRDTSTSTVYMELSSLRSEDTAVYYCAR			98	
JH4	1	YFDY	WGQGTLVTVSS	15	

Bold; different residues between mouse and human

Underline; grafted CDRs

Bold Italic; substituted residues with mouse residues

FIG. 6B

Light chain							
Kabat definition		<----------FWR1--------->	<---CDR1-->	<-------FWR2---->	<-CDR2>	<--------------	
Chothia definition			<---CDR1-->		<-CDR2>		
mouse 36F7C10	1	DIQMTQSPASLSASVGETVTITC	GASENIYGALN	WYQRKQGKSPQLLIY	GATNLAD	GMSSRFSGSGSGRQ	70
LC3	1	DIQMTQSPSSLSASVGDRVTITC	RASENIYGALN	WYQQKPGKAPKLLIY	GATNLAD	GVPSRFSGSGSGRQ	70
LK3	1	DIQMTQSPSSLSASVGDRVTITC	GASENIYGALN	WYQRKPGKAPKLLIY	GATNLAD	GVPSRFSGSGSGRD	70
BAH04867.1	1	DIQMTQSPSSLSASVGDRVTITC	RASQSISSYLN	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTD	70
IGKV1D-39*01	1	DIQMTQSPSSLSASVGDRVTITC	RASQSISSYLN	WYQQKPGKAPKLLIY	AASSLQS	GVPSRFSGSGSGTD	70

Kabat definition		-FWR3------------->	<-CDR3-->	<--FWR4--->	
Chothia definition			<-CDR3-->		
mouse 36F7C10	71	YSLKISSLKPDDVATYYC	QNVLITPYT	FGGGTKLEIK	107
LC3	71	YSLTISSLQPEDFATYYC	QNVLITPYT	FGGGTKLEIK	107
LK3	71	YTLTISSLQPEDFATYYC	QNVLITPYT	FGQGTKLEIK	107
BAH04867.1	71	FTLTISSLQPEDFATYYC	QQSYSTPYT	FGQGTKLEIK	107
IGKV1D-39*01	71	FTLTISSLQPEDFATYYC	QQSYSTPP		96

Bold; different residues between mouse and human

Underline; grafted CDRs

Bold Italic; substituted residues with mouse residues

Underline, Italic; substituted residues with human residues

FIG. 6C

IC50 (nM, mean±SD)				
OR: out of range				
If no SD value is shown, the assay was single.				
		Heavy chain		
		36HCM1	40HCM1	42HCM1
Light chain	36LM1	3.297±2.303	15.483±12.423	1.903±0.707
	40LM1	OR	3.423±0.975	8.580±1.858
	42LM1	OR	OR	3.297±1.330
		Heavy chain		
		36HKK1	36HKK2	36HKK3
Light chain	36LK1	1.870±0.281	1.323±0.258	0.987±0.219
	36LK2	2.513±0.828	1.220±0.474	1.107±0.474
	36LK3	1.240±0.192	1.233±0.075	1.313±0.245
		Heavy chain		
		42HKK1	42HKK2	42HKK3
Light chain	42LK1	13.51	18.22	18.97
	42LK2	9.72	13.35	13.6
	42LK3	2.38	1.99	3.09
		Heavy chain		
		42HKK1	42HKK2	42HKK3
Light chain	36LK1	1.39	2.62	1.86
	36LK2	1.33	1.08	1.59
	36LK3	1.357±0.250	1.663±0.475	1.467±0.160
		Heavy chain		
		36HKK3	36HC2	36HC3
Light chain	36LK3	1.210±0.306	1.014±0.330	1.128±0.041
	36LC3	1.265±0.266	1.117±0.193	1.327±0.346

FIG. 7A

IC90 (nM, mean±SD)				
OR: out of range				
		Heavy chain		
		36HCM1	40HCM1	42HCM1
Light chain	36LM1	OR	OR	28.173±18.382
	40LM1	OR	OR	OR
	42LM1	OR	OR	OR
		Heavy chain		
		36HKK1	36HKK2	36HKK3
Light chain	36LK1	34.300±12.049	21.040±12.576	6.957±1.059
	36LK2	OR	OR	16.360±4.492
	36LK3	8.75±1.121	5.613±0.882	4.130±1.769
		Heavy chain		
		42HKK1	42HKK2	42HKK3
Light chain	42LK1	OR	OR	OR
	42LK2	OR	OR	OR
	42LK3	40.74	53.08	OR
		Heavy chain		
		42HKK1	42HKK2	42HKK3
Light chain	36LK1	8.74	44.04	15.99
	36LK2	14.31	25.33	18.65
	36LK3	4.857±0.403	6.540±2.040	5.647±1.107
		Heavy chain		
		36HKK3	36HC2	36HC3
Light chain	36LK3	4.376±0.651	4.564±1.928	4.603±1.521
	36LC3	4.327±0.816	5.251±0.591	6.339±0.614

FIG. 7B

IC95 (nM, mean±SD)				
OR: out of range				
		Heavy chain		
		36HCM1	40HCM1	42HCM1
Light chain	36LM1	OR	OR	OR
	40LM1	OR	OR	OR
	42LM1	OR	OR	OR
		Heavy chain		
		36HKK1	36HKK2	36HKK3
Light chain	36LK1	OR	OR	OR
	36LK2	OR	OR	OR
	36LK3	OR	OR	7.587±3.511
		Heavy chain		
		42HKK1	42HKK2	42HKK3
Light chain	42LK1	OR	OR	OR
	42LK2	OR	OR	OR
	42LK3	OR	OR	OR
		Heavy chain		
		42HKK1	42HKK2	42HKK3
Light chain	36LK1	OR	OR	OR
	36LK2	32.31	54.83	60.93
	36LK3	9.020±0.762	15.337±9.599	10.780±3.938
		Heavy chain		
		36HKK3	36HC2	36HC3
Light chain	36LK3	6.886±1.274	8.972±4.417	8.381±4.053
	36LC3	9.747±3.758	(11.77, >66.7, 10.33)	OR

FIG. 7C

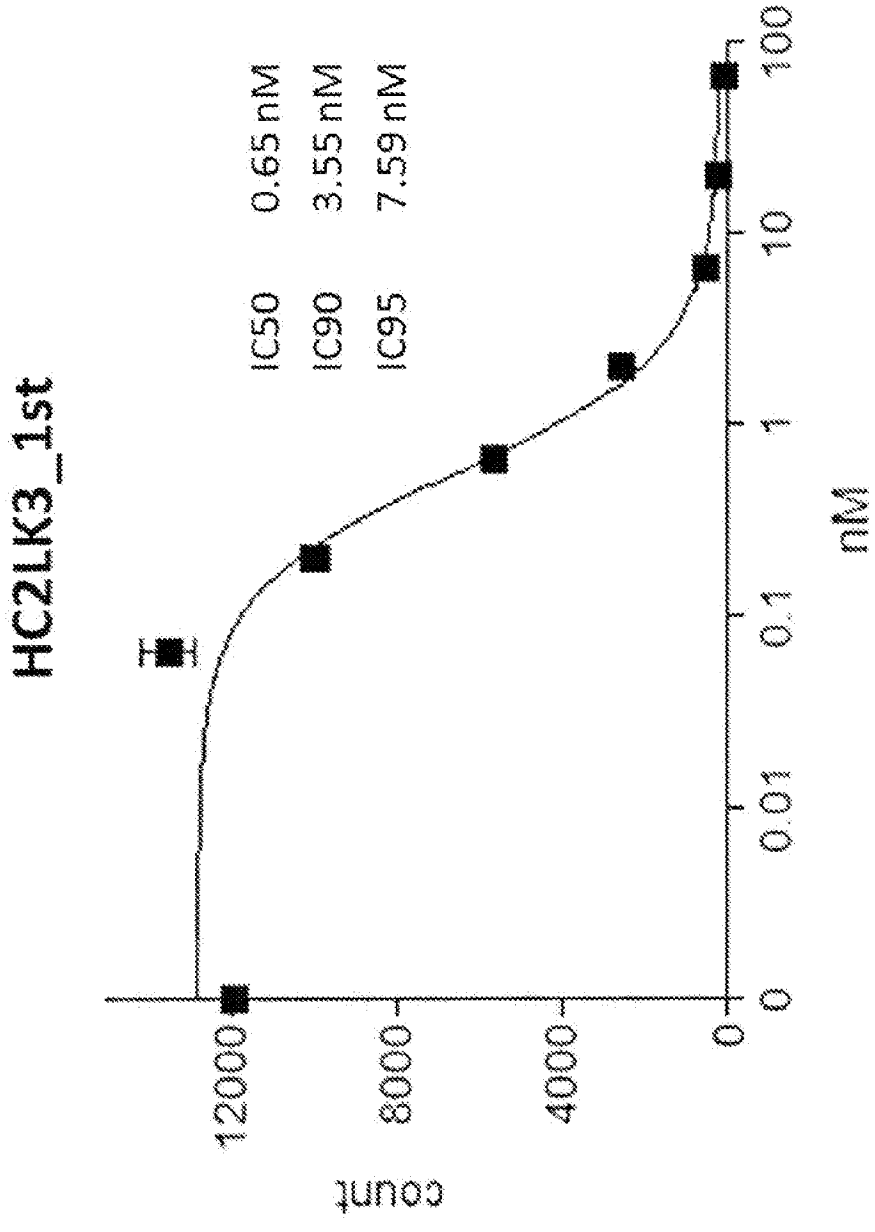

HC2LK3_1st
IC50 0.65 nM
IC90 3.55 nM
IC95 7.59 nM
count
12000
8000
4000
0
0
0.01
0.1
1
10
100
nM

FIG. 8A

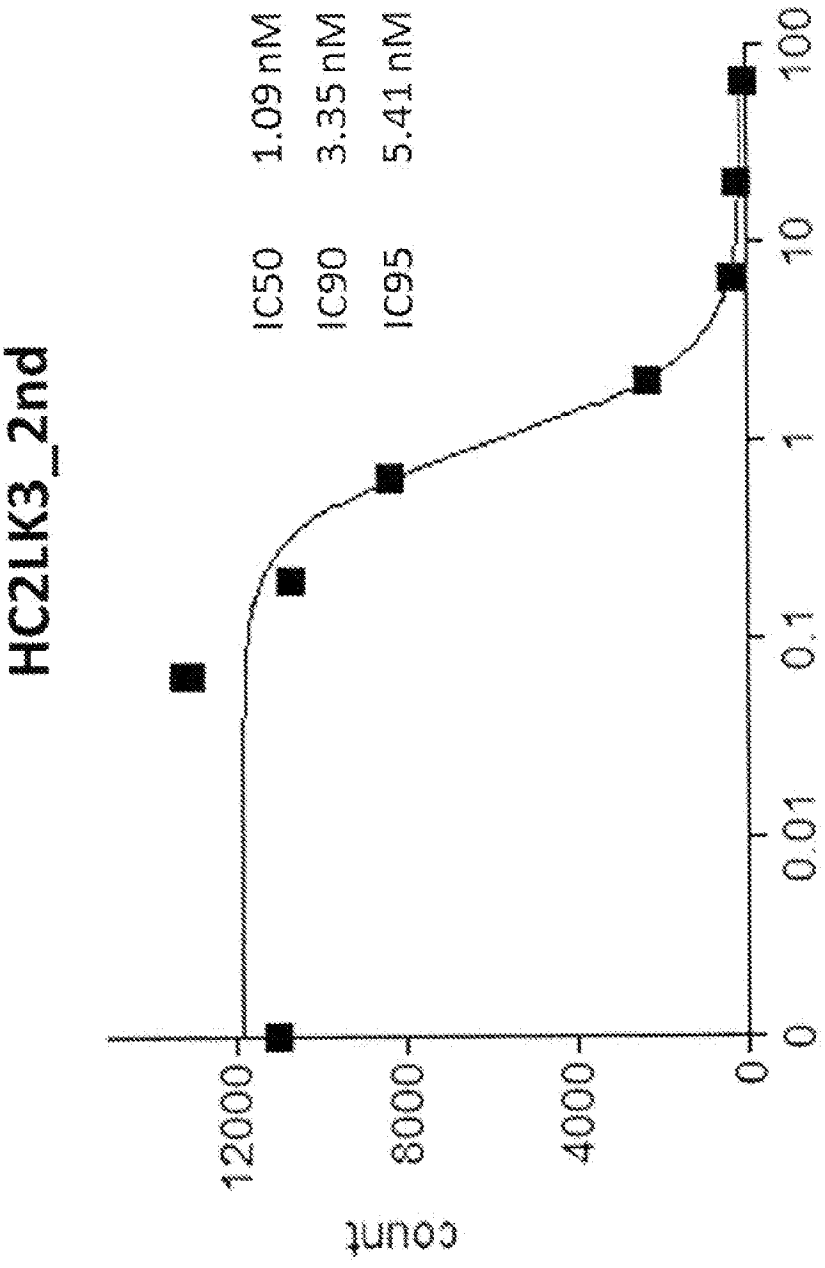

HC2LK3_2nd
IC50 1.09 nM
IC90 3.35 nM
IC95 5.41 nM
count
12000
8000
4000
0
0
0.01
0.1
1
10
100
nM

FIG. 8B

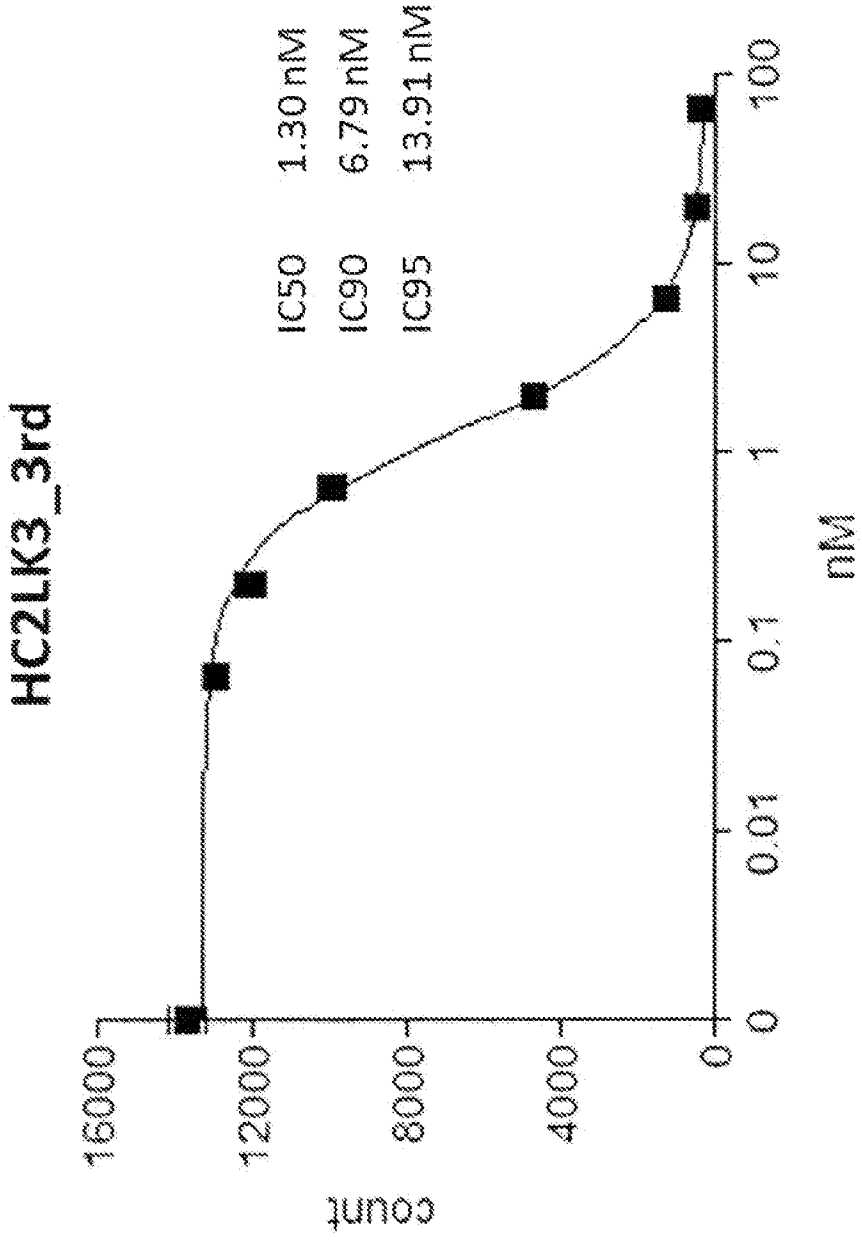

HC2LK3_3rd
IC50 1.30 nM
IC90 6.79 nM
IC95 13.91 nM
count
16000
12000
8000
4000
0
0
0.01
0.1
1
10
100
nM

FIG. 8C

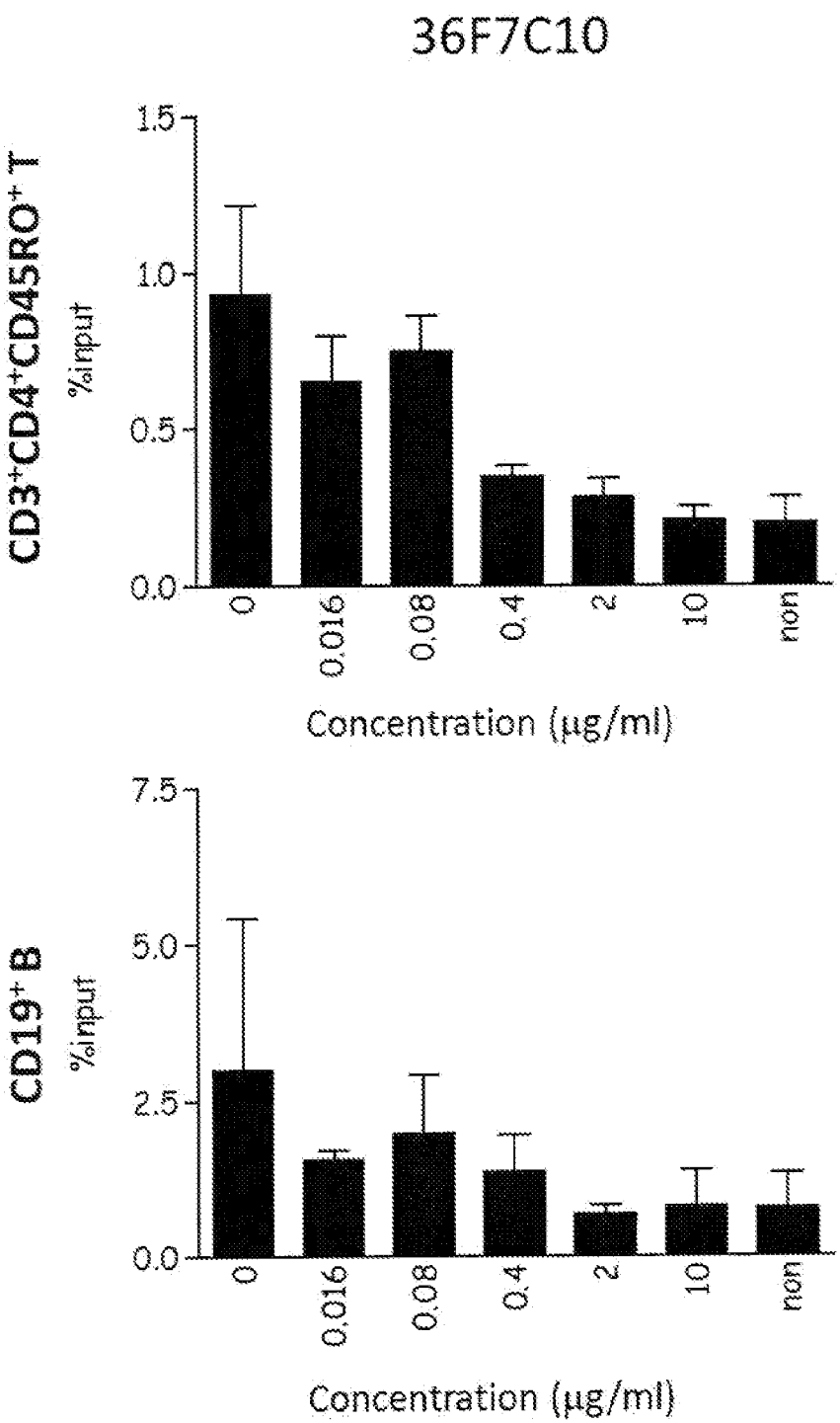

36F7C10
CD3+CD4+CD45RO+ T
%input
1.5
1.0
0.5
0.0
0
0.016
0.08
0.4
2
10
non
Concentration (μg/ml)
CD19+ B
%input
7.5
5.0
2.5
0.0
0
0.016
0.08
0.4
2
10
non
Concentration (μg/ml)

FIG. 9A

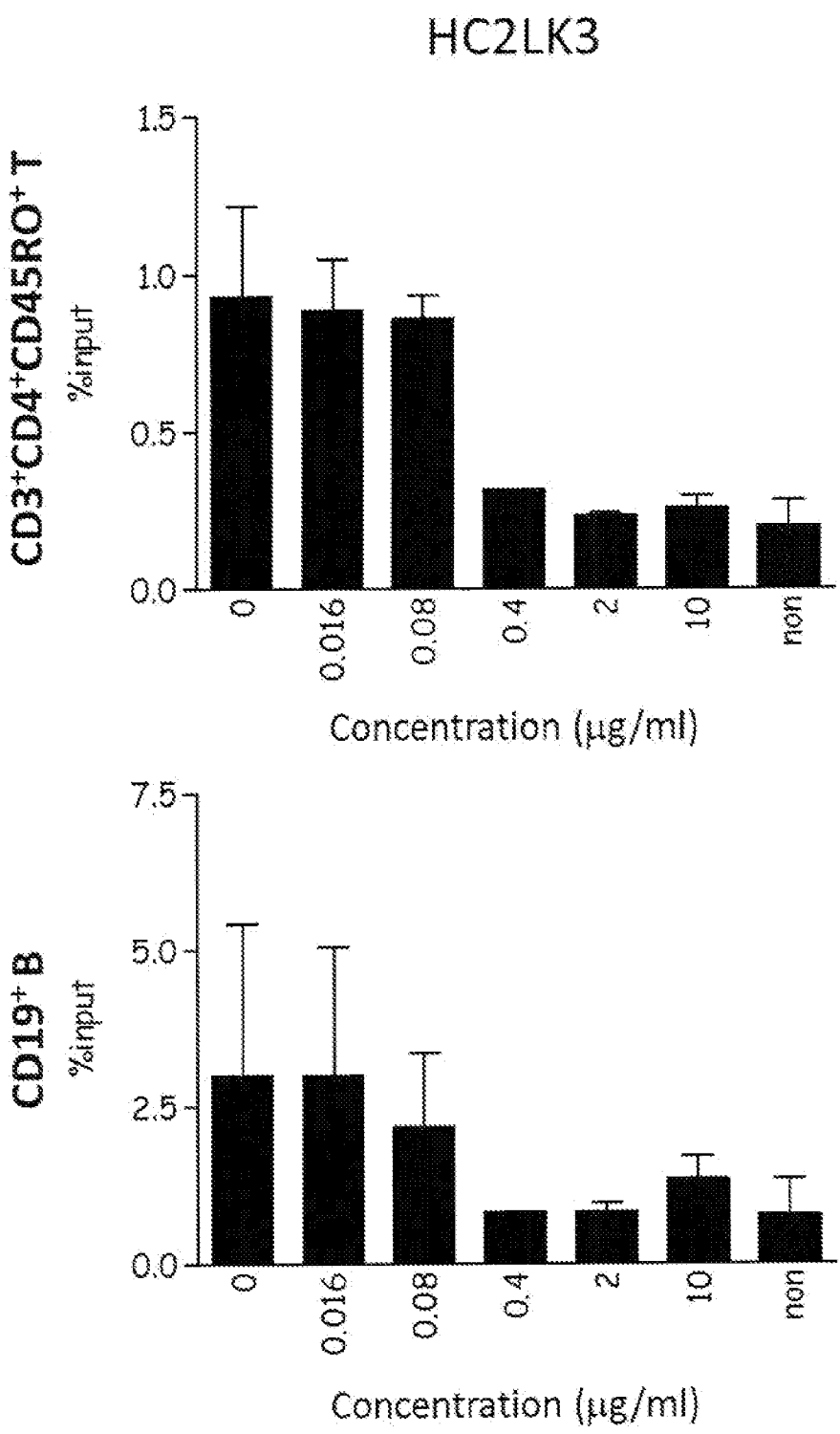

HC2LK3
CD3+CD4+CD45RO+ T
%input
1.5
1.0
0.5
0.0
0
0.016
0.08
0.4
2
10
non
Concentration (μg/ml)
CD19+ B
%input
7.5
5.0
2.5
0.0
0
0.016
0.08
0.4
2
10
non
Concentration (μg/ml)

FIG. 9B

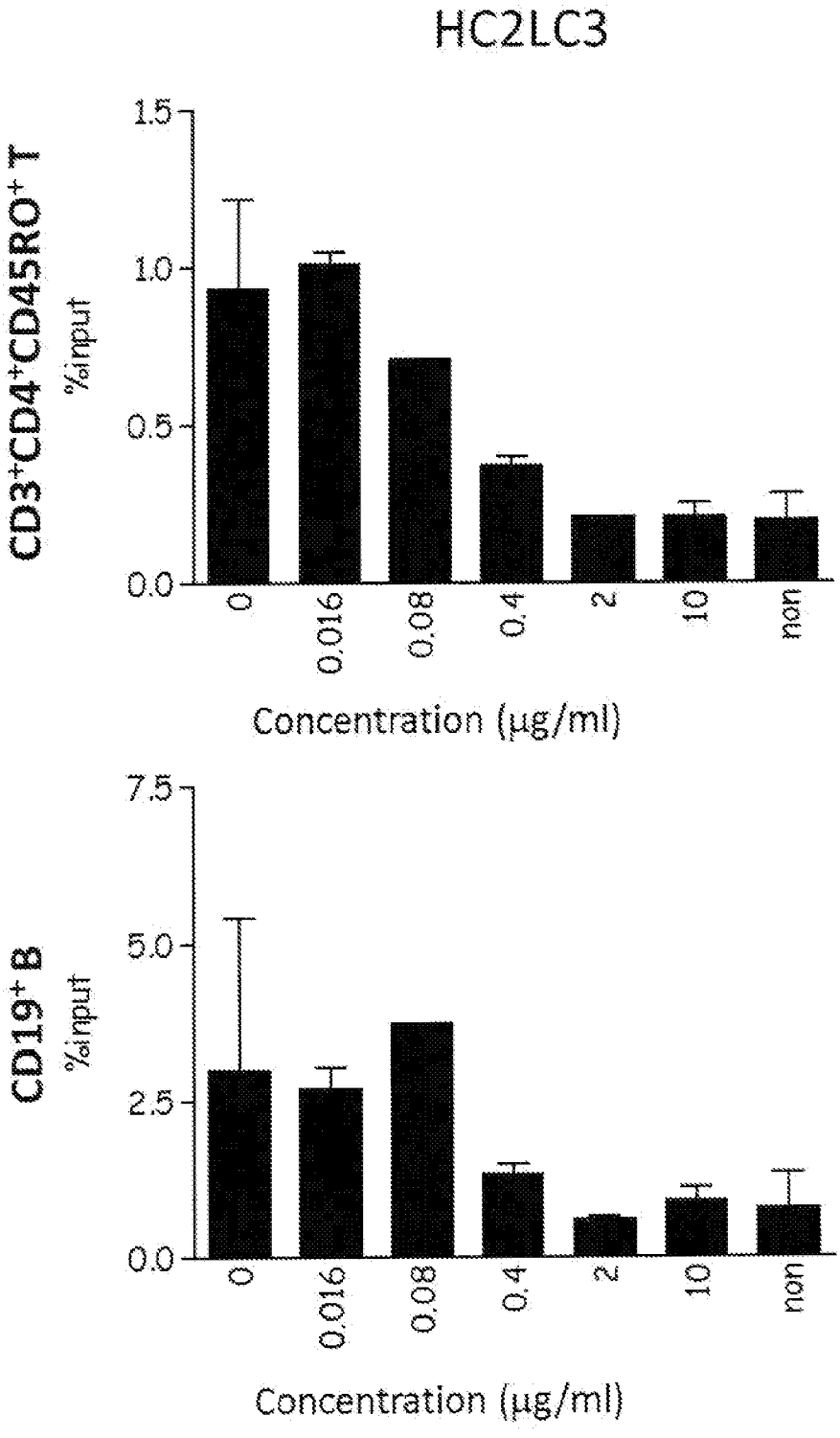

HC2LC3
CD3+CD4+CD45RO+ T
%input
1.5
1.0
0.5
0.0
0
0.016
0.08
0.4
2
10
non
Concentration (µg/ml)
CD19+ B
%input
7.5
5.0
2.5
0.0
0
0.016
0.08
0.4
2
10
non
Concentration (µg/ml)

FIG. 9C

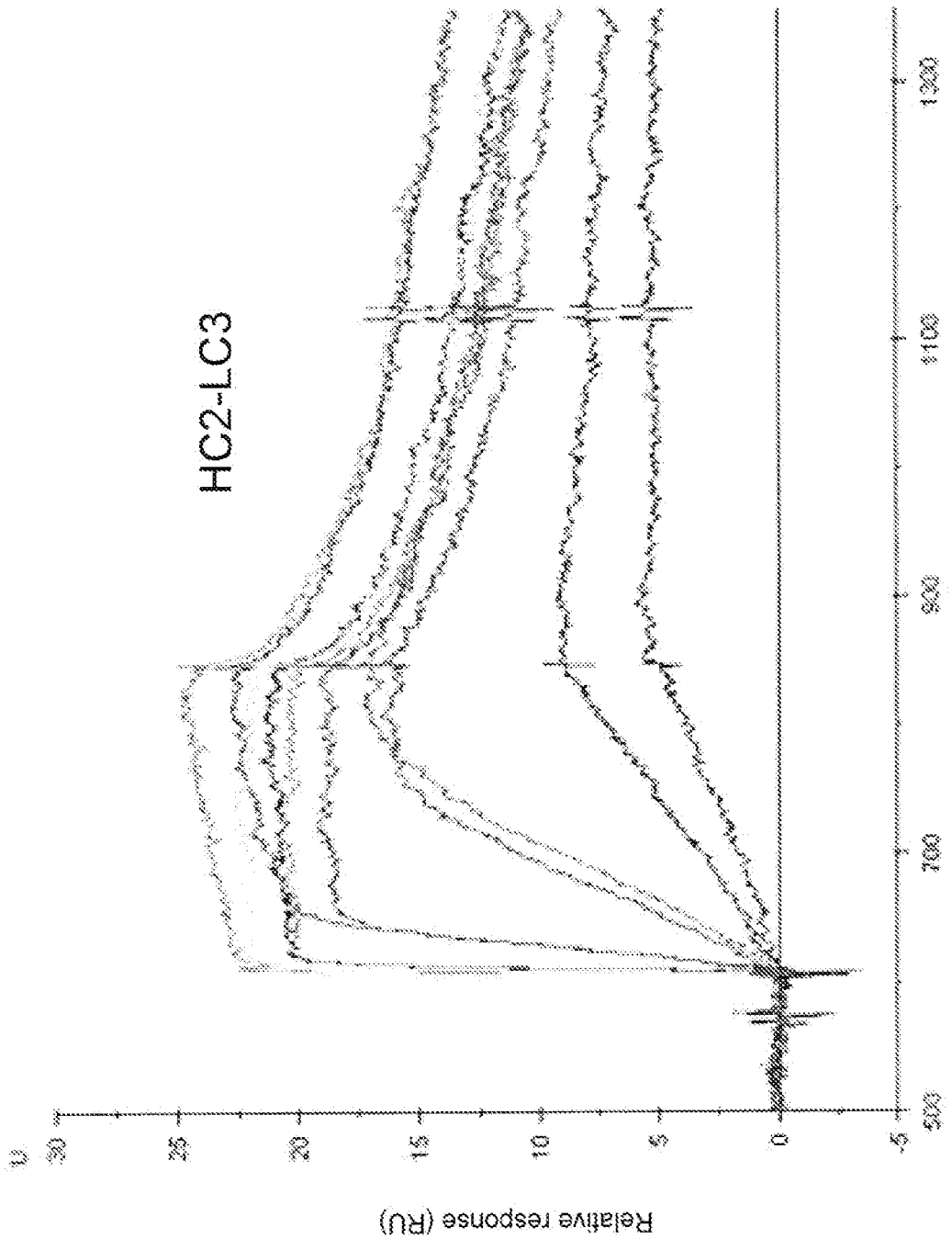

HC2-LC3
Relative response (RU)
30
25
20
15
10
5
0
-5
500
700
900
1100
1300
Time (sec)

FIG. 10A

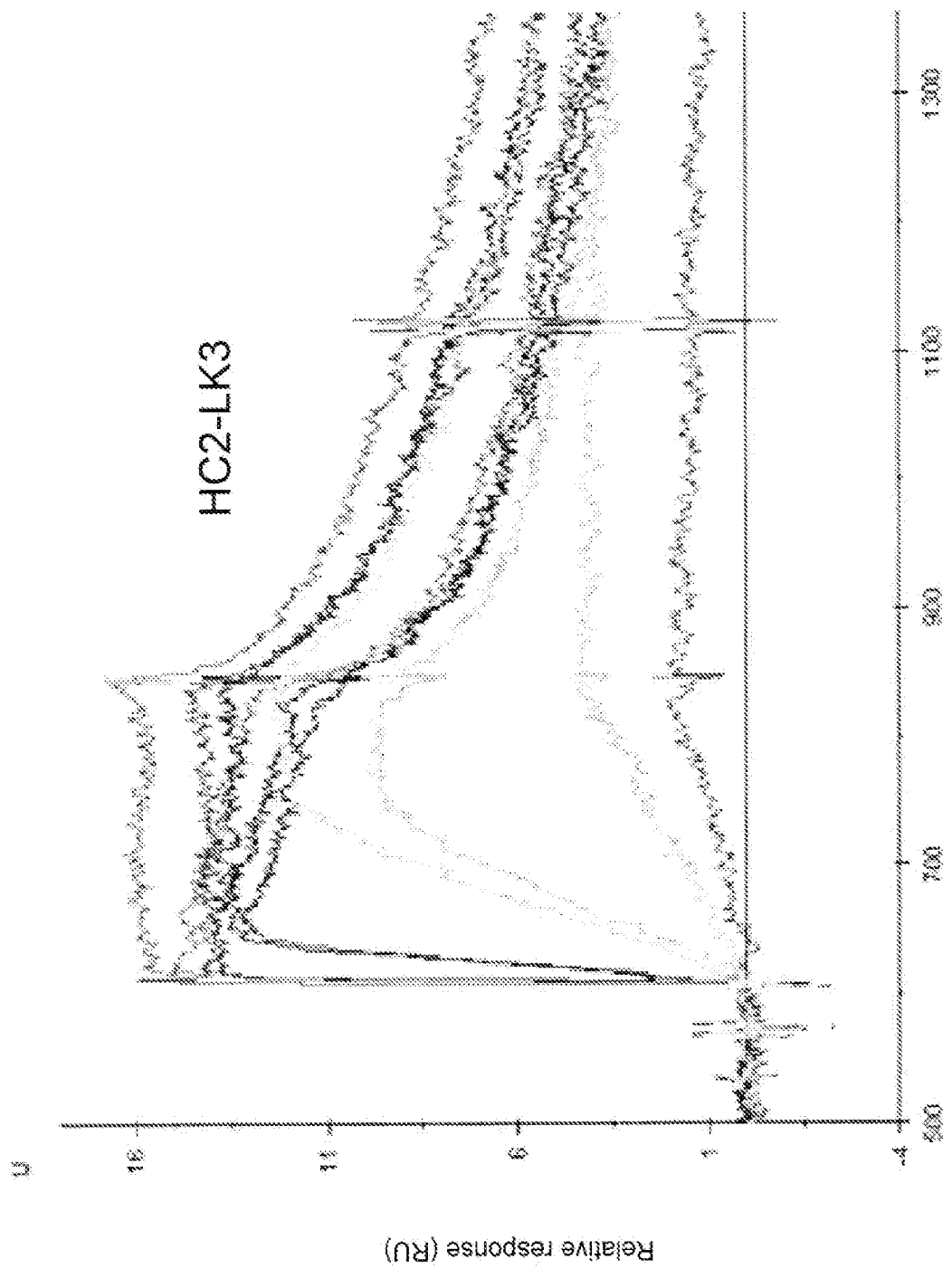

HC2-LK3
Relative response (RU)
Time (sec)
16
11
6
1
-4
500
700
900
1100
1300

FIG. 10B

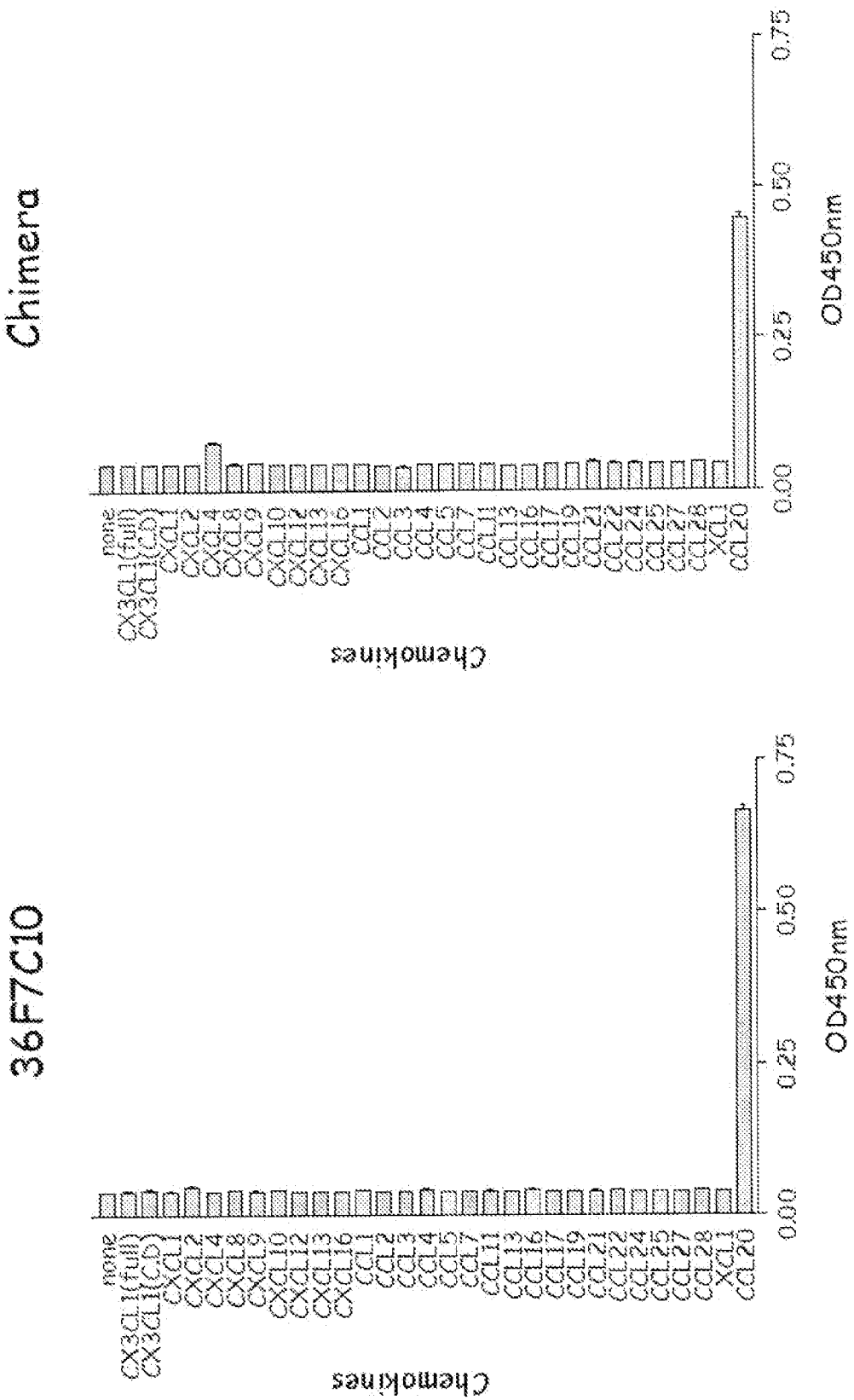

Chimera
36F7C10
Chemokines
OD450nm
0.00
0.25
0.50
0.75
none
CX3CL1(Full)
CX3CL1(CD)
CXCL1
CXCL2
CXCL4
CXCL8
CXCL9
CXCL10
CXCL12
CXCL13
CX
CCL1
CCL2
CCL3
CCL4
CCL5
CCL7
CCL11
CCL13
CCL16
CCL17
CCL19
CCL21
CCL22
CCL24
CCL25
CCL27
CCL28
XCL1
CCL20

FIG. 11A

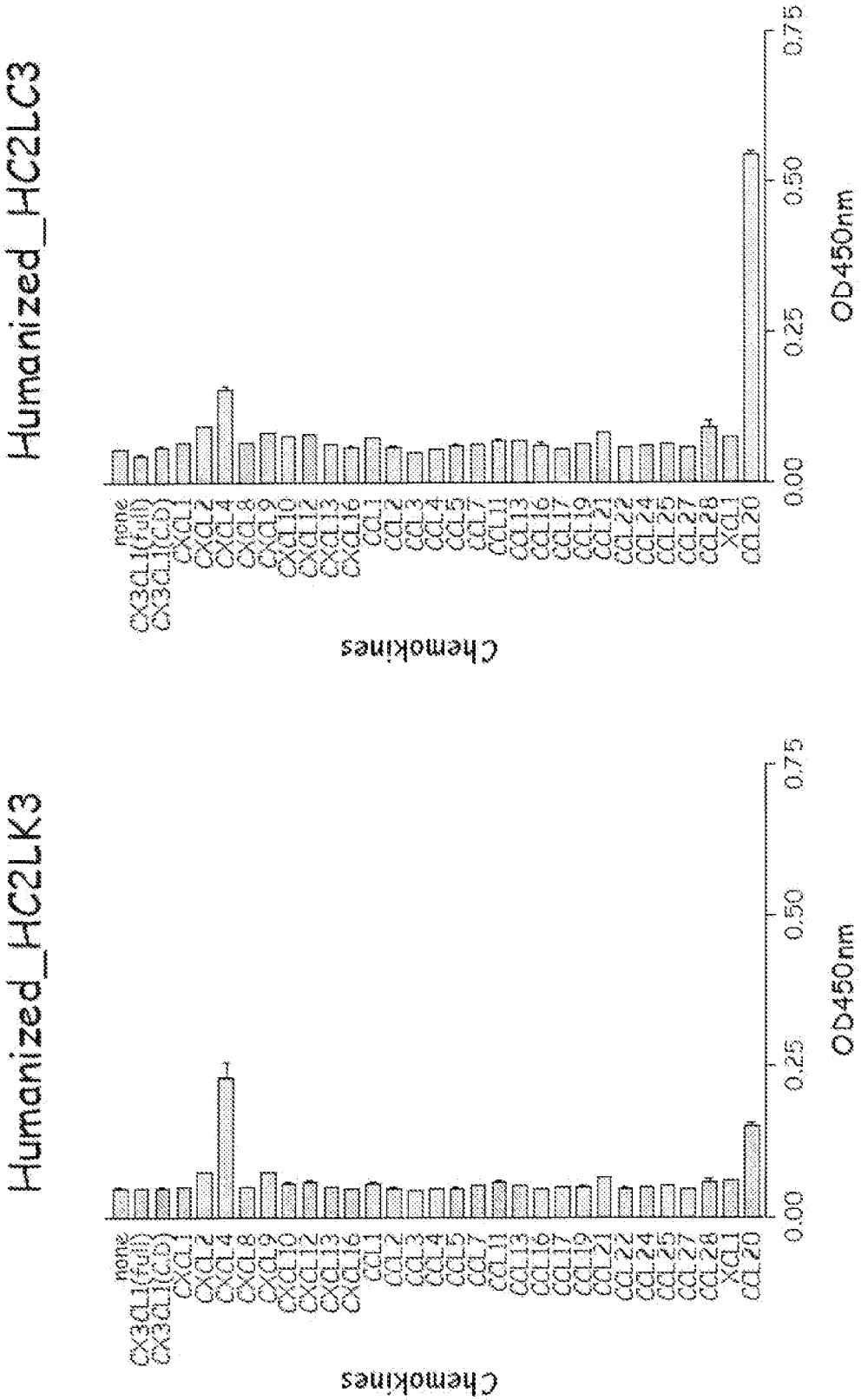

Humanized_HC2LC3
Humanized_HC2LK3
Chemokines
OD450nm
0.00
0.25
0.50
0.75
none
CX3CL1(full)
CX3CL1(CD)
CXCL1
CXCL2
CXCL4
CXCL8
CXCL9
CXCL10
CXCL12
CXCL13
CXCL16
CCL1
CCL2
CCL3
CCL4
CCL5
CCL7
CCL11
CCL13
CCL16
CCL17
CCL19
CCL21
CCL22
CCL24
CCL25
CCL27
CCL28
XCL1
CCL20

FIG. 11B

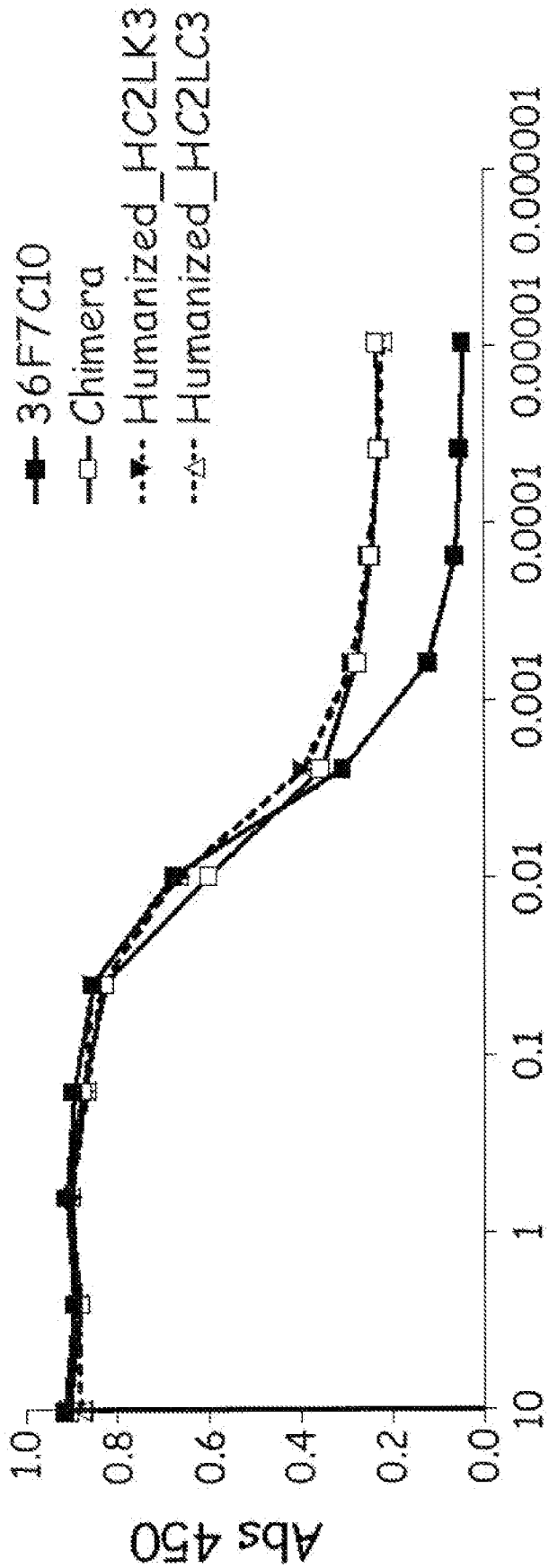

36F7C10
Chimera
Humanized_HC2LK3
Humanized_HC2LC3
Abs 450
1.0
0.8
0.6
0.4
0.2
0.0
10
1
0.1
0.01
0.001
0.0001
0.00001
0.000001
Ab conc. (ug/ml)

FIG. 11C

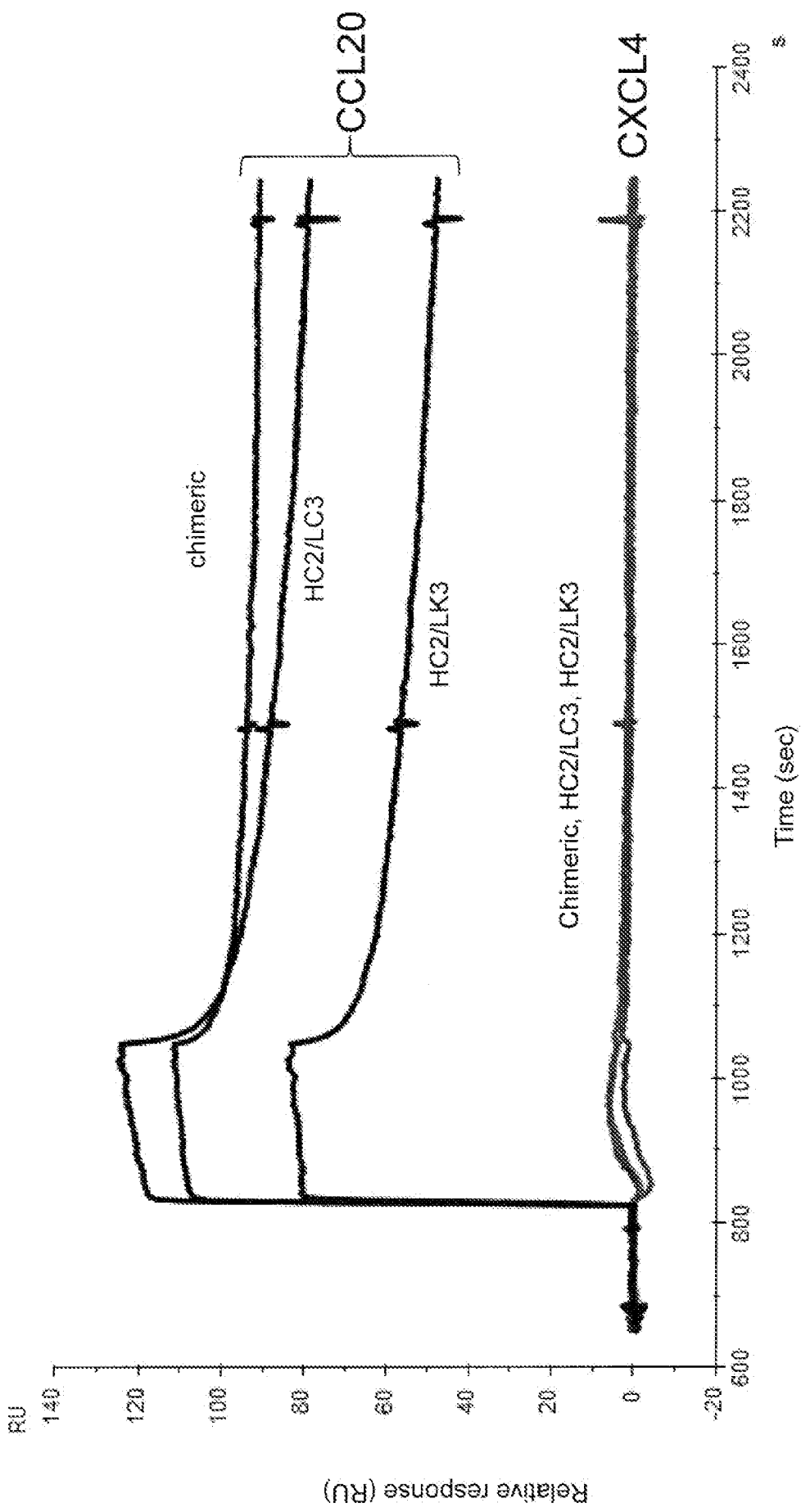

CCL20
CXCL4
chimeric
HC2/LC3
HC2/LK3
Chimeric, HC2/LC3, HC2/LK3
RU
140
120
100
80
60
40
20
0
-20
600
800
1000
1200
1400
1600
1800
2000
2200
2400
s
Time (sec)
Relative response (RU)

FIG. 11D

```
37.5% identity in 56 residues overlap; Score: 106.0; Gap frequency: 5.4%

CCL16,      37 CCLKYYEKVLPRRLVVGYRKAL--NCHLPAIIFVTKRNREVCTNPNDDWVQEYIK
CCL20,      32 CCLGYTDRILHPKFIVGFTRQLANEGCDINAIIFHTKKKLSVCANPKQTWVKYIVR
               **** * *   *   **   *   *   **** ** ** ** **   **
```

FIG. 12

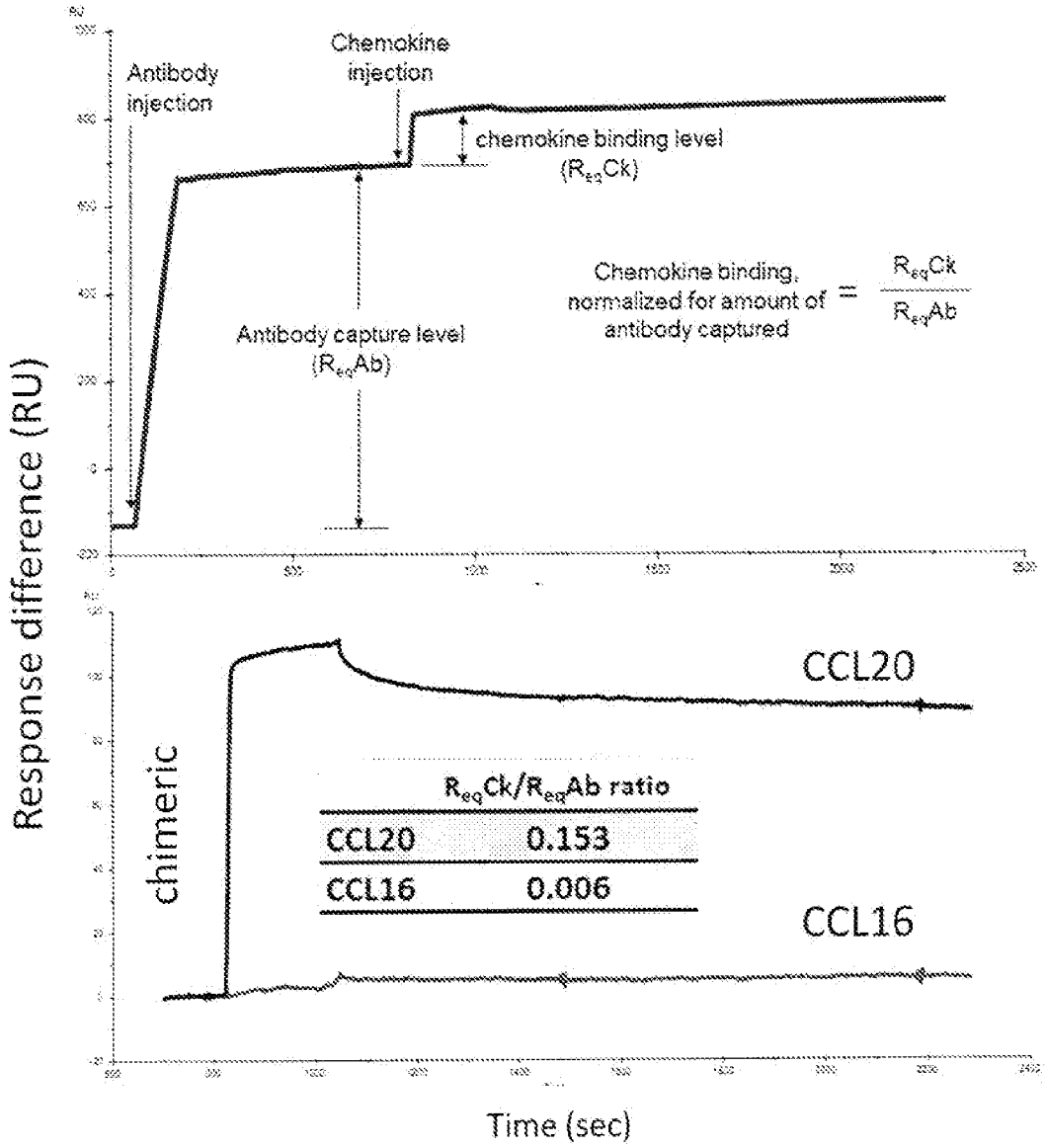

Antibody injection
Chemokine injection
chemokine binding level ($R_{eq}Ck$)
Antibody capture level ($R_{eq}Ab$)
Chemokine binding, normalized for amount of antibody captured = $\frac{R_{eq}Ck}{R_{eq}Ab}$
Response difference (RU)
chimeric
CCL20
CCL16
$R_{eq}Ck/R_{eq}Ab$ ratio
CCL20 0.153
CCL16 0.006
Time (sec)

FIG. 13A

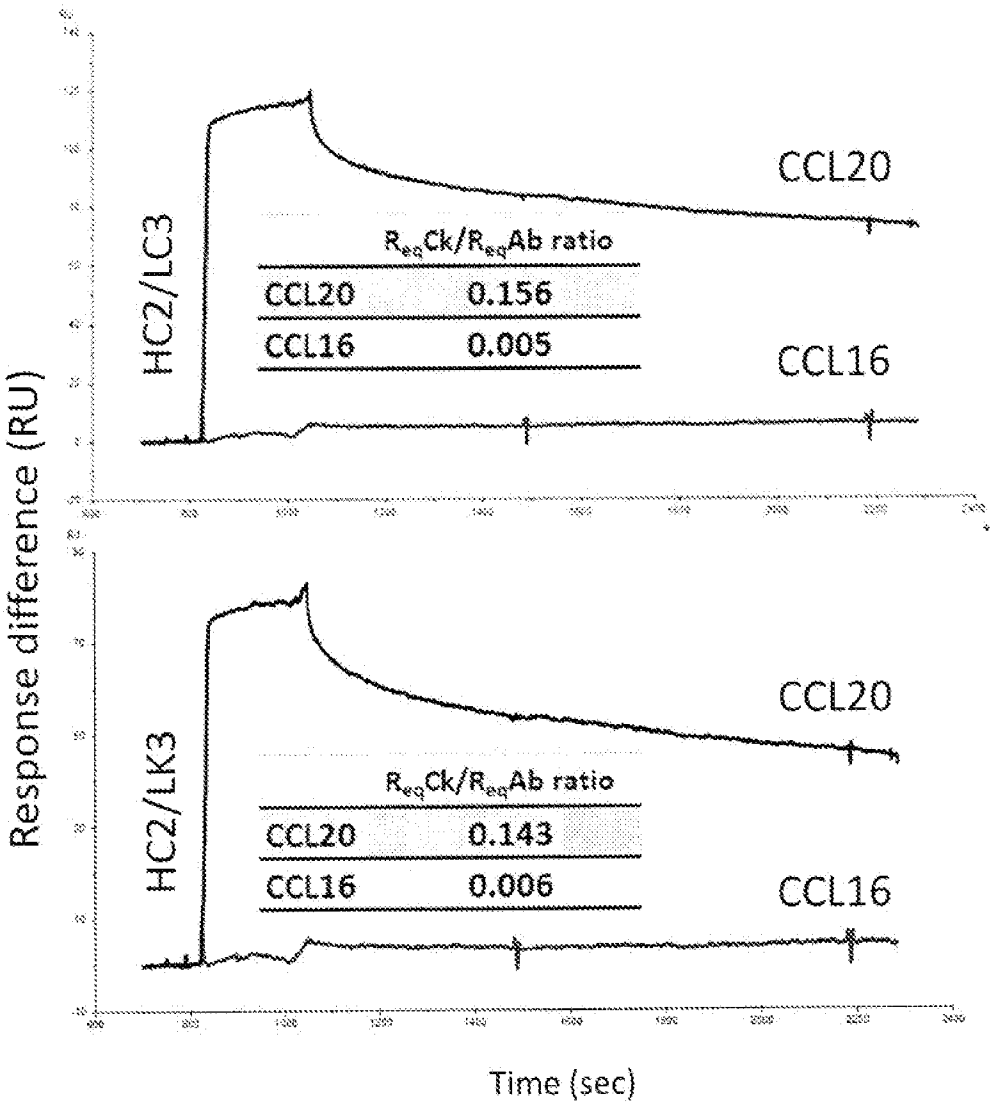

Response difference (RU)
HC2/LC3
CCL20
R_{eq}Ck/R_{eq}Ab ratio
CCL20 0.156
CCL16 0.005
CCL16
HC2/LK3
CCL20
R_{eq}Ck/R_{eq}Ab ratio
CCL20 0.143
CCL16 0.006
CCL16
Time (sec)

FIG. 13B

```
86.5% identity in 96 residues overlap; Score: 441.0; Gap frequency: 0.0%

hum_CCL20         1 MCCTKSLLLAALMSVLLLHLCGESEAASNFDCCLGYTDRILHPKFIVGFTRQLANEGCDI
rhe_CCL20         1 MCCTKSLLLAALMSVLLLYLCSESEAASNFDCCLRYTDRILHPKFIVGFTQQLANETCDI
                    ****************** ** ************ *************** ***** ***

hum_CCL20        61 NAIIFHTKKKLSVCANPKQTWVKYIVRLLSKKVKNM
rhe_CCL20        61 NAVVFHTKKGLSVCANPKQTWVKLIVRRLSKKINKM
                    **   ***** ************** *** ****    *
```

```
97.9% identity in 96 residues overlap; Score: 497.0; Gap frequency: 0.0%

rhe_CCL20         1 MCCTKSLLLAALMSVLLLYLCSESEAASNFDCCLRYTDRILHPKFIVGFTQQLANETCDI 60
cyn_CCL20         1 MCCSKSLLLAALMSVLLLYLCSESEAASNFDCCLRYTDRILHPKFIVGFTQQLANETCDI 60
                    *** ************************************************************

rhe_CCL20        61 NAVVFHTKKGLSVCANPKQTWVKLIVRRLSKKINKM 96
cyn_CCL20        61 NAVIFHTKKGLSVCANPKQTWVKLIVRRLSKKINKM 96
                    *** ********************************
```

```
64.2% identity in 95 residues overlap; Score: 310.0; Gap frequency: 0.0%
hum_CCL20         2 CCTKSLLLAALMSVLLLHLCGESEAASNFDCCLGYTDRILHPKFIVGFTRQLANEGCDIN
mou_CCL20         3 CGGKRLLFLALAWVLLAHLCSQAEAASNYDCCLSYIQTPLPSRAIVGFTRQMADEACDIN
                    *  * **  **  *** ***   ***** **** *    *    ******* * * ****

hum_CCL20        62 AIIFHTKKKLSVCANPKQTWVKYIVRLLSKKVKNM 96
mou_CCL20        63 AIIFHTKKRKSVCADPKQNWVKRAVNLLSLRVKKM 97
                    ********  **** *** ***  * ***  ** *
```

FIG. 14

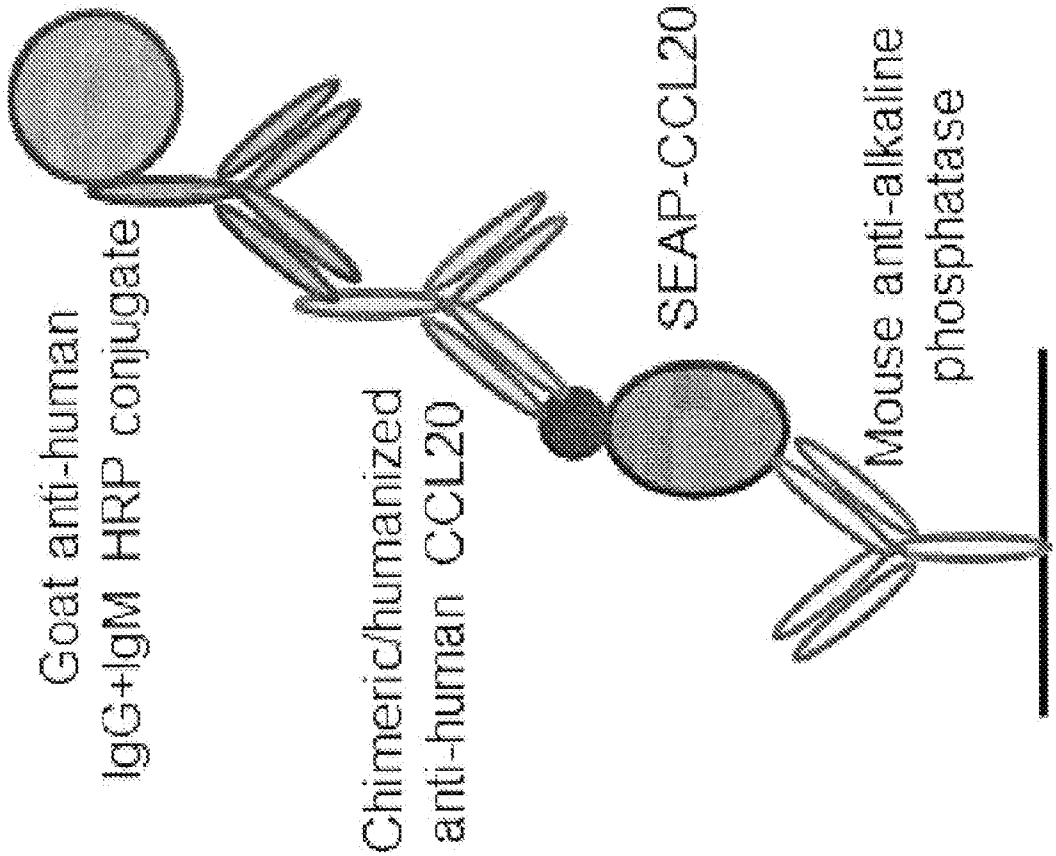

Goat anti-human
IgG+IgM HRP conjugate
Chimeric/humanized
anti-human CCL20
SEAP-CCL20
Mouse anti-alkaline
phosphatase

FIG. 15

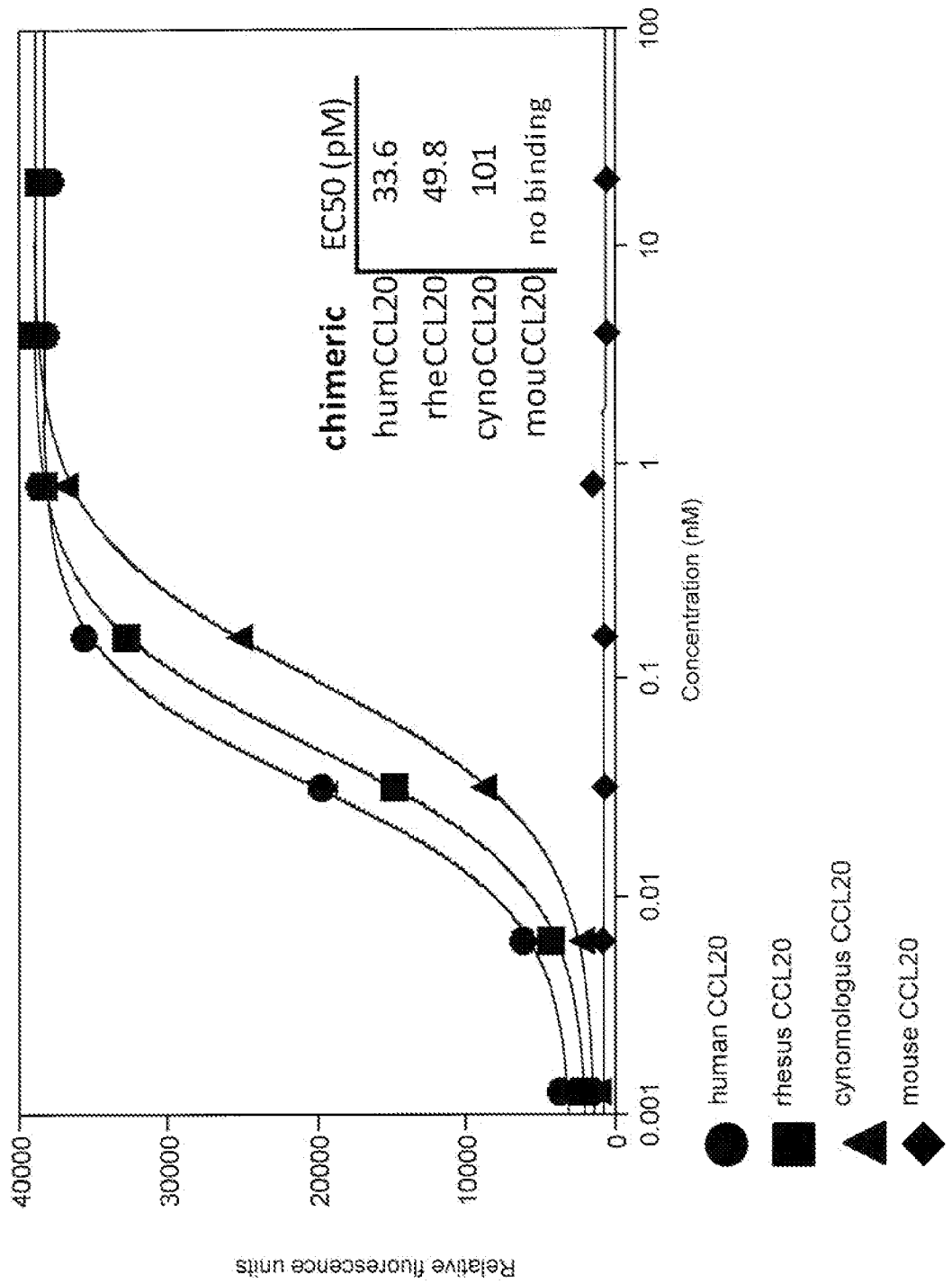

chimeric
EC50 (pM)
humCCL20 33.6
rheCCL20 49.8
cynoCCL20 101
mouCCL20 no binding
Relative fluorescence units
40000
30000
20000
10000
0
0.001
0.01
0.1
1
10
100
Concentration (nM)
human CCL20
rhesus CCL20
cynomologus CCL20
mouse CCL20

FIG. 16A

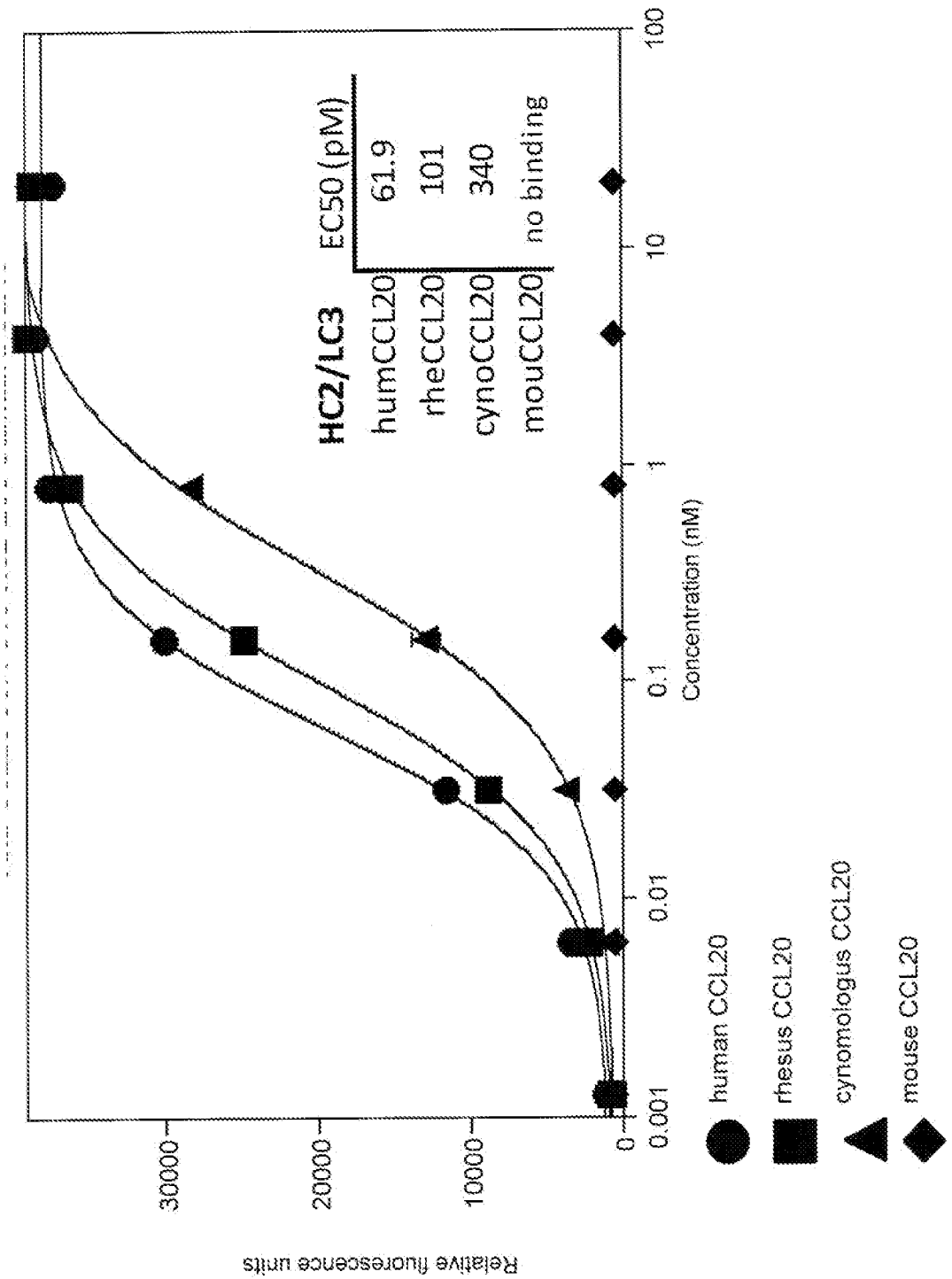

HC2/LC3
EC50 (pM)
humCCL20 61.9
rheCCL20 101
cynoCCL20 340
mouCCL20 no binding
Relative fluorescence units
30000
20000
10000
0
0.001
0.01
0.1
1
10
100
Concentration (nM)
human CCL20
rhesus CCL20
cynomologus CCL20
mouse CCL20

FIG. 16B

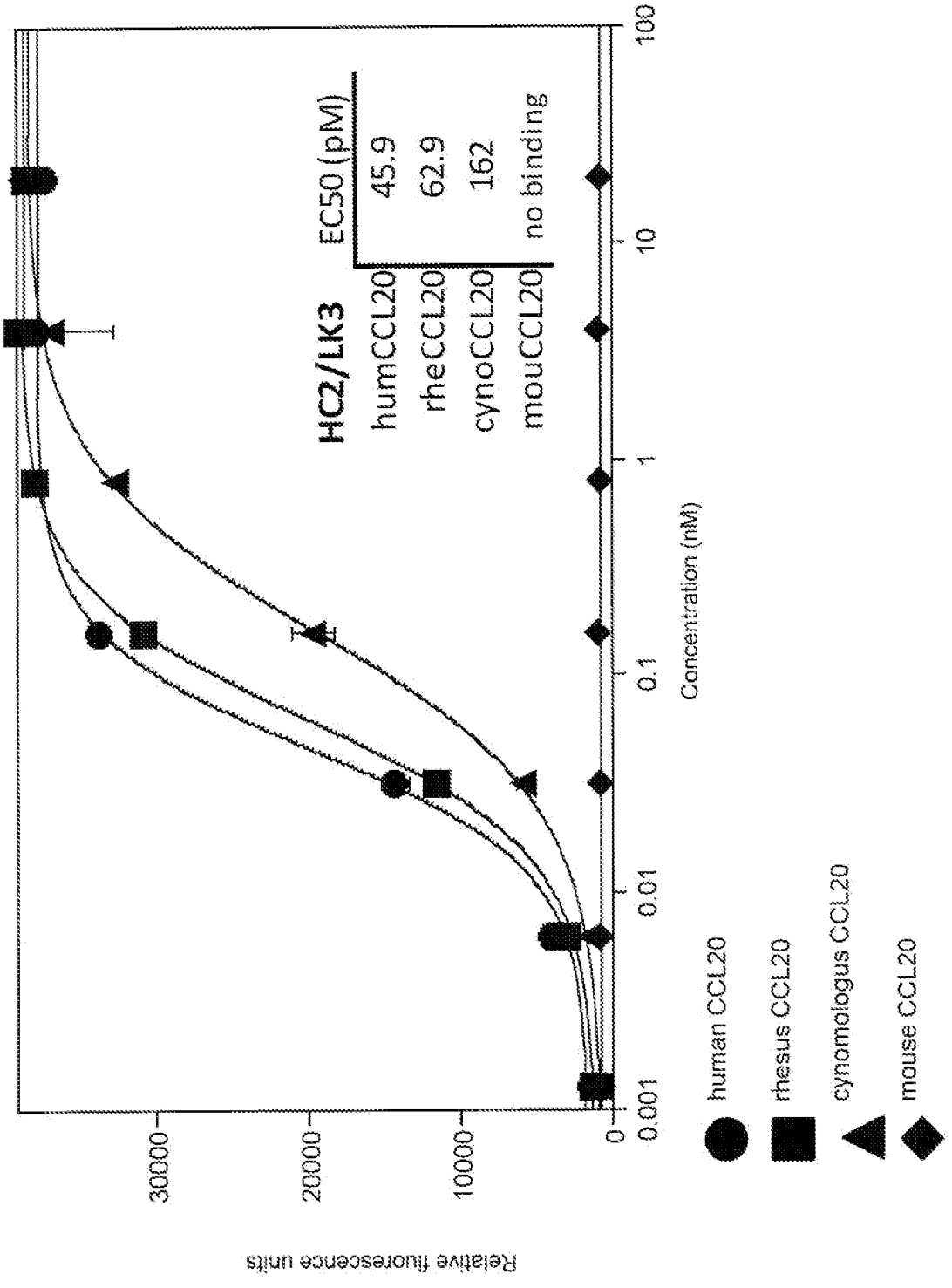

HC2/LK3
EC50 (pM)
humCCL20 45.9
rheCCL20 62.9
cynoCCL20 162
mouCCL20 no binding
Relative fluorescence units
30000
20000
10000
0
0.001
0.01
0.1
1
10
100
Concentration (nM)
human CCL20
rhesus CCL20
cynomologus CCL20
mouse CCL20

FIG. 16C

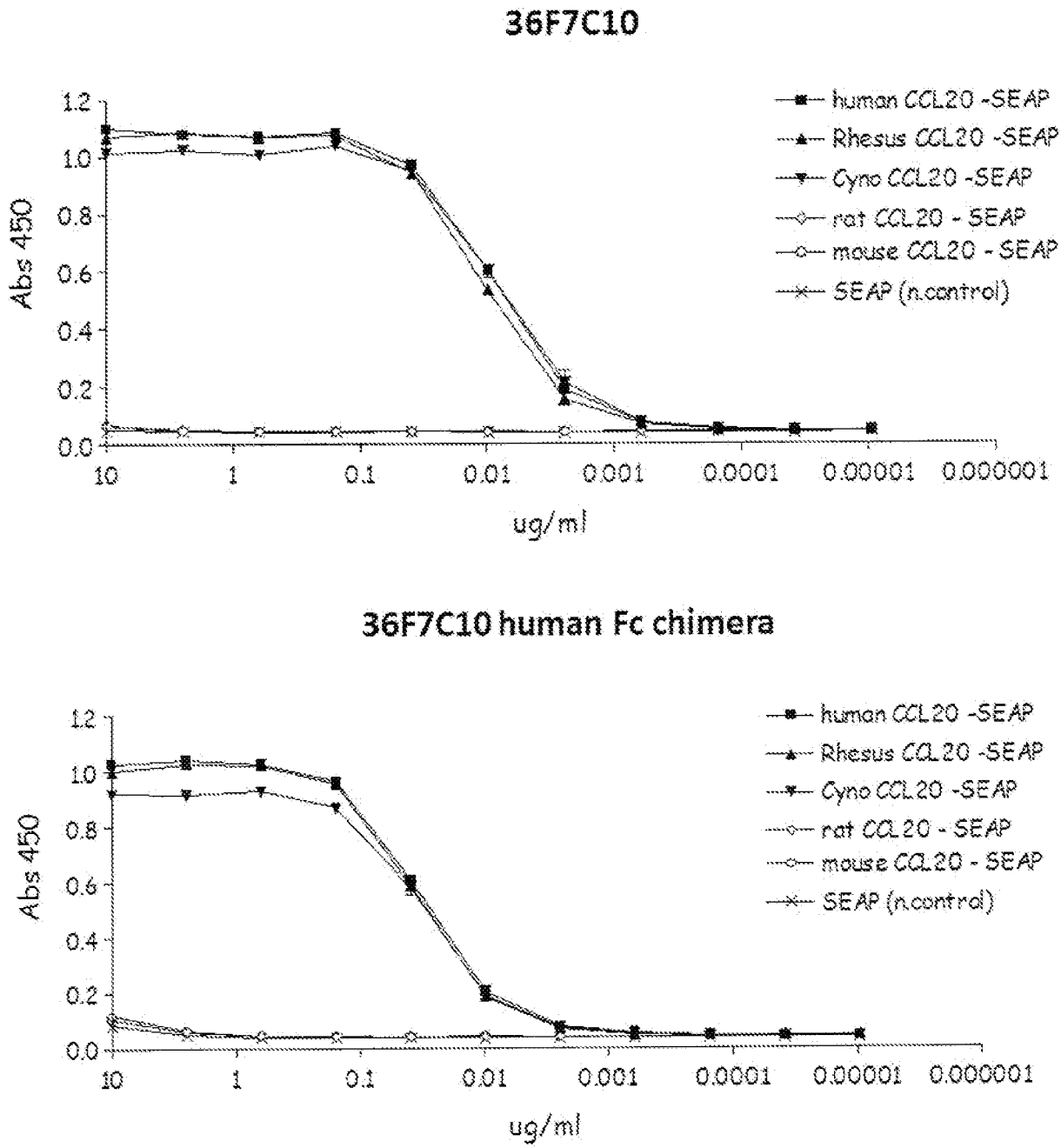

36F7C10
Abs 450
1.2
1.0
0.8
0.6
0.4
0.2
0.0
10
1
0.1
0.01
0.001
0.0001
0.00001
0.000001
ug/ml
human CCL20 -SEAP
Rhesus CCL20 -SEAP
Cyno CCL20 -SEAP
rat CCL20 - SEAP
mouse CCL20 - SEAP
SEAP (n.control)
36F7C10 human Fc chimera
Abs 450
1.2
1.0
0.8
0.6
0.4
0.2
0.0
10
1
0.1
0.01
0.001
0.0001
0.00001
0.000001
ug/ml
human CCL20 -SEAP
Rhesus CCL20 -SEAP
Cyno CCL20 -SEAP
rat CCL20 - SEAP
mouse CCL20 - SEAP
SEAP (n.control)

FIG. 17A

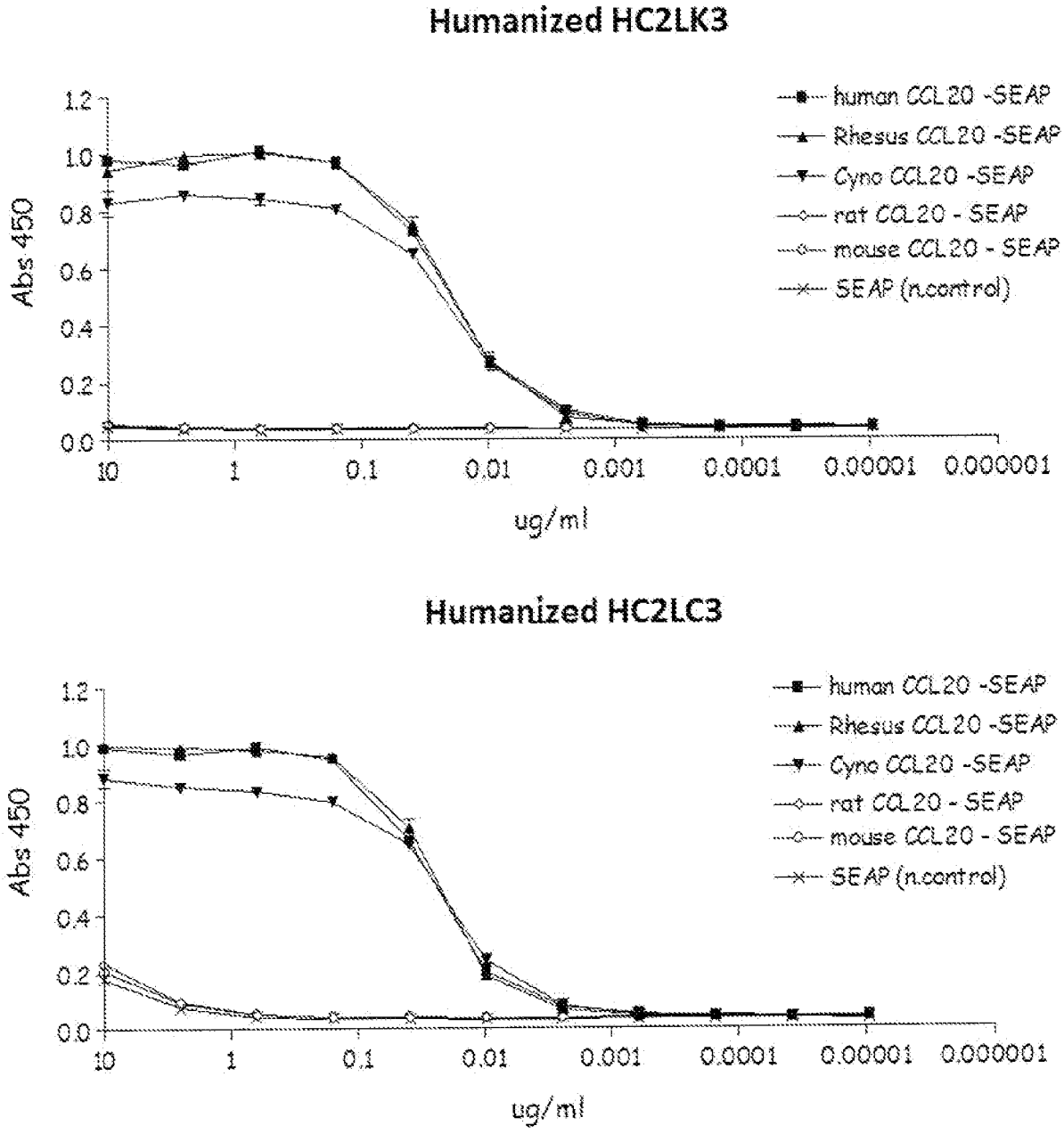

Humanized HC2LK3
Abs 450
1.2
1.0
0.8
0.6
0.4
0.2
0.0
10
1
0.1
0.01
0.001
0.0001
0.00001
0.000001
ug/ml
human CCL20 -SEAP
Rhesus CCL20 -SEAP
Cyno CCL20 -SEAP
rat CCL20 - SEAP
mouse CCL20 - SEAP
SEAP (n.control)
Humanized HC2LC3
Abs 450
1.2
1.0
0.8
0.6
0.4
0.2
0.0
10
1
0.1
0.01
0.001
0.0001
0.00001
0.000001
ug/ml
human CCL20 -SEAP
Rhesus CCL20 -SEAP
Cyno CCL20 -SEAP
rat CCL20 - SEAP
mouse CCL20 - SEAP
SEAP (n.control)

FIG. 17B

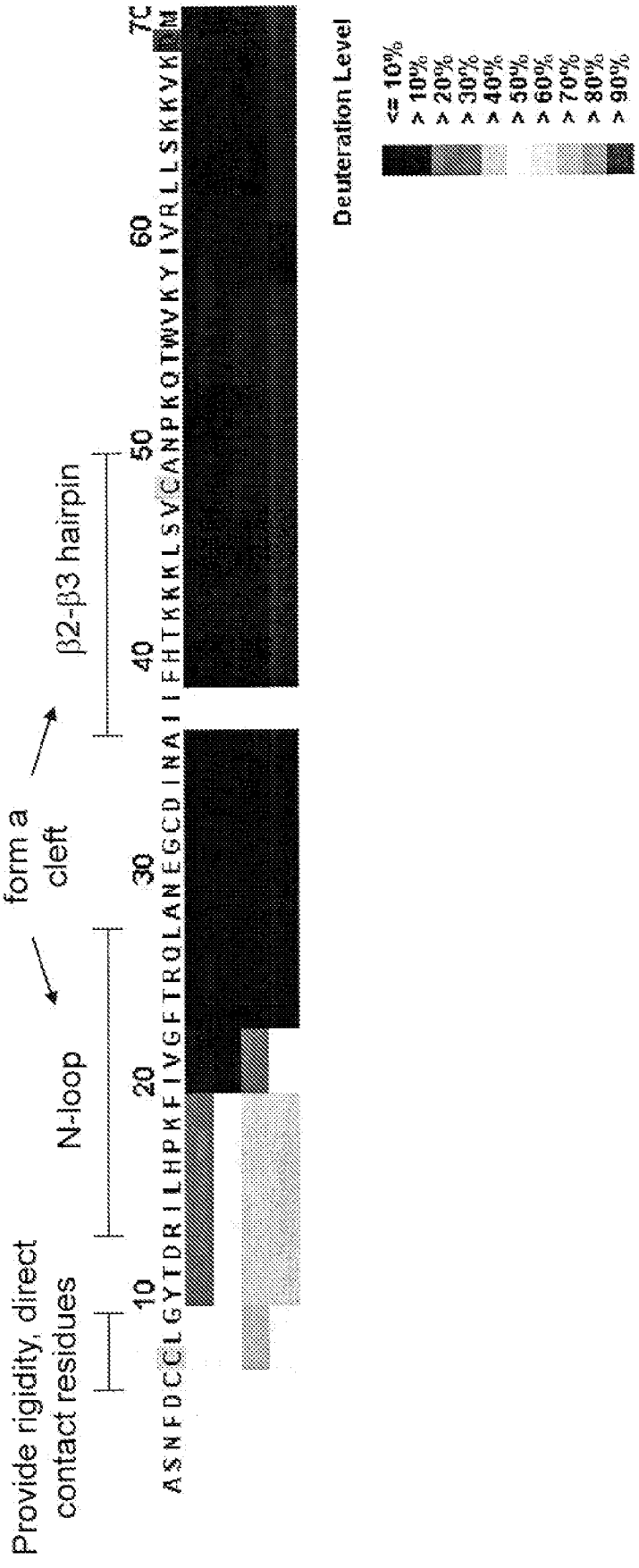

Deuteration Level
<= 10%
> 10%
> 20%
> 30%
> 40%
> 50%
> 60%
> 70%
> 80%
> 90%
Provide rigidity, direct contact residues
N-loop
form a cleft
β2-β3 hairpin
ASNFDCCLGYTDRILHPKFIVGFTROLANEGGCDINAIIFHTKKKLSVCANPKQTWVKYIVRLLSKKVK
10
20
30
40
50
60
7C

FIG. 18A

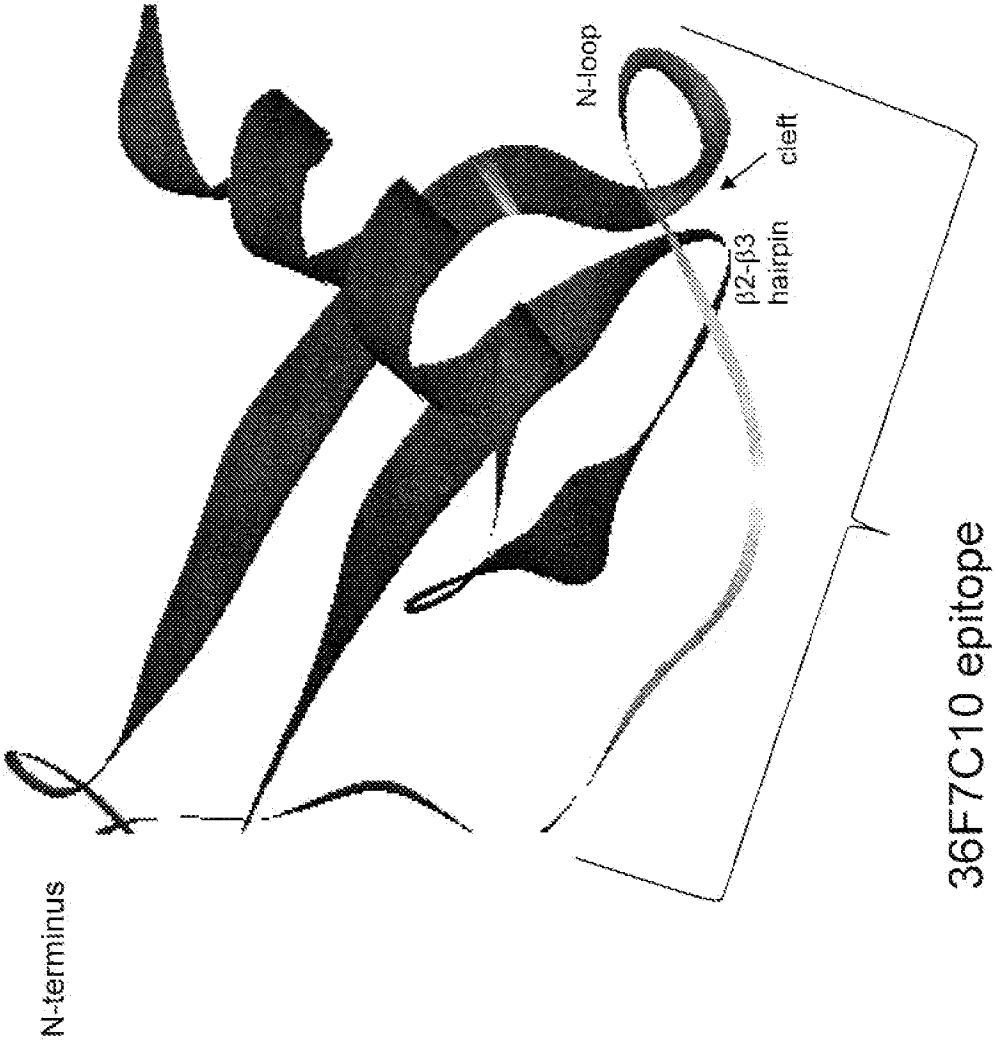
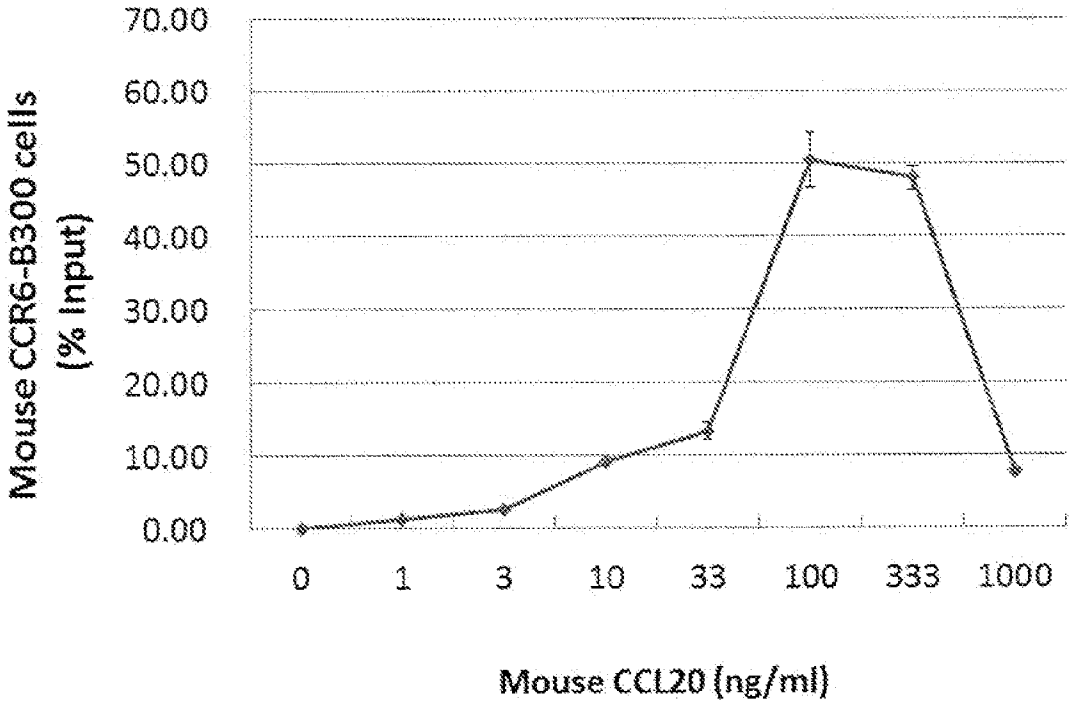


FIG. 18B


70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Mouse CCR6-B300 cells (% Input)
0
1
3
10
33
100
333
1000
Mouse CCL20 (ng/ml)

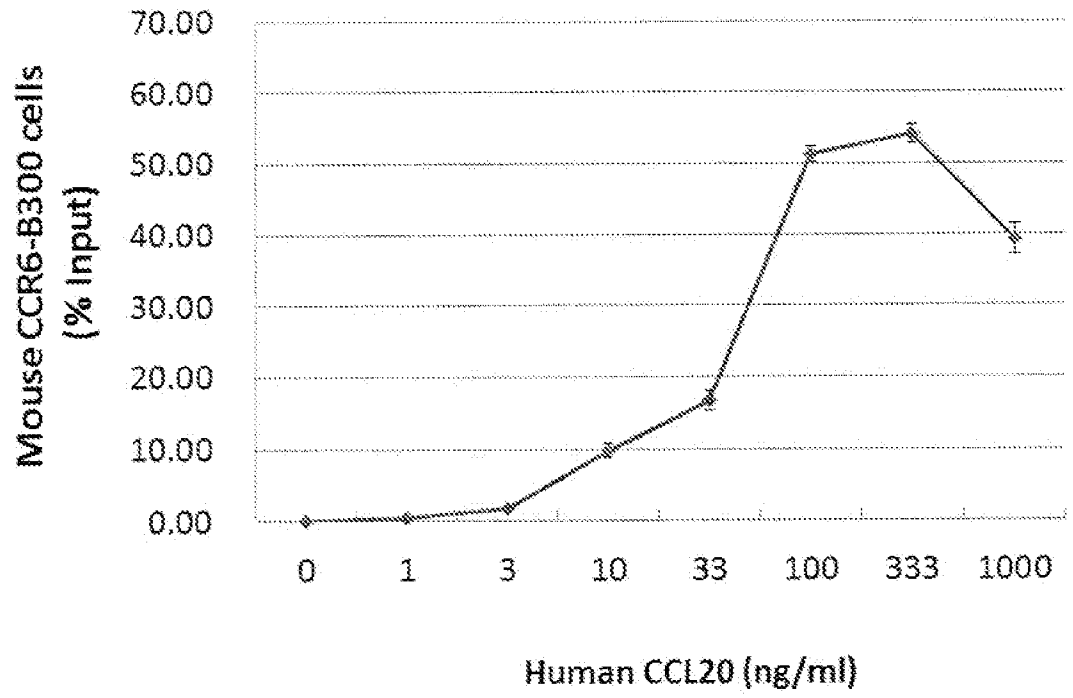

70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
Mouse CCR6-B300 cells (% Input)
0
1
3
10
33
100
333
1000
Human CCL20 (ng/ml)

FIG. 19

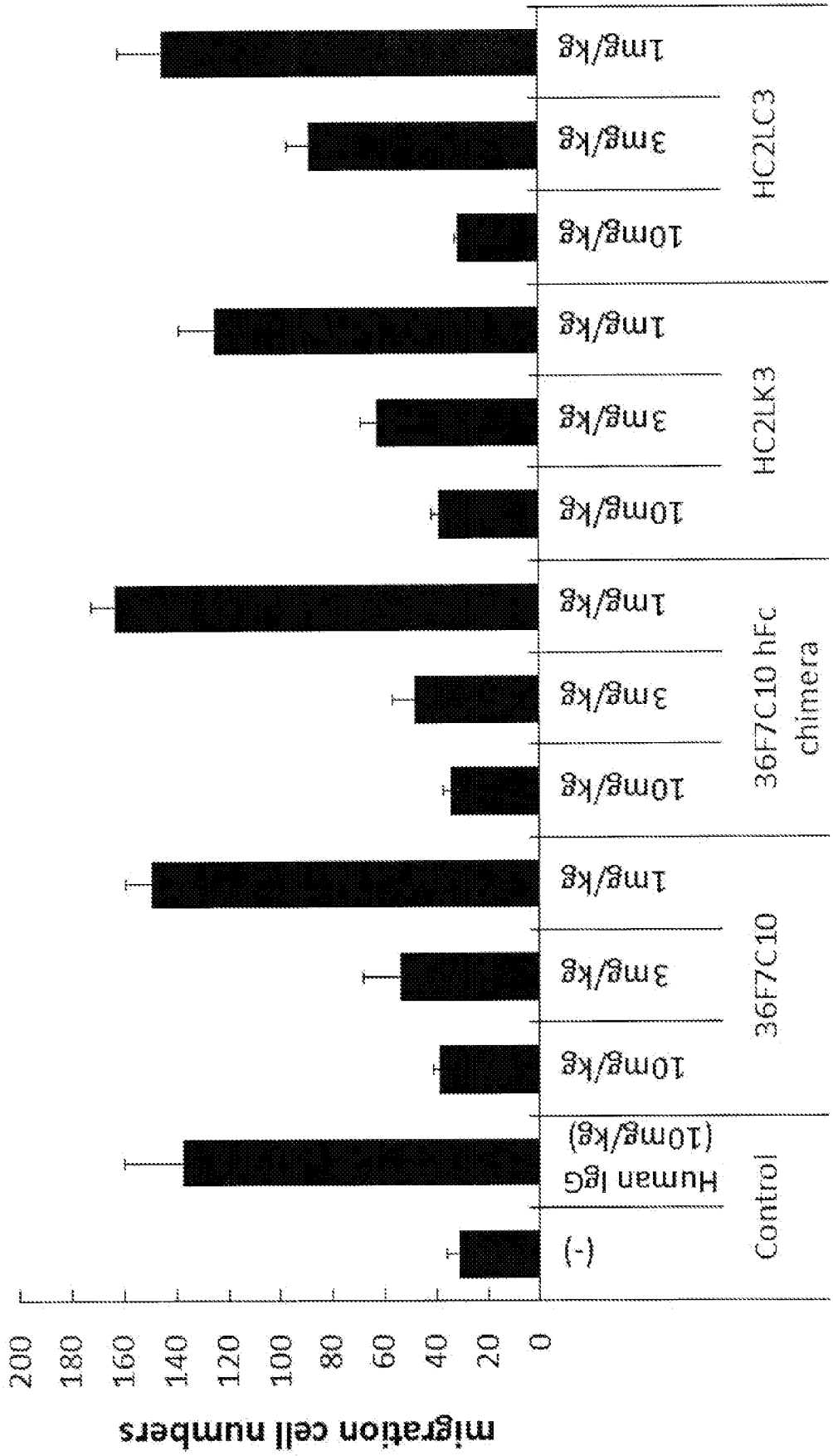

migration cell numbers
200
180
160
140
120
100
80
60
40
20
0
Control
(-)
Human IgG (10mg/kg)
36F7C10
10mg/kg
3mg/kg
1mg/kg
36F7C10 hFc chimera
10mg/kg
3mg/kg
1mg/kg
HC2LK3
10mg/kg
3mg/kg
1mg/kg
HC2LC3
10mg/kg
3mg/kg
1mg/kg

FIG. 20

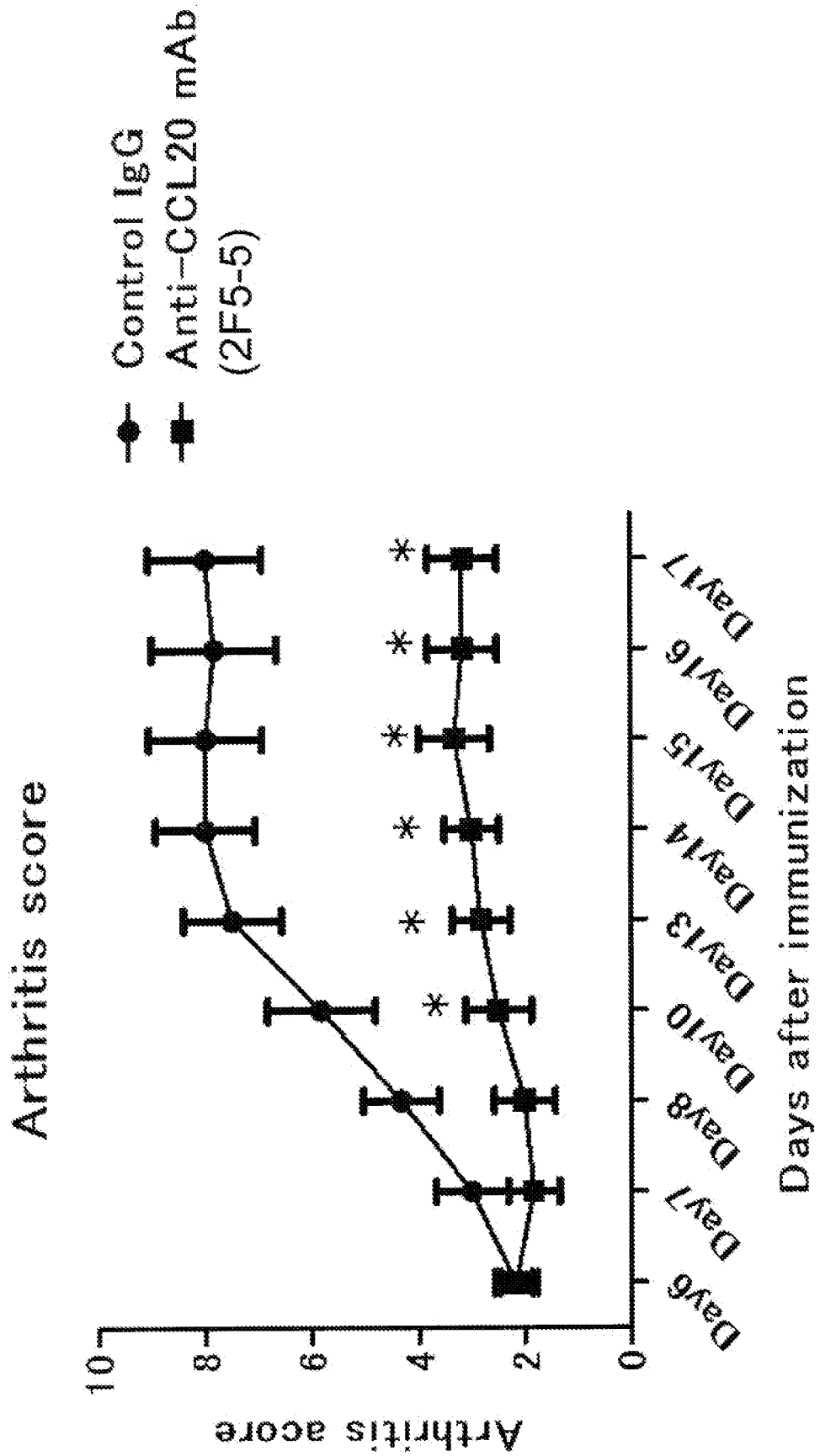

Control IgG
Anti-CCL20 mAb (2F5-5)
Arthritis score
Arthritis score
10
8
6
4
2
0
Day6
Day7
Day8
Day10
Day13
Day14
Day15
Day16
Day17
Days after immunization
*
*
*
*
*
*
*

FIG. 21

Control IgG	Day								
Animal	6	7	8	10	13	14	15	16	17
1	1	1	2	2	9	9	10	10	10
2	2	2	4	5	6	7	7	7	7
3	3	6	7	8	8	8	8	8	8
4	2	3	3	5	5	6	5	4	5
5	3	3	5	9	11	12	12	12	12
6	2	3	5	6	6	6	6	6	6
Average	2.2	3.0	4.3	5.8	7.5	8.0	8.0	7.8	8.0
ST DEV	0.3	0.7	0.7	1.0	0.9	0.9	1.1	1.2	1.1
2F5-5									
Animal									
1	3	3	3	4	5	5	6	6	6
2	3	2	2	2	2	3	3	3	3
3	3	3	4	4	3	3	4	3	3
4	1	1	2	3	3	3	3	3	3
5	2	2	1	2	3	3	3	3	3
6	1	0	0	0	1	1	1	1	1
Average	2.2	1.8	2.0	2.5	2.8	3.0	3.3	3.2	3.2
ST DEV	0.4	0.5	0.6	0.6	0.5	0.5	0.7	0.7	0.7

FIG. 22

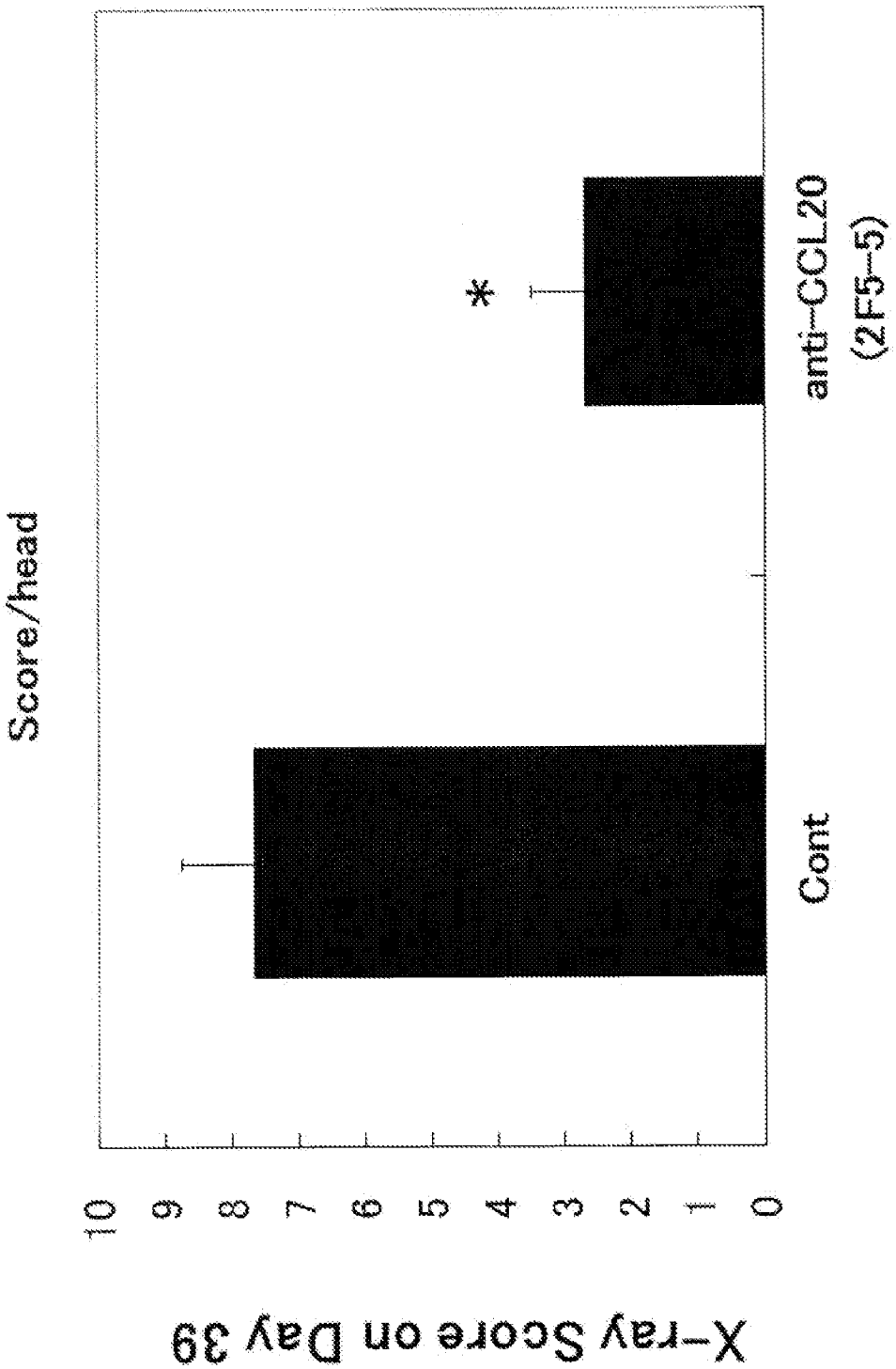

X-ray Score on Day 39
Score/head
10
9
8
7
6
5
4
3
2
1
0
Cont
anti-CCL20
(2F5-5)
*

FIG. 23

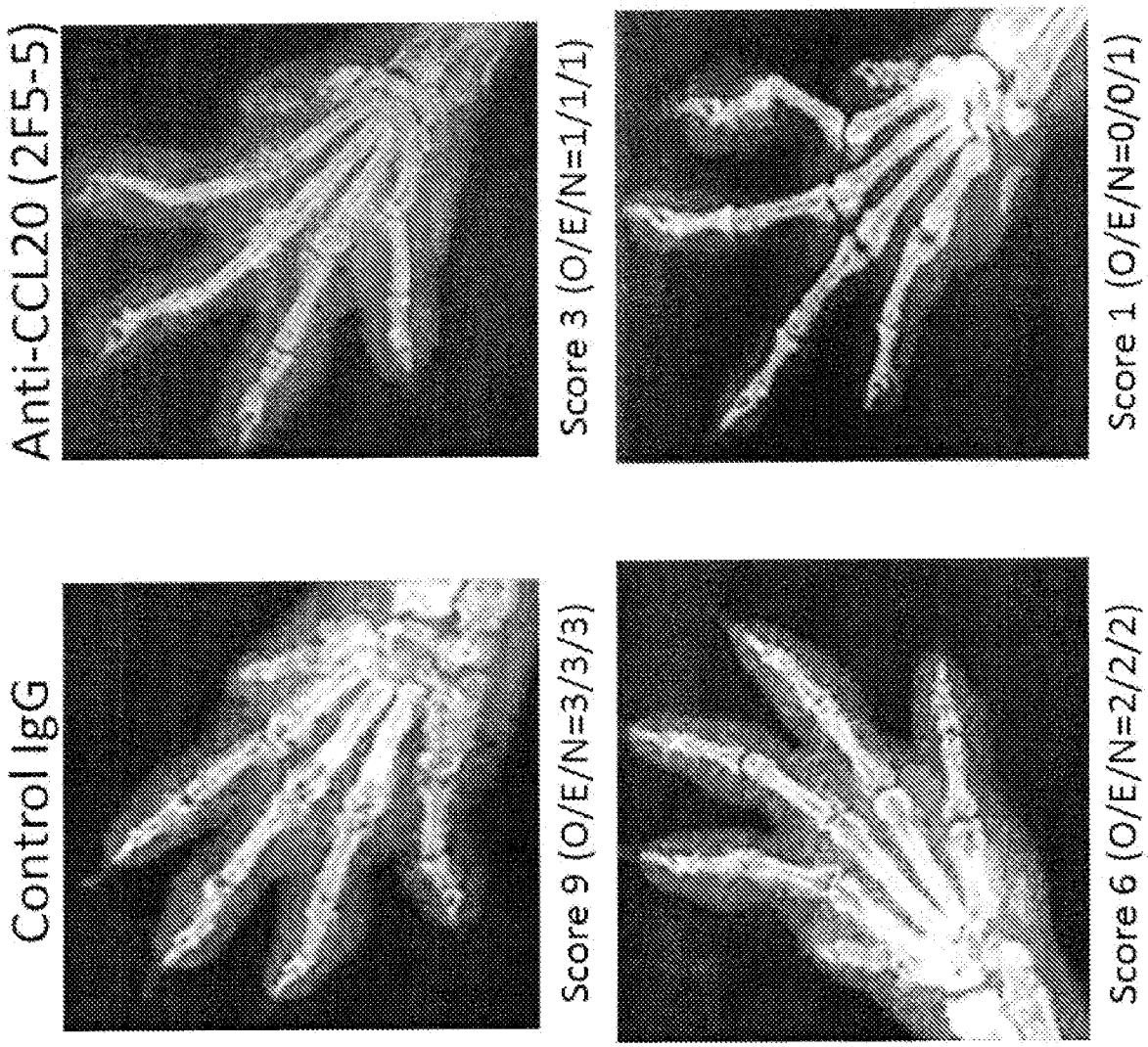

Control IgG
Anti-CCL20 (2F5-5)
Score 9 (O/E/N=3/3/3)
Score 3 (O/E/N=1/1/1)
Score 6 (O/E/N=2/2/2)
Score 1 (O/E/N=0/0/1)

FIG. 24

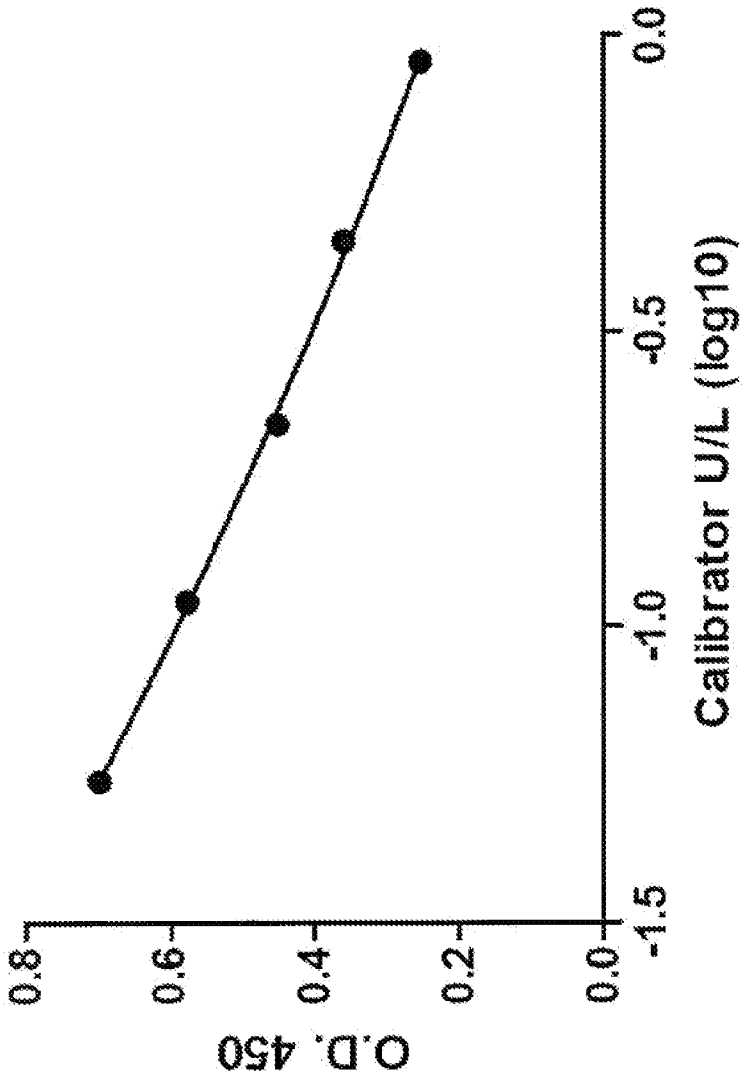


FIG. 25A

				CIA with IFA (Incomplete Freund adjuvant)				CIA with CFA (Complete Freund adjuvant)			
	1	2	3	4	5	6	7	8	9	10	11
A	1	normal_1		Hamster control IgG		anti-CCL20 (2F5-5)		Hamster control IgG		anti-CCL20 (2F5-5)	
B	2	normal_2		Hamster control IgG		anti-CCL20 (2F5-5)		Hamster control IgG		anti-CCL20 (2F5-5)	
C	3	normal_3		Hamster control IgG		anti-CCL20 (2F5-5)		Hamster control IgG		anti-CCL20 (2F5-5)	
D	4	normal_4		Hamster control IgG		anti-CCL20 (2F5-5)		Hamster control IgG		anti-CCL20 (2F5-5)	
E	5	normal_5		Hamster control IgG		anti-CCL20 (2F5-5)		Hamster control IgG		anti-CCL20 (2F5-5)	
F	6	normal_6		Hamster control IgG		anti-CCL20 (2F5-5)		Hamster control IgG		anti-CCL20 (2F5-5)	
G	Calibrator U/L	0	0.9	0.45	0.22	0.11	0.055	blank	blank	blank	blank
H								blank	blank	blank	blank

	1	2	3	4	5	6	7	8	9	10	11
A	1	0.669	0.637	0.504	0.483	0.558	0.585	0.508	0.512	0.552	0.566
B	2	0.577	0.559	0.493	0.468	0.541	0.552	0.442	0.445	0.614	0.618
C	3	0.592	0.550	0.559	0.484	0.551	0.566	0.475	0.482	0.520	0.549
D	4	0.551	0.537	0.460	0.432	0.512	0.523	0.437	0.461	0.513	0.524
E	5	0.600	0.557	0.515	0.480	0.481	0.487	0.529	0.518	0.554	0.581
F	6	0.589	0.577	0.431	0.400	0.518	0.538	0.443	0.456	0.537	0.561
G	Calibrator OD450	0.800	0.250	0.360	0.450	0.575	0.692	0.848	0.840	0.855	0.897
H		0.807	0.257	0.361	0.456	0.581	0.706	0.873	0.890	0.876	0.941

OD450 values were obtained by regular colorimetric assay

FIG. 25B

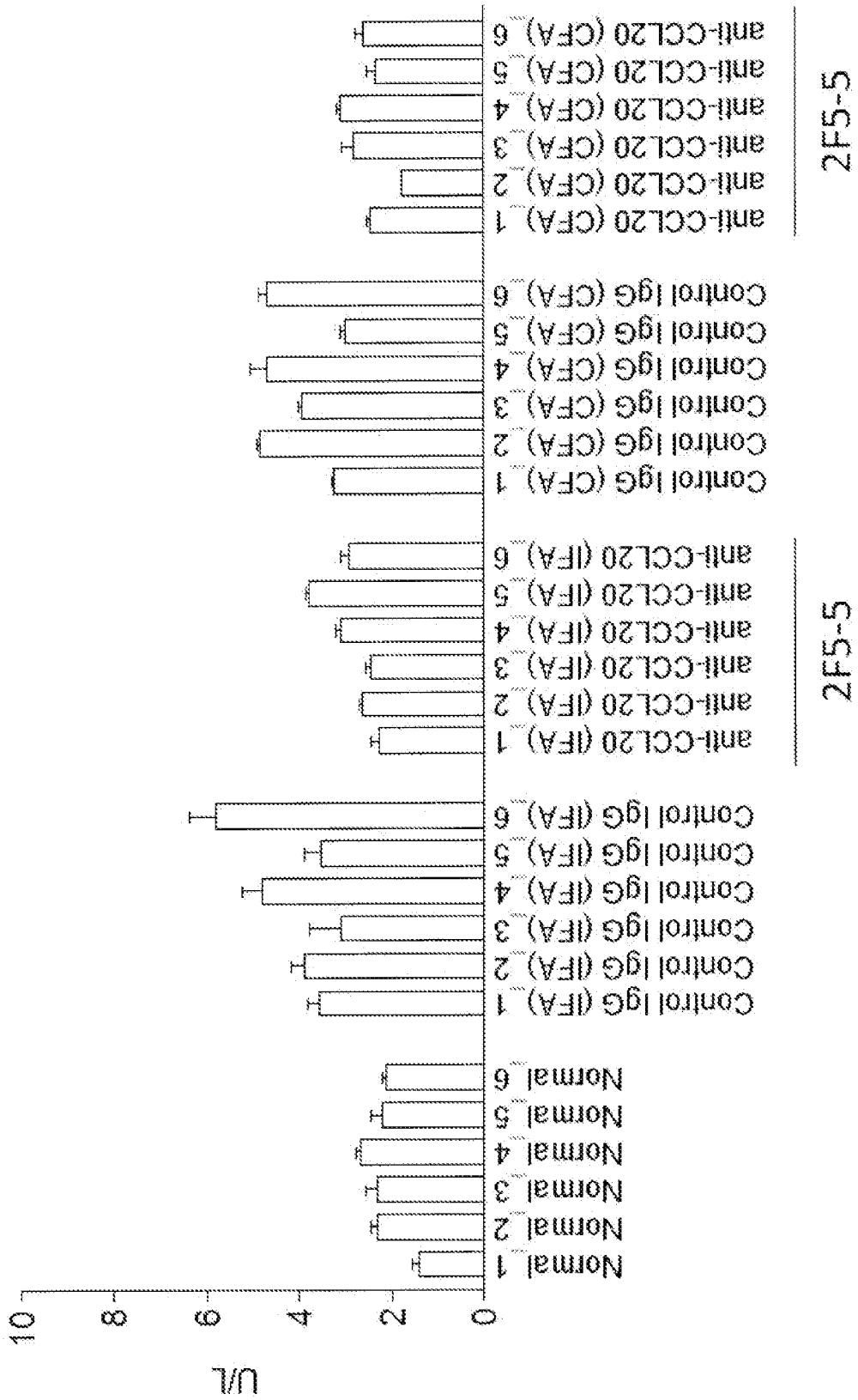

U/L
10
8
6
4
2
0
Normal_1
Normal_2
Normal_3
Normal_4
Normal_5
Normal_6
Control IgG (IFA)_1
Control IgG (IFA)_2
Control IgG (IFA)_3
Control IgG (IFA)_4
Control IgG (IFA)_5
Control IgG (IFA)_6
anti-CCL20 (IFA)_1
anti-CCL20 (IFA)_2
anti-CCL20 (IFA)_3
anti-CCL20 (IFA)_4
anti-CCL20 (IFA)_5
anti-CCL20 (IFA)_6
2F5-5
Control IgG (CFA)_1
Control IgG (CFA)_2
Control IgG (CFA)_3
Control IgG (CFA)_4
Control IgG (CFA)_5
Control IgG (CFA)_6
anti-CCL20 (CFA)_1
anti-CCL20 (CFA)_2
anti-CCL20 (CFA)_3
anti-CCL20 (CFA)_4
anti-CCL20 (CFA)_5
anti-CCL20 (CFA)_6
2F5-5

FIG. 25C

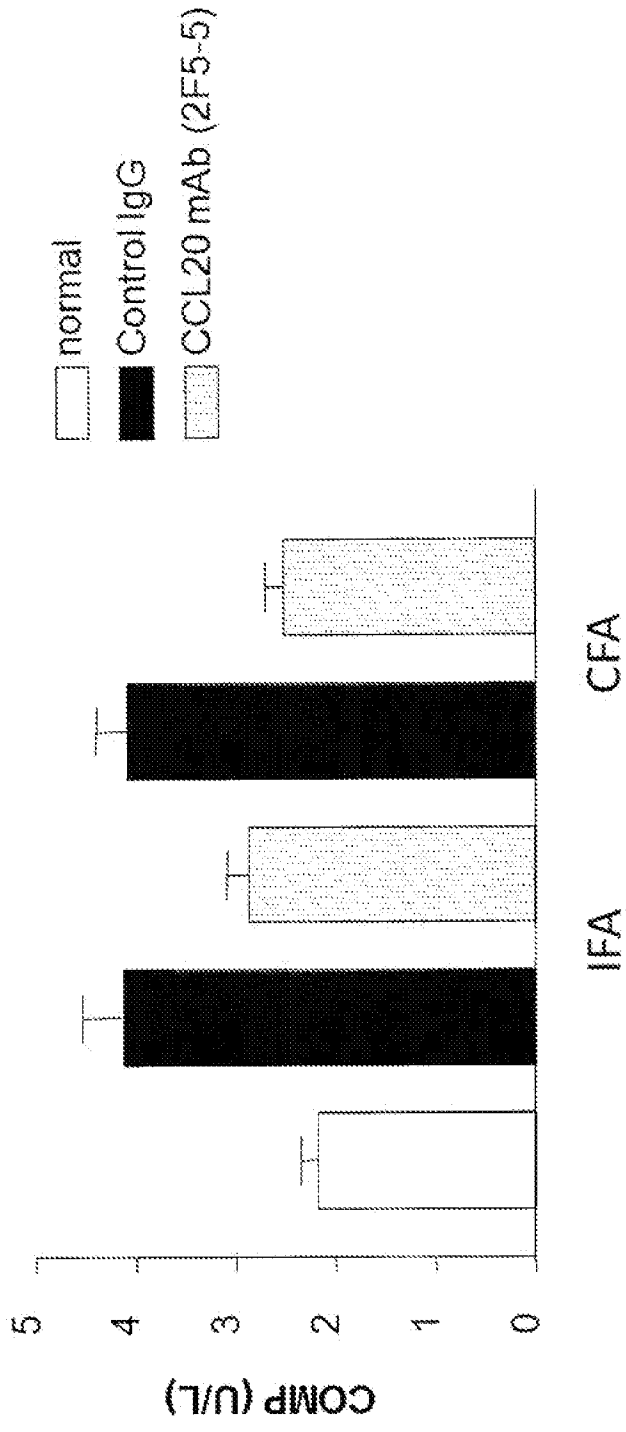


FIG. 26

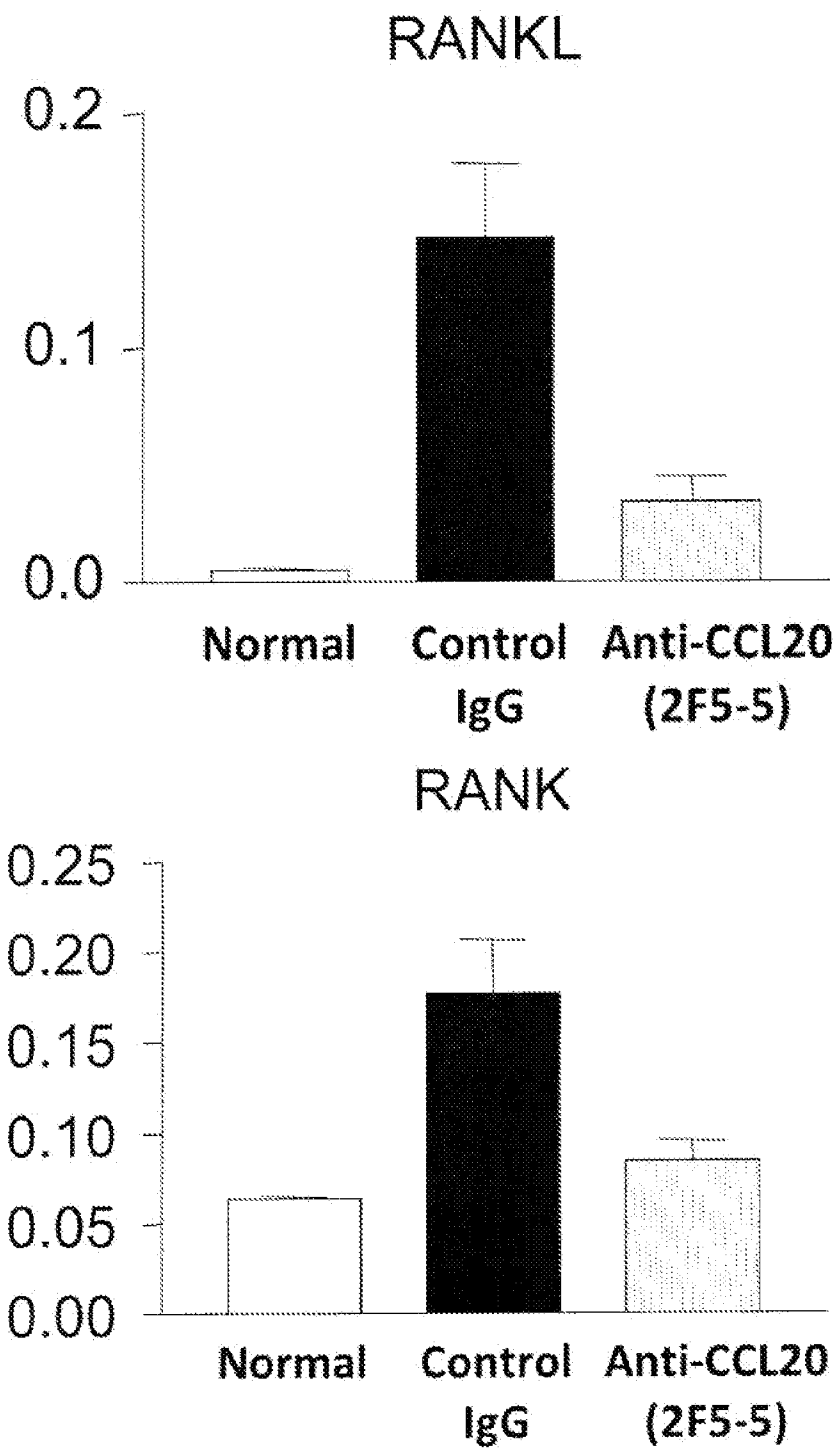

RANKL
0.2
0.1
0.0
Normal
Control IgG
Anti-CCL20 (2F5-5)
RANK
0.25
0.20
0.15
0.10
0.05
0.00
Normal
Control IgG
Anti-CCL20 (2F5-5)

FIG. 27A

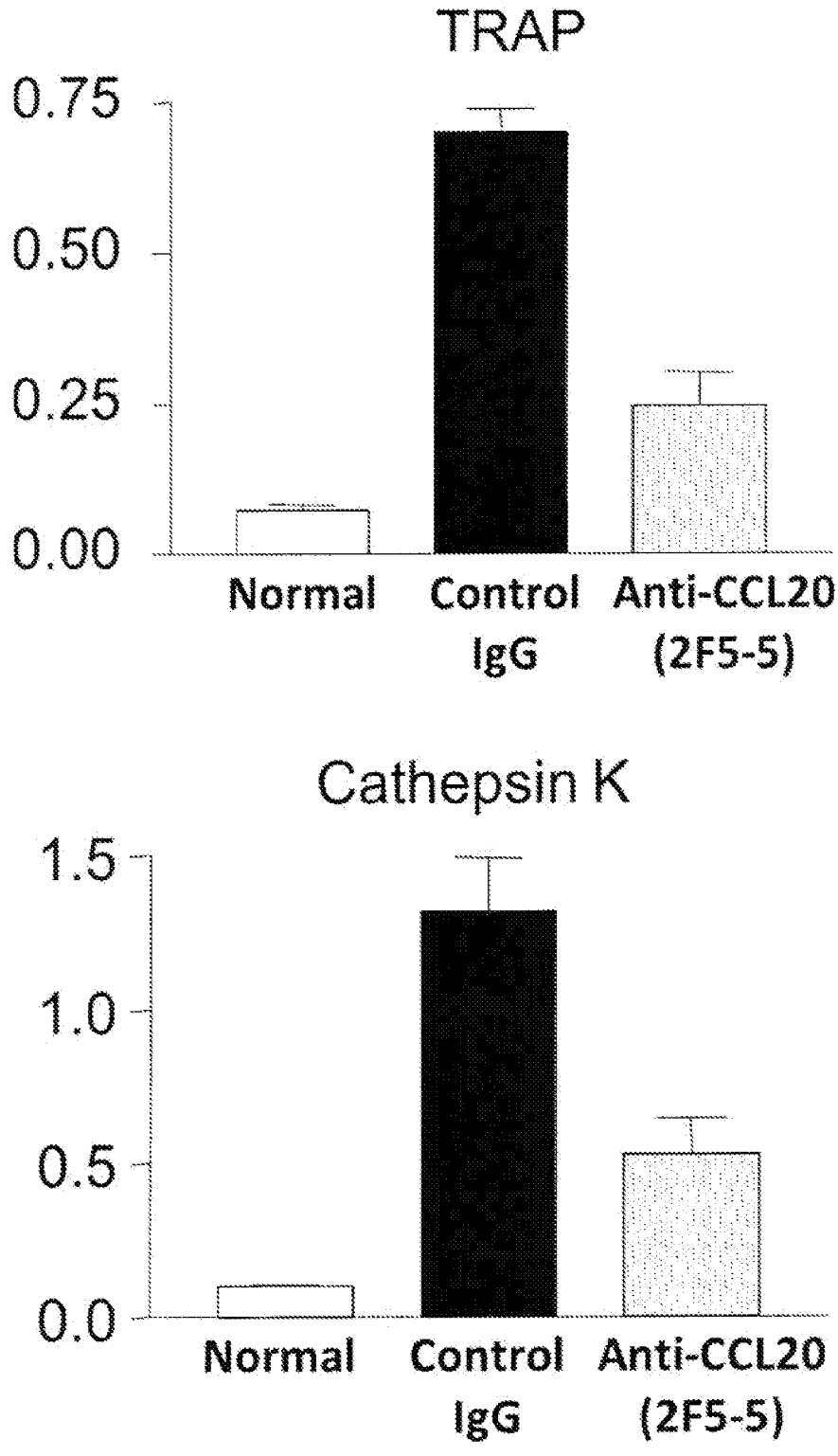

TRAP
0.75
0.50
0.25
0.00
Normal
Control IgG
Anti-CCL20 (2F5-5)
Cathepsin K
1.5
1.0
0.5
0.0
Normal
Control IgG
Anti-CCL20 (2F5-5)

FIG. 27B

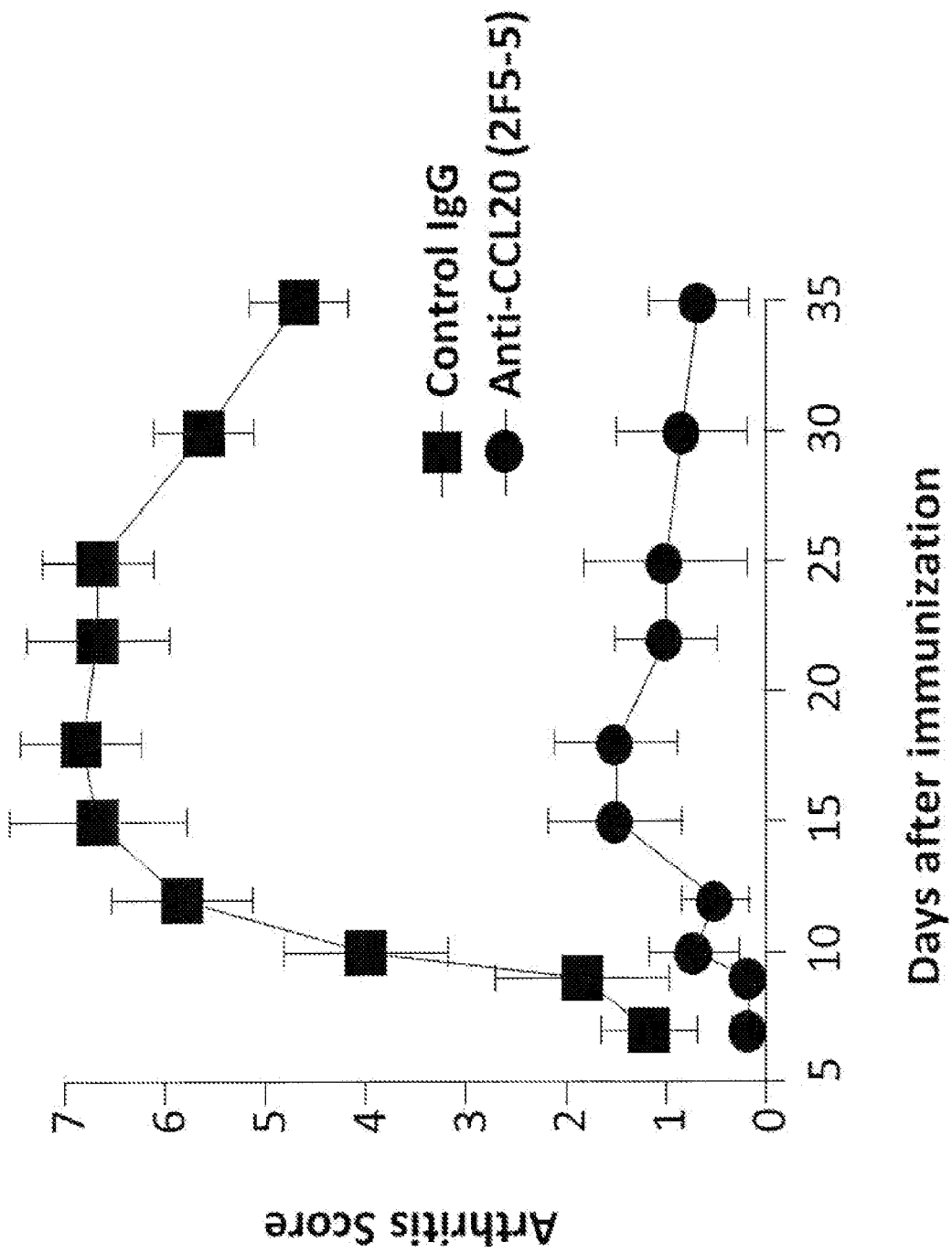

Arthritis Score
0
1
2
3
4
5
6
7
5
10
15
20
25
30
35
Days after immunization
Control IgG
Anti-CCL20 (2F5-5)

FIG. 28

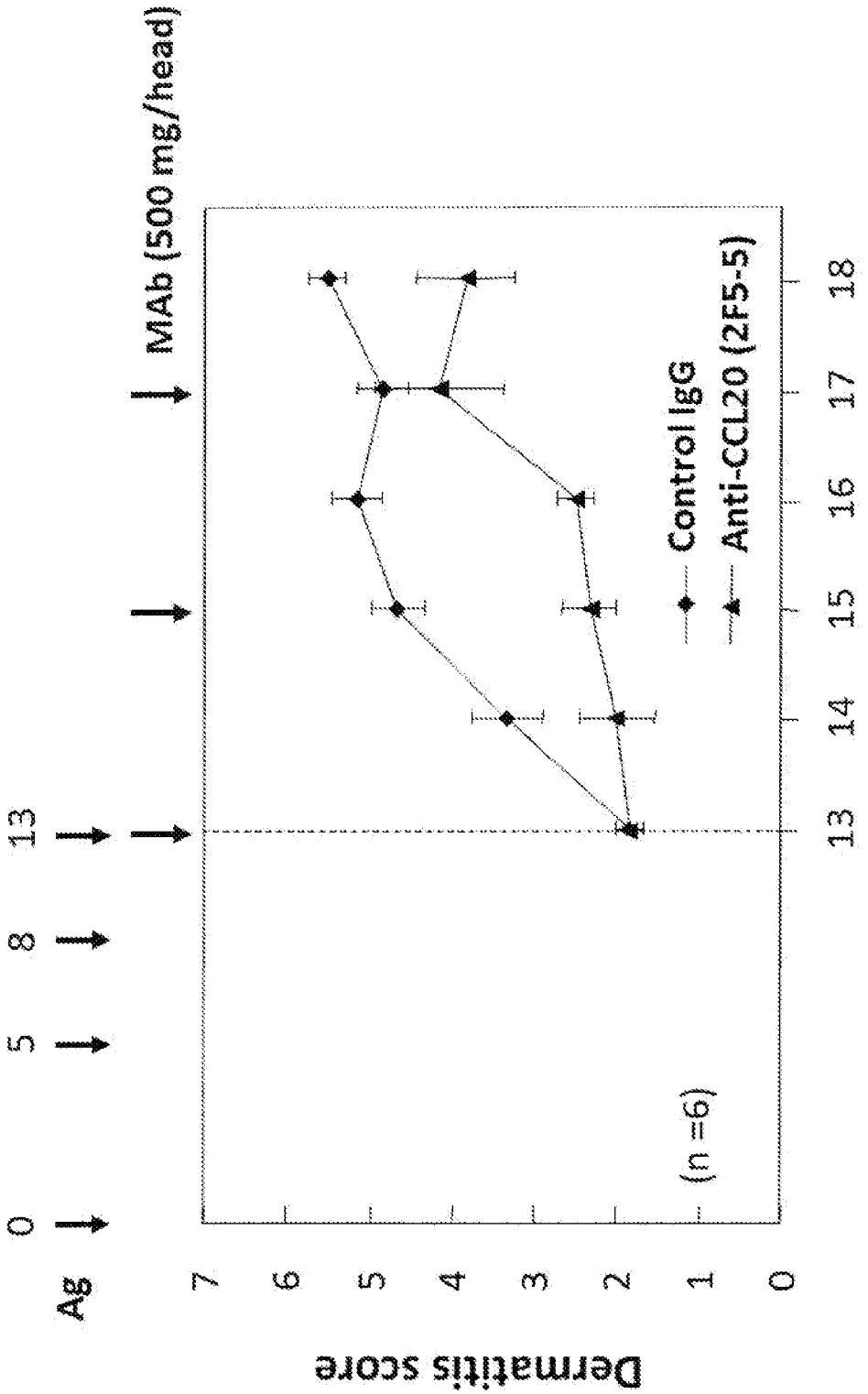

Ag
0
5
8
13
MAb (500 mg/head)
Dermatitis score
0
1
2
3
4
5
6
7
(n =6)
Control IgG
Anti-CCL20 (2F5-5)
13
14
15
16
17
18
Day

FIG. 29

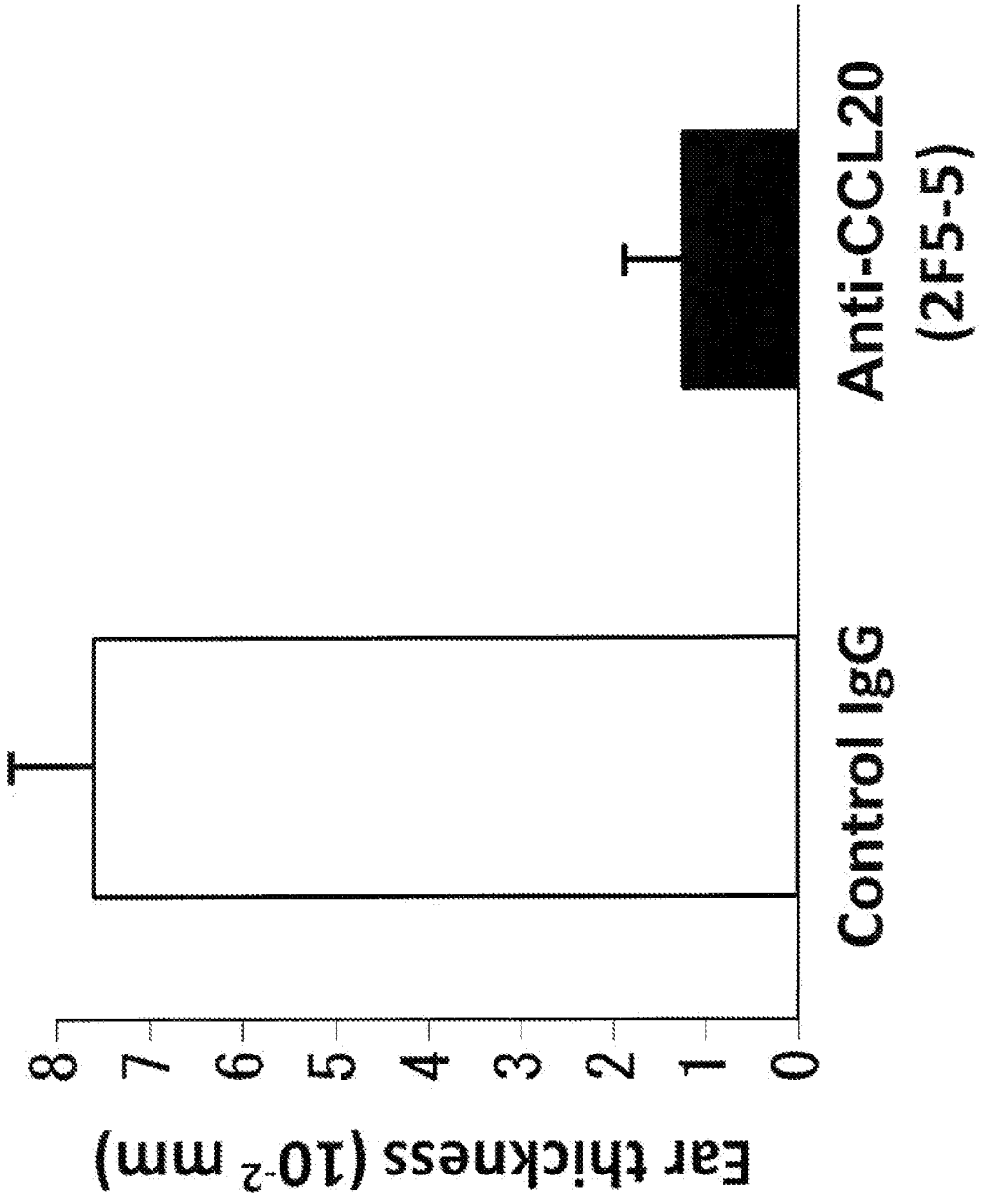


FIG. 30