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(71) **Applicant** *(for all designated States except US):* **NOVIMMUNE** S.A. *[CWCH];* 14 Ch. Des AuIx, CH-1228 Plan-les-ouates (CH).

(72) **Inventors; and**

(75) **Inventors/Applicants** *(for US only):* **FISCHER, Nicolas** *[CWCH];* 16, Ch. Des Ouches, CH-1203 Geneva (CH). **KOSCO-VILBOIS, Marie** [US/FR]; Le Cret, F-74270 Minzier (FR). **MACH, Francois** [CH/CH]; 24 Chemin Petrey, CH-1222 Vesenaz (CH).

(74) **Agents: ELRIFI, Ivor, R.** et al.; Mintz, Levin, Cohn, Ferris, Glovsky And Popeo PC, One Financial Center, Boston, MA 021 11 (US).

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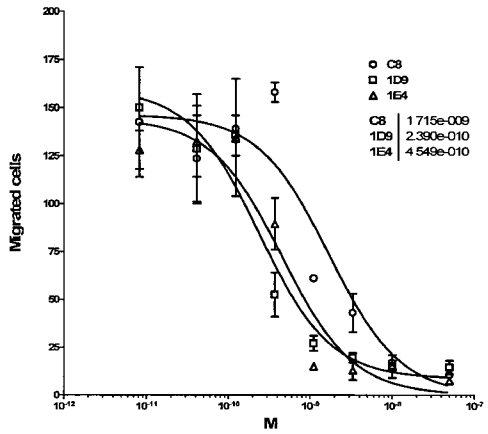
(54) **Title:** ANTI-RANTES ANTIBODIES AND METHODS OF USE THEREOF

Fig. 1 A



(57) **Abstract:** The invention relates to fully human monoclonal antibodies, and fragments thereof, that bind to the chemokine Regulated upon Activation, Normal T-cell Expressed, and Secreted (RANTES, CCL5), thereby modulating the interaction between RANTES and one of more of its receptors, such as, e.g., CCRl, CCR3, CCR4 and CCR5, and/or modulating the biological activities of RANTES. The invention also relates to the use of these or any anti-RANTES antibodies in the prevention or treatment of immune-related disorders and in the amelioration of one or more symptoms associated with an immune-related disorder.

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Anti-RANTES Antibodies and Methods of Use Thereof

RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application No. 60/963,271, filed August 2, 2007, the contents of which are hereby incorporated by reference in their entirety.

FIELD OF THE INVENTION

This invention relates generally to fully human monoclonal antibodies that bind to RANTES (Regulated upon Activation, Normal T-cell Expressed, and Secreted) as well as to methods for use thereof.

BACKGROUND OF THE INVENTION

RANTES (Regulated upon Activation, Normal T-cell Expressed, and Secreted, CCL5) is a chemokine that is a chemoattractant for eosinophils, monocytes, and lymphocytes.

Elevated levels of RANTES expression has been implicated in a variety of diseases and disorders. Accordingly, there exists a need for therapies that target RANTES activity.

SUMMARY OF THE INVENTION

The present invention provides monoclonal antibodies, such as fully human monoclonal antibodies, that specifically bind Regulated upon Activation, Normal T-cell Expressed, and Secreted (RANTES, also referred to herein as CCL5). Exemplary monoclonal antibodies include the antibodies referred to herein as 1D9, 1E4, C8, 3E7, 4D8, 5El, 6A8, 7B5, CGl 1, BGl 1, A9, E6, H6, G2, ElO, ClO, 2Dl, A5, Hl 1, Dl and/or E7. Alternatively, the monoclonal antibody is an antibody that binds to the same epitope as 1D9, 1E4, C8, 3E7, 4D8, 5El, 6A8, 7B5, CGl 1, BGl 1, A9, E6, H6, G2, ElO, ClO, 2Dl, A5, Hl 1, Dl and/or E7. The antibodies are respectively referred to herein as huRANTES antibodies. huRANTES antibodies include fully human monoclonal antibodies, as well as humanized monoclonal antibodies and chimeric antibodies.

huRANTES antibodies of the invention also include antibodies that include a heavy chain variable amino acid sequence that is at least 90%, 92%, 95%, 97%, 98%, 99% or more identical the amino acid sequence of SEQ ID NO: 2, 18, 22, 38, 48, 52, 56, 60, 68, 84, 100, 116, 132, 148, 164, 180, 200, 216, 232, or 248 and/or a light chain variable amino acid that is at least 90%, 92%, 95%, 97%, 98%, 99% or more identical the amino acid sequence of SEQ ID NO: 4, 24, 40, 62, 70, 86, 102, 118, 134, 150, 166, 182, 196, 202, 218, 234, or 250.

Preferably, the three heavy chain complementarity determining regions (CDRs) include an amino acid sequence at least 90%, 92%, 95%, 97%, 98%, 99% or more identical to each of: (i) a VH CDRl sequence selected from SEQ ID NO: 8, 28, 44, 74, 90, 106, 122, 138, 154, and 222; (ii) a VH CDR2 sequence selected from SEQ ID NO: 9, 29, 45, 75, 91, 107, 123, 139, 155, 207, 223, 239, and 255; (iii) a VH CDR3 sequence selected from SEQ ID NOs: 10, 20, 30, 46, 50, 54, 58, 64, 76, 92, 108, 124, 140, 156, 188, 208, 224, 240 and 256; and a light chain with three CDR that include an amino acid sequence at least 90%, 92%, 95%, 97%, 98%, 99% or more identical to each of (iv) a VL CDRl sequence selected from SEQ ID NO: 14, 34, 80, 96, 112, 128, 144, 160, 176, 192, 212, 228, 244 and 260; (v) a VL CDR2 sequence selected from SEQ ID NO: 15, 35, 97, 113, 129, 145, 161, 177, 193, 213, 229, 245 and 261; and (vi) a VL CDR3 sequence selected from SEQ ID NO: 16, 36, 66, 82, 98, 114, 130, 146, 162, 178, 194, 198, 214, 230, 246 and 262.

Preferably, the huRANTES antibodies are formatted in an IgG isotype. More preferably, the huRANTES antibodies are formatted in an IgGl isotype.

Exemplary IgGl -formatted antibody are the IgG 1-formatted 1D9, 1E4 and C8 antibodies comprising the heavy chain sequence and light chain sequence shown below, and the CDR sequences are shown in boxes:

> 1D9 Heavy chain amino acid sequence (SEQ ID NO: 263)

QVQLVQSGAEVKKPGASVKVSCKVSGYTLT[EFAMH]WVRQAPGKGLEWMGG[FVPEDGETIYA
[QKFQG]RVTMTEDTSTDTAYMELSSLRSEDTAVYYCAT[DPLYTPGLEP]WGQGTTVTVSSAST
KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS
LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFL
FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV
SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC
SVMHEALHNHYTQKSLSLSPGK

> 1D9 Light chain amino acid sequence (SEQ ID NO: 264)

SYVLTQPPSVSVAPGQTARITCGGNNIESKSVHWYQQKPGQAPVLVVYDDSDRPSGIPERF
SGSNSGNTATLTISRVEAGDEADYYCQVWDSNTDHWVFGGGTKLTVLGQPKAAPSVTLFPP
SSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLT
PEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

> 1E4 Heavy chain amino acid sequence (SEQ ID NO:238)

QVQLVQSGAEVKKPGASVKVSCKVSGYTLTEFAMHWVRQAPGKGLEWMGGFVPEDGETIYA
QKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATDPLYEGSFSVWGQGTTVTVSSAST
KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS
LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFL
FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV
SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC
SVMHEALHNHYTQKSLSLSPGK

> 1E4 Light chain amino acid sequence (SEQ ID NO:254)

SYVLTQPPSVSVAPGQTARITCGGNNIESKSVHWYQQKPGQAPVLVVYDDSDRPSGIPERF
SGSNSGNTATLTISRVEAGDEADYYCQVWDSNTDHWVFGGGTKLTVLGQPKAAPSVTLFPP
SSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLT
PEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

> C8 Heavy chain amino acid sequence (SEQ ID NO: 186)

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYYA
DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARETFPHYYYYYMDVWGRGTLVTVSS
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV
FSCSVMHEALHNHYTQKSLSLSPGK

> C8 Light chain amino acid sequence (SEQ ID NO: 187)

SYVLTQPPSVSVAPGQTARITCEGDDTDIGTVNWYQQKPGQAPVLVISEDGYRPSGIPERF
SGSNSGNTATLTISRVEAGDEADYYCQFWDVDSDHPVFGGGTQLTVLGQPKAAPSVTLFPP
SSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLT
PEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

The closest germline for the huRANTES antibodies described herein are shown below in Table 1:

Table 1. Closest germlines for the huRANTES antibodies. Antibodies marked in italic were derived by an affinity maturation process from antibody 2Dl (Lower part of the table).

Clone ID	VH dp number	VL dp number
CG11	Vh1_DP-3_(1-f)	Vlambda1_DPL8_(1e)
BG11	Vh1_DP-5_(1-24)	Vlambda3_DPL16_(3l
A9	Vh3_DP-47_(3-23)	Vlambda6_6a
E6	Vh1_DP-5_(1-24)	Vlambda6_6a
H6	Vh1_DP-5_(1-24)	Vlambda1_DPL8_(1e)
G2	Vh1_DP-5_(1-24)	Vlambda2_DPL11_(2a2)
E10	Vh3_DP-46_(3-30.3)	Vlambda3_3h
C10	Vh3_DP-47_(3-23)	Vlambda3_3h
2D1	Vh1_DP-5_(1-24)	Vlambda3_3h
A5	Vh1_DP-5_(1-24)	Vlambda3_3h
H11	Vh1_DP-10_(1-69)	Vlambda1_DPL8_(1e)
D1	Vh1_DP-3_(1-f)	Vlambda1_DPL8_(1e)
E7	Vh1_DP-10_(1-69)	Vlambda1_DPL9_(1f)
C8	Vh3_DP-46_(3-30.3)	Vlambda3_3h
1D9	Vh1_DP-5_(1-24)	Vlambda3_3h
1E4	Vh1_DP-5_(1-24)	Vlambda3_3h
3E7	Vh1_DP-5_(1-24)	Vlambda3_3h
4D8	Vh1_DP-5_(1-24)	Vlambda3_3h
5E1	Vh1_DP-5_(1-24)	Vlambda3_3h
6A8	Vh1_DP-5_(1-24)	Vlambda3_3h
7B5	Vh1_DP-5_(1-24)	Vlambda3_3h

The invention also provides antibodies that bind human RANTES when human RANTES is bound to glycosaminoglycan (GAG), i.e., bind human RANTES in the context of GAG. In a preferred embodiment, these antibodies include (a) a V_H CDRl region comprising the amino acid sequence of SEQ ID NO: 8, 28, 44, 90, 106, 122 or 154; (b) a V_H CDR2 region comprising the amino acid sequence of SEQ ID NO: 9, 29, 45, 91, 107, 123, 155, or 207; (c) a V_H CDR3 region comprising the amino acid sequence of SEQ ID NO: 10, 20, 30, 64, 92, 124, 156, 188, or 208, (d) a V_L CDRl region comprising the amino acid sequence of SEQ ID NO: 14, 34, 96, 128, 160, 176, 192, or 212; (e) a V_L CDR2 region comprising the amino acid sequence of SEQ ID NO: 15, 35, 97, 129, 161, 177, 193, or 213; and (f) a V_L CDR3 region comprising the amino acid sequence of SEQ ID NO: 16, 36, 98, 130, 162, 178, 194, or 214.

In some embodiments, the antibody is a monoclonal antibody or an antigen-binding fragment thereof. In some embodiments, the antibody is a fully human monoclonal

antibody or an antigen-binding fragment thereof. In some embodiments, the antibody is an IgG isotype, such as, for example, an IgGl isotype.

The invention also provides antagonist molecules of human RANTES, and in particular, antagonists of human RANTES proteins, polypeptides and/or peptides that include at least amino acid residues 16-18 of the mature amino acid sequence of human RANTES, e.g., SEQ ID NO: 170 shown in Figure 6. The anti-human RANTES antagonists bind to, or otherwise interact with, a human RANTES protein, polypeptide, and/or peptide to modulate, e.g., reduce, inhibit or otherwise interfere, partially or completely with a biological function of a human RANTES protein, such as for example, the binding of RANTES to a receptor such as CCRl , CCR3, CCR4 and/or CCR5, or the binding of RANTES to glycosaminoglycans (GAG).

In a preferred embodiment, the ability of the anti-human RANTES antagonists to bind to, or otherwise interact with, human RANTES protein to modulate one or more biological functions of human RANTES is dependent upon the presence of amino acid residues 16-18 of the mature human RANTES sequence such as SEQ ID NO: 170. In this embodiment, the antagonist molecules do not bind a human RANTES polypeptide that lacks amino acid residues 16-18 of SEQ ID NO: 170.

The anti-RANTES antagonist molecules provided herein completely or partially reduce or otherwise modulate RANTES expression or activity upon binding to, or otherwise interacting with, human RANTES. The reduction or modulation of a biological function of RANTES is complete or partial upon interaction between the antagonist and the human RANTES protein, polypeptide and/or peptide. The anti-huRANTES antagonists are considered to completely inhibit RANTES expression or activity when the level of RANTES expression or activity in the presence of the anti-huRANTES antagonist is decreased by at least 95%, *e.g.*, by 96%, 97%, 98%, 99% or 100% as compared to the level of RANTES expression or activity in the absence of interaction, e.g., binding with an anti-huRANTES antagonist described herein. The anti-huRANTES antagonists are considered to partially inhibit RANTES expression or activity when the level of RANTES expression or activity in the presence of the anti-huRANTES antagonist is decreased by less than 95%, *e.g.,* 10%, 20%, 25%, 30%, 40%, 50%, 60%, 75%, 80%, 85% or 90% as compared to the level of RANTES expression or activity in the absence of interaction, e.g., binding with an anti-huRANTES antagonist described herein.

In some embodiments, the anti-RANTES antagonist molecule is selected from a small molecule inhibitor; a polypeptide, a peptide, a RANTES-derived mutant polypeptide, a RANTES-derived polypeptide variant, a RANTES receptor-derived mutant polypeptide, e.g., a mutated CCRl, CCR3, CCR4 or CCR5 protein, polypeptide or peptide, a RANTES receptor-derived polypeptide variant, e.g., a CCRl, CCR3, CCR4 or CCR5 variant peptide, polypeptide or protein, and a nucleic acid-based antagonist.

In some embodiments, the anti-RANTES antagonist molecule is an isolated monoclonal anti-human RANTES antibody or antigen-binding fragment thereof. Preferably, the antibody (or antigen-binding fragment thereof) binds to amino acid residues 16-18 of the mature amino acid sequence of human RANTES, e.g., SEQ ID NO: 170 shown in Figure 6. In some embodiments, the anti-RANTES antibody is a fully human monoclonal anti-human RANTES antibody or antigen-binding fragment thereof. In some embodiments, the antibody is an IgG isotype, such as an IgGl isotype.

In some embodiments, the anti-RANTES antagonist molecule is a mutated RANTES polypeptide or RANTES-derived variant polypeptide or a mutated RANTES receptor, for example, selected from CCRl, CCR3, CCR4, and CCR5, or a variant of a RANTES receptor polypeptide, such as CCRl, CCR3, CCR4, or CCR5, that modulates an activity of RANTES selected from the ability of RANTES to bind to a receptor selected from CCRl, CCR3, CCR4, and CCR5, the ability of RANTES to bind a glycosaminoglycan and the ability of RANTES to form oligomers.

In some embodiments, the anti-RANTES antagonist molecule is a nucleic acid-based antagonist such as, for example, an aptamer or other oligonucleotide capable of interacting with targets, such as proteins, polypeptides, small molecules, carbohydrates, peptides or any other biological molecules, through interactions other than Watson-Crick base pairing.

The invention also provides methods of treating, preventing, alleviating a symptom of, or otherwise mitigating ischemia, a clinical indication associated with ischemia and/or reperfusion injury in a subject. The invention is based on the discovery that modulation, particularly, inhibition or other reduction of RANTES expression or activity inhibits ischemia and/or reperfusion injury in an animal model for ischemia and reperfusion. Accordingly, the invention provides methods of preventing or inhibiting ischemia, a clinical indication associated with ischemia, reperfusion injury, in a subject, in a bodily tissue and/or in a tissue or organ to be transplanted. In the methods provided herein, the subject to

be treated is administered an antagonist of RANTES. Likewise, in the treatment of organs to be transplanted, the organ, or a portion thereof, is contacted with an antagonist of RANTES. The methods provided herein are useful *in vivo* and *ex vivo.*

Suitable antagonists of RANTES include any antibody or fragment thereof that inhibits, neutralizes or otherwise interferes with the expression and/or activity of RANTES, such as, *e.g.*, the huRANTES antibodies provided herein; small molecule inhibitors; proteins, polypeptides, peptides; protein-, polypeptide- and/or peptide-based antagonists such as RANTES mutants and/or other RANTES variants and or RANTES receptor-based mutants and/or variants, such as, for example, mutated or variant versions of CCRl, CCR3, CCR4 or CCR5 polypeptides; nucleic acid based antagonists such as siRNA and/or anti-sense RNA, and/or aptamers; and/or fragments thereof that inhibit, neutralize or otherwise interfere with the expression and/or activity of RANTES.

Examples of polypeptide-based antagonists of RANTES include modified variants of RANTES that inhibit, neutralize or otherwise interfere with the expression and/or activity of RANTES. Variants of RANTES that are known to antagonize RANTES, for example, by decreasing the ability of RANTES to bind to glycosaminoglycans (GAG), include the RANTES mutants and variants described in PCT Publication Nos. WO 2004/062688; WO 2003/0844562; WO 2003/051921; WO 2002/028419; WO 2000/016796 and WO 1996/017935, each of which is hereby incorporated by reference in its entirety.

Examples of nucleic acid-based antagonists of RANTES include short interfering RNA (siRNA) mediated gene silencing where expression products of a RANTES gene are targeted by specific double stranded RANTES derived siRNA nucleotide sequences that are complementary to a segment of the RANTES gene transcript, *e.g.*, at least 19-25 nucleotides long, including the 5' untranslated (UT) region, the ORF, or the 3' UT region. *See, e.g.*, PCT applications WO00/44895, WO99/32619, WO01/75164, WO01/92513, WO 01/29058, WO01/89304, WO02/16620, and WO02/29858, each incorporated by reference herein in their entirety. Nucleic-acid based antagonists of RANTES also include antisense nucleic acids. An antisense nucleic acid comprises a nucleotide sequence that is complementary to a "sense" nucleic acid encoding a RANTES protein or fragment thereof. For example, antisense RANTES antagonists comprise a sequence complementary to at least about 10, 25, 50, 100, 250 or 500 nucleotides or an entire RANTES coding strand, or to only a portion thereof.

Preferably, the RANTES antagonist inhibits, partially or completely, a function of RANTES selected from the ability of RANTES to bind to a corresponding receptor *(e.g.,* CCRl, CCR3, CCR4, and/or CCR5), the ability of RANTES to bind glycosaminoglycans and/or the ability of RANTES to form oligomers. Suitable RANTES antagonists are identified, for example, using the assays and models provided in the Examples below.

The anti-huRANTES antagonists are considered to completely inhibit RANTES expression or activity when the level of RANTES expression or activity in the presence of the anti-huRANTES antagonist is decreased by at least 95%, *e.g.,* by 96%, 97%, 98%, 99% or 100% as compared to the level of RANTES expression or activity in the absence of interaction, e.g., binding with an anti-huRANTES antagonist described herein. The anti-huRANTES antagonists are considered to partially inhibit RANTES expression or activity when the level of RANTES expression or activity in the presence of the anti-huRANTES antagonist is decreased by less than 95%, *e.g.,* 10%, 20%, 25%, 30%, 40%, 50%, 60%, 75%, 80%, 85% or 90% as compared to the level of RANTES expression or activity in the absence of interaction, e.g., binding with an anti-huRANTES antagonist described herein.

In one aspect, the invention provides methods of treating, preventing or alleviating a symptom of ischemia or a clinical indication associated with ischemia by administering a RANTES antagonist, such as a huRANTES antibody, to a subject in need thereof or by contacting an organ in need thereof with a RANTES antagonist, such as a huRANTES antibody. The ischemia to be treated includes cardiac ischemia, cerebral ischemia, renal ischemia, and related ischemic diseases or events. Clinical indications associated with ischemia and reperfusion include, for example, coronary artery disease, cerebral vascular disease, cardiac ischemia, myocardial ischemia, renal ischemia and peripheral vascular disease. Ischemia is a feature of heart diseases including atherosclerosis, myocardial infarction, transient ischemic attacks, cerebrovascular accidents, ruptured arteriovenous malformations, and peripheral artery occlusive disease. The heart, the kidneys, and the brain are among the organs that are the most sensitive to inadequate blood supply. Ischemia in brain tissue is due, for example, to stroke or head injury. Use of a RANTES antagonist, such as a huRANTES antibody, is also envisioned as part of a protocol for optimizing tissue health during extra-corporeal perfusion of organs and/or tissue prior to transplantation, including, for example, heart, lung, and kidney. The organs to be treated using the methods provided herein are contacted *in vivo* or *ex vivo.*

The antibodies and compositions provided herein are useful in treating, preventing or otherwise delaying the progression of tissue injury or other damage caused by ischemia or a clinical indication associated with ischemia. For example, a huRANTES antibody or other RANTES antagonist of the invention is administered to a subject in need thereof before an ischemic event, during an ischemic event, after an ischemic event or any combination thereof.

The antibodies, RANTES antagonists and compositions provided herein are also useful in methods of treating, preventing or alleviating a symptom of a reperfusion injury or other tissue damage that occurs in a subject when blood supply returns to a tissue site after a period of ischemia. For example, a RANTES antagonist, such as a huRANTES antibody of the invention, is administered to a subject in need thereof, *e.g.,* during an ischemic event, after an ischemic event or both during and after an ischemic event. In some cases, restoration of blood flow after a period of ischemia can be more damaging than the ischemia. Reintroduction of oxygen causes a greater production of damaging free radicals, resulting in reperfusion injury. With reperfusion injury, tissue damage and/or necrosis can be greatly accelerated. Reperfusion injuries to be treated or prevented include injuries caused by an inflammatory response in the damaged tissue or tissues.

The subject or organ to be transplanted is suffering from or is predisposed to developing ischemia, an ischemic-related disorder, and/or reperfusion related tissue damage. Preferably, the subject is a mammal, and more preferably, the subject is a human.

In another aspect, the invention provides methods of treating, preventing or alleviating a symptom of an immune-related disorder by administering a huRANTES antibody to a subject. For example, the huRANTES antibodies are used to treat, prevent or alleviate a symptom associated with an autoimmune disease or inflammatory disorder. Optionally, the subject is further administered with a second agent such as, but not limited to, an anti-cytokine reagent, anti-chemokine reagent, an anti-cytokine reagent or an anti-chemokine receptor that recognizes the ligand or receptor for proteins such as interleukin 1 (IL-I), IL-2, IL-4, IL-6, IL-12, IL-13, IL-15, IL-17, IL-18, IL-20, IL-21, IL-22, IL-23, IL-27, IL-31, MIPl alpha, MIPl beta, IP-IO, MCPl, ITAC, MIG, SDF and fractalkine.

The subject is suffering from or is predisposed to developing an immune related disorder, such as, for example, an autoimmune disease or an inflammatory disorder. Preferably, the subject is a mammal, and more preferably, the subject is a human.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a series of graphs depicting the activity of anti-huRANTES antibodies in chemotaxis assays using L1.2 cells transfected with hCCR5 and 1 nM or 0.2 nM of recombinant human RANTES (Figure IA and B respectively) as well as native human RANTES (Figures 1C).

Figure 2 is a graph depicting the capacity of anti-huRANTES antibodies to bind to huRANTES in the context of glycosaminoglycans in and ELISA assay.

Figure 3 is a series of graphs depicting the activity of anti-huRANTES antibody 1E4 in calcium flux assays using: L1.2 cells expressing hCCRl and 25nM recombinant human RANTES (Fig. 3A); L1.2 cells expressing hCCR3 and 25nM recombinant human RANTES (Fig. 3B); L1.2 cells expressing hCCR5 and 4nM recombinant human RANTES (Fig. 3C).

Figure 4 is a series of graphs depicting the activity of anti-huRANTES antibody 1E4 in chemotaxis assays using: L1.2 cells expressing hCCRl an d 2nM of recombinant human RANTES (Fig. 4A); L1.2 cells expressing hCCR3 and 1OnM of recombinant human RANTES (Fig. 4B); L1.2 cells expressing hCCR5 and InM of recombinant human RANTES (Fig. 4C); L1.2 cells expressing hCCR5 and about InM of native human RANTES (Fig. 4D).

Figure 5 is a graph depicting the cross-reactivity profile of antibody 1E4 against a panel of human, *cynomolgus,* mouse and rat chemokines in an ELISA.

Figure 6 is a sequence alignment of mature RANTES protein from human (SEQ ID NO: 170), *cynomolgus* monkey (SEQ ID NO: 171), mouse (SEQ ID NO: 172) and rat (SEQ ID NO: 206). The arrows indicate positions that are conserved in human and *cynomolgus* RANTES but not in the mouse or rat sequences and that were targeted by site-directed mutagenesis.

Figure 7 is a graph depicting the binding of antibody 1E4 (open bars) or of a polyclonal antibody raised against mouse RANTES (hatched bars) to human RANTES, mouse RANTES and variants of mouse RANTES in which the indicated mouse amino acids have been replaced by the amino acids found in the human sequence at the same position.

Figure 8 is an illustration depicting the protocol of a murine ischemia reperfusion model provided herein.

Figure 9 is a series of graphs depicting that anti-RANTES treatment decreased infarct size in a murine model of ischemia reperfusion. The data represents 20 mice per group.

Figure 10 is a series of graphs depicting that anti-RANTES treatment decreased infarct size in a murine model of ischemia reperfusion in a dose-dependent manner. Data represents 3 mice per group.

Figure 11 is an illustration depicting the protocol of a murine ischemia model provided herein.

Figure 12 is a series of graphs depicting that anti-RANTES treatment decreased infarct size in a murine model of ischemia. The data represents 10 mice per group.

Figure 13 is a series of graphs depicting that anti-RANTES treatment decreased infarct size in a murine model of ischemia in a dose-dependent manner. Data represents 3 mice per group.

DETAILED DESCRIPTION

The present invention provides fully human monoclonal antibodies specific for the chemokine Regulated upon Activation, Normal T-cell Expressed, and Secreted (RANTES, CCL5). The terms "RANTES" and "CCL5" are used interchangeably herein. The antibodies are collectively referred to herein as huRANTES antibodies. The huRANTES antibodies specifically bind RANTES. As used herein, the terms "specific for", "specific binding", "directed against" (and all grammatical variations thereof) are used interchangeably in the context of antibodies that recognize and bind to a RANTES epitope when the equilibrium binding constant (K_d) is ≤1 μM, e.g., ≤ 100 nM, preferably ≤ 10 nM, and more preferably ≤ 1 nM. For example, the huRANTES antibodies provided herein exhibit a K_d in the range approximately between ≤ 10 nM to about 100 pM.

The huRANTES antibodies are, for example, RANTES antagonists or inhibitors that modulate at least one biological activity of RANTES. Biological activities of RANTES include, for example, binding a RANTES receptor such as, for example, CCRl, CCR3, CCR4, and/or CCR5; chemoattraction of eosinophils, monocytes, and lymphocytes; binding of RANTES to glycosaminoglycans as well as RANTES oligomerization. For example, the huRANTES antibodies completely or partially inhibit RANTES activity by partially or completely blocking the binding of RANTES to a RANTES receptor *(e.g.,* CCRl, CCR3, CCR4, and/or CCR5). The RANTES antibodies are considered to completely inhibit

RANTES activity when the level of RANTES activity in the presence of the huRANTES antibody is decreased by at least 95%, *e.g.,* by 96%, 97%, 98%, 99% or 100% as compared to the level of RANTES activity in the absence of binding with a huRANTES antibody described herein. The RANTES antibodies are considered to partially inhibit RANTES activity when the level of RANTES activity in the presence of the huRANTES antibody is decreased by less than 95%, *e.g.,* 10%, 20%, 25%, 30%, 40%, 50%, 60%, 75%, 80%, 85% or 90% as compared to the level of RANTES activity in the absence of binding with a huRANTES antibody described herein.

The huRANTES antibodies of the invention are produced by immunizing an animal with RANTES, such as, for example, murine or human RANTES or an immunogenic fragment, derivative or variant thereof. Alternatively, the animal is immunized with cells transfected with a vector containing a nucleic acid molecule encoding RANTES, such that RANTES is expressed and associated with the surface of the transfected cells. Alternatively, the antibodies are obtained by screening a library that contains antibody or antigen binding domain sequences for binding to RANTES. This library is prepared, *e.g.*, in bacteriophage as protein or peptide fusions to a bacteriophage coat protein that is expressed on the surface of assembled phage particles and the encoding DNA sequences contained within the phage particles *(i.e.,* "phage displayed library").

huRANTES antibodies of the invention include, for example, the heavy chain complementarity determining regions (CDRs) shown below in Table 2, the light chain CDRs shown in Table 3, and combinations thereof.

Table 2. VH CDR sequences from antibody clones that bind and neutralize RANTES. Antibodies marked in italic were derived by an affinity maturation process from antibody 2Dl (Lower part of the table).

Clone ID	Heavy CDR1	Heavy CDR2	Heavy CDR3
CG11	DYYIH (SEQ NO: 74)	LIDPKDGEIQYAEKFQA (SEQ NO: 75)	EVLSGIRVFPFDP (SEQ NO: 76)
BG11	ELSMH (SEQ NO: 90)	GFDPEDGETIYAQKFQG (SEQ NO: 91)	YSGSSGWWAFDI (SEQ NO: 92)
A9	SYAMS (SEQ NO: 106)	AISGSGGSTYYADSVKG (SEQ NO: 107)	DLGYCTNGVCWGIDY (SEQ NO: 108)
E6	EIAIH (SEQ NO: 122)	SFEPEDAEAIYAQRFQG (SEQ NO: 123)	DPYYASSGSNYMEV (SEQ NO: 124)
H6	KQSMH (SEQ NO: 138)	SSNPEDDETLYAKKFQG (SEQ NO: 139)	DSQGFYYYYGMDV (SEQ NO: 140)
G2	ELSIH (SEQ NO: 154)	GFDPEDGETIYAQNFQG (SEQ NO: 155)	DLTGSRDS (SEQ NO: 156)
E10	SYAMH (SEQ NO: 28)	VISYDGSNKYYADSVKG (SEQ NO: 29)	ETFPHYYYYYMDV (SEQ NO: 30)

C10	SYAMS (SEQ NO: 106)	AISGSGGSTYYADSVKG (SEQ NO: 107)	VRGSSQYDFWSGSEFDY (SEQ NO: 188)
2D1	DFAMH (SEQ NO: 44)	GYVPEDGDTIYAQKFQG (SEQ NO: 45)	DPLYSGSLSY (SEQ NO: 64)
A5	ELSIH (SEQ NO: 154)	YIDPEDGEPIYAQKFQG (SEQ NO: 207)	VTGSTSDAFDL (SEQ NO: 208)
H11	NYALS (SEQ NO: 222)	GFIPLVDTTNYAQRFQG (SEQ NO: 223)	EQVAVGPGPTSDRGPDGLDV (SEQ NO: 224)
D1	DYYIH (SEQ NO: 74)	LVDSEEDGETLFAETFRG (SEQ NO: 239)	EYGEYGFFQS (SEQ NO: 240)
E7	NYALS (SEQ NO: 222)	AVIPLVETTSYAQRFQG (SEQ NO: 255)	EQVAVGPGPTSNRGPDGLDV (SEQ NO: 256)
C8	SYAMH (SEQ NO: 28)	VISYDGSNKYYADSVKG (SEQ NO: 29)	ETFPHYYYYYMDV (SEQ NO: 30)
1D9	EFAMH (SEQ NO: 8)	GFVPEDGETIYAQKFQG (SEQ NO: 9)	DPLYTPGLEP (SEQ NO: 10)
1E4	EFAMH (SEQ NO: 8)	GFVPEDGETIYAQKFQG (SEQ NO: 9)	DPLYEGSFSV (SEQ NO: 20)
3E7	DFAMH (SEQ NO: 44)	GYVPEDGDTIYAQKFQG (SEQ NO: 45)	DPLYPPGLSP (SEQ NO: 46)
4D8	DFAMH (SEQ NO: 44)	GYVPEDGDTIYAQKFQG (SEQ NO: 45)	DPLYTPGLYV (SEQ NO: 50)
5E1	DFAMH (SEQ NO: 44)	GYVPEDGDTIYAQKFQG (SEQ NO: 45)	DYLYIPSLSY (SEQ NO: 54)
6A8	DFAMH (SEQ NO: 44)	GYVPEDGDTIYAQKFQG (SEQ NO: 45)	DPLYPPGLQP (SEQ NO: 58)
7B5	DFAMH (SEQ NO: 44)	GYVPEDGDTIYAQKFQG (SEQ NO: 45)	DPLYSGSLSY (SEQ NO: 64)

Table 3. VL CDR sequences from antibody clones that bind and neutralize RANTES. Antibodies marked in italic were derived by an affinity maturation process from antibody 2Dl (Lower part of the table).

Clone ID	Light CDR1	Light CDR2	Light CDR3
CG11	TGSSSNIGAGYDVY (SEQ NO: 77)	DTNNRPP (SEQ NO: 81)	QSYDIALSNSNVV (SEQ NO: 82)
BG11	QGDSLRSYYAS (SEQ NO: 96)	GKNNRPS (SEQ NO: 97)	QTWGTGIWV (SEQ NO: 98)
A9	TRSSGSIADNYVQ (SEQ NO: 112)	DDDQRLS (SEQ NO: 113)	QSYDDSNDV (SEQ NO: 114)
E6	TGSGGSISSNYVQ (SEQ NO: 128)	EDDQRPS (SEQ NO: 129)	HSYDGNNRWV (SEQ NO: 130)
H6	TGSSSNIGADYDVH (SEQ NO: 144)	DNINRPS (SEQ NO: 145)	QSYDSSLSGVL (SEQ NO: 146)
G2	TGSRSDIGYYNYVS (SEQ NO: 160)	DVTERPS (SEQ NO: 161)	SSFSSGDTFVV (SEQ NO: 162)
E10	GGGNFDDEGVH (SEQ NO: 176)	DDTGRPS (SEQ NO: 177)	QAWDSSNDHPV (SEQ NO: 178)
C10	GGDNIGGQNVH (SEQ NO: 192)	YDTDRPS (SEQ NO: 193)	QVWDVDSDHPWV (SEQ NO: 194)
2D1	GGNNIESKSVH (SEQ NO: 14)	DDSDRPS (SEQ NO: 15)	QVWDSNTDHWV (SEQ NO: 16)
A5	GGANLWGLGVH (SEQ NO: 212)	DNSDRAS (SEQ NO: 213)	QVWDSSSDHWV (SEQ NO: 214)

H11	TGSNSNLGADYDVH (SEQ NO: 228)	DNNIRPS (SEQ NO: 229)	QSYDTGLTSSDVI (SEQ NO: 230)
D1	TGSSSNIGADYDVN (SEQ NO: 244)	GDINRPS (SEQ NO: 245)	QSFDNSLSGSVI (SEQ NO: 246)
E7	TGSSSNIGDGYDVH (SEQ NO: 260)	GNSNRPS (SEQ NO: 261)	GTWDDILNGWV (SEQ NO: 262)
C8	EGDDTDIGTVN (SEQ NO: 34)	EDGYRPS (SEQ NO: 35)	QFWDVDSDHPV (SEQ NO: 36)
1D9	GGNNIESKSVH (SEQ NO: 14)	DDSDRPS (SEQ NO: 15)	QVWDSNTDHWV (SEQ NO: 16)
1E4	GGNNIESKSVH (SEQ NO: 14)	DDSDRPS (SEQ NO: 15)	QVWDSNTDHWV (SEQ NO: 16)
3E7	GGNNIESKSVH (SEQ NO: 14)	DDSDRPS (SEQ NO: 15)	QVWDSNTDHWV (SEQ NO: 16)
4D8	GGNNIESKSVH (SEQ NO: 14)	DDSDRPS (SEQ NO: 15)	QVWDSNTDHWV (SEQ NO: 16)
5E1	GGNNIESKSVH (SEQ NO: 14)	DDSDRPS (SEQ NO: 15)	QVWDSNTDHWV (SEQ NO: 16)
6A8	GGNNIESKSVH (SEQ NO: 14)	DDSDRPS (SEQ NO: 15)	QVWDSNTDHWV (SEQ NO: 16)
7B5	GGNNIESKSVH (SEQ NO: 14)	DDSDRPS (SEQ NO: 15)	QVWDSGPVWWI (SEQ NO: 16)

An exemplary huRANTES monoclonal antibody is the 1D9 antibody described herein. As shown below, the 1D9 antibody includes a heavy chain variable region (SEQ ID NO:2) encoded by the nucleic acid sequence shown in SEQ ID NO:1, and a light chain variable region (SEQ ID NO:4) encoded by the nucleic acid sequence shown in SEQ ID NO:3. The CDR sequences are shown in boxes.

> 1D9 Heavy chain variable domain nucleic acid sequence (SEQ ID NO: 1):

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTTT
CCTGCAAGGTTTCCGGATACACCCTCACTGAGTTCGCCATGCACTGGGTGCGACAGGCTCC
TGGAAAAGGGCTTGAGTGGATGGGAGGTTTTGTTCCTGAAGATGGTGAGACAATCTACGCG
CAGAAGTTCCAGGGCAGAGTCACCATGACCGAGGACACATCTACAGACACAGCCTACATGG
AGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCAACAGATCCCCTGTA
TACTCCGGGTCTTGAGCCTTGGGGGCAGGGGACCACGGTCACCGTCTCGAGT

> 1D9 Heavy chain variable domain amino acid sequence (SEQ ID NO: 2)

QVQLVQSGAEVKKPGASVKVSCKVSGYTLTEFAMHWVRQAPGKGLEWMGGFVPEDGETIYA
QKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATDPLYTPGLEPWGQGTTVTVSS

> D9 Light chain variable domain nucleic acid sequence (SEQ ID NO: 3):

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGACAGACGGCCAGGATTA
CCTGTGGGGGAAACAACATTGAAAGTAAAAGTGTGCACTGGTACCAGCAGAAGCCAGGCCA
GGCCCCTGTGCTGGTGGTCTATGATGATAGCGACCGGCCCTCAGGGATCCCTGAGCGATTC
TCTGGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAAGCCGGGGATG

AGGCCGACTATTACTGTCAGGTGTGGGATAGTAATACTGATCATTGGGTGTTCGGCGGAGG
GACCAAGCTCACCGTCCTA

>1 D9 Light chain variable domain amino acid sequence (SEQ ID NO: 4)

SYVLTQPPSVSVAPGQTARITCGGNNIESKSVHWYQQKPGQAPVLVVYDDSDRPSGIPERF
SGSNSGNTATLTISRVEAGDEADYYCQVWDSNTDHWVFGGGTKLTVL

The amino acids encompassing the complementarity determining regions (CDR) are as defined by Chothia et al. and E.A. Kabat et al. {*See* Chothia, C, *et al*, Nature 342:877-883 (1989); Kabat, EA, *et al*, Sequences of Protein of immunological interest, Fifth Edition, US Department of Health and Human Services, US Government Printing Office (1991)). The heavy chain CDRs of the 1D9 antibody have the following sequences: EFAMH (SEQ ID NO:8), encoded by the nucleic acid sequence GAGTTCGCCATGCAC (SEQ ID NO: 5); GFVPEDGETIY AQKFQG (SEQ ID NO:9), encoded by the nucleic acid sequence GGTTTTGTTCCTGAAGATGGTGAGACAATCTACGCGCAGAAGTTCCAGGGC (SEQ ID NO: 6); and DPLYTPGLEP (SEQ ID NO: 10), encoded by the nucleic acid sequence GATCCCCTGTATACTCCGGGTCTTGAGCCT (SEQ ID NO: 7). The light chain CDRs of the 1D9 antibody have the following sequences: GGNNIESKSVH (SEQ ID NO: 14), encoded by the nucleic acid sequence GGGGGAAACAACATTGAAAGTAAAAGTGTGCAC (SEQ ID NO: 11); DDSDRPS (SEQ ID NO: 15), encoded by the nucleic acid sequence GATGATAGCGACCGGCCCTCA (SEQ ID NO: 12); and QVWDSNTDHWV (SEQ ID NO: 16), encoded by the nucleic acid sequence CAGGTGTGGGATAGTAATACTGATCATTGGGTG (SEQ ID NO: 13).

An exemplary huRANTES monoclonal antibody is the 1E4 antibody described herein. As shown below, the 1E4 antibody includes a heavy chain variable region (SEQ ID NO: 18) encoded by the nucleic acid sequence shown in SEQ ID NO: 17, and a light chain variable region (SEQ ID NO: 4) encoded by the nucleic acid sequence shown in SEQ ED NO:3. The CDR sequences are shown in boxes.

> 1E4 Heavy Chain variable domain nucleic acid sequence (SEQ ID NO:17):

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTTT
CCTGCAAGGTTTCCGGATACACCCTCACTGAGTTCGCCATGCACTGGGTGCGACAGGCTCC
TGGAAAAGGGCTTGAGTGGATGGGAGGTTTTGTTCCTGAAGATGGTGAGACAATCTACGCG
CAGAAGTTCCAGGGCAGAGTCACCATGACCGAGGACACATCTACAGACACAGCCTACATGG

AGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCAACAGATCCCCTGTA
TGAGGGTTCGTTTTCTGTTTGGGGGCAGGGGACCACGGTCACCGTCTCGAGT

> 1E4 Heavy chain variable domain amino acid sequence (SEQ ID NO:18)

QVQLVQSGAEVKKPGASVKVSCKVSGYTLTEFAMHWVRQAPGKGLEWMGGFVPEDGETIYA
QKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATDPLYEGSFSVWGQGTTVTVSS

>1E4 Light chain nucleic acid sequence (SEQ ID NO:3):

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGACAGACGGCCAGGATTA
CCTGTGGGGGAAACAACATTGAAAGTAAAAGTGTGCACTGGTACCAGCAGAAGCCAGGCCA
GGCCCCTGTGCTGGTGGTCTATGATGATAGCGACCGGCCCTCAGGGATCCCTGAGCGATTC
TCTGGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAAGCCGGGGATG
AGGCCGACTATTACTGTCAGGTGTGGGATAGTAATACTGATCATTGGGTGTTCGGCGGAGG
GACCAAGCTCACCGTCCTA

> 1E4 Light chain variable domain amino acid sequence (SEQ ID NO:4)

SYVLTQPPSVSVAPGQTARITCGGNNIESKSVHWYQQKPGQAPVLVVYDDSDRPSGIPERF
SGSNSGNTATLTISRVEAGDEADYYCQVWDSNTDHWVFGGGTKLTVL

The amino acids encompassing the complementarity determining regions (CDR) are as defined by Chothia et al. and E.A. Kabat et al. *{See* Chothia, C, *et al.,* Nature 342:877-883 (1989); Kabat, EA, *et al,* Sequences of Protein of immunological interest, Fifth Edition, US Department of Health and Human Services, US Government Printing Office (1991)). The heavy chain CDRs of the 1E4 antibody have the following sequences: EFAMH (SEQ ID NO:8), encoded by the nucleic acid sequence GAGTTCGCCATGCAC (SEQ ID NO: 5); GFVPEDGETIY AQKFQG (SEQ ID NO: 9), encoded by the nucleic acid sequence GGTTTTGTTCCTGAAGATGGTGAGACAATCTACGCGCAGAAGTTCCAGGGC (SEQ ID NO: 6); and DPLYEGSFSV (SEQ ID NO:20), encoded by the nucleic acid sequence GATCCCCTGTATGAGGGTCCGTTTTCTGTT (SEQ ID NO: 19). The light chain CDRs of the 1E4 antibody have the following sequences: GGNNIESKS VH (SEQ ID NO: 14), encoded by the nucleic acid sequence GGGGGAAACAACATTGAAAGTAAAAGTGTGCAC (SEQ ID NO: 11); DDSDRPS (SEQ ID NO: 15), encoded by the nucleic acid sequence GATGATAGCGACCGGCCCTCA (SEQ ID NO: 12); and QVWDSNTDHWV (SEQ ID NO: 16), encoded by the nucleic acid sequence CAGGTGTGGGATAGTAATACTGATCATTGGGTG (SEQ ID NO: 13).

An exemplary huRANTES monoclonal antibody is the C8 antibody described herein. As shown below, the C8 antibody includes a heavy chain variable region (SEQ ID

NO:22) encoded by the nucleic acid sequence shown in SEQ ID NO: 21, and a light chain variable region (SEQ ID NO:24) encoded by the nucleic acid sequence shown in SEQ ID NO: 23. The CDR sequences are shown in boxes.

> C8 Heavy chain variable domain nucleic acid sequence (SEQ ID NO: 21)

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTCT
CCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGCTATGCACTGGGTCCGCCAGGCTCC
AGGCAAGGGGCTAGAGTGGGTGGCAGTTATATCATATGATGGAAGTAATAAATACTACGCA
GACTCCGTGAAGGGCCGATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGC
AAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGAAACTTTCCC
CCACTACTACTACTACTACATGGACGTCTGGGGCCGGGGCACCCTGGTCACCGTCTCGAGT

> C8 Heavy chain variable domain amino acid sequence (SEQ ID NO: 22)

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYYA
DSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARETFPHYYYYYMDVWGRGTLVTVSS

> C8 Light chain variable domain nucleic acid sequence (SEQ ID NO: 23):

TCCTATGTGCTGACTCAGCCCCCCTCGGTGTCAGTGGCCCCAGGGCAGACGGCCCGCATTA
CCTGTGAGGGAGACGACACTGACATTGGTACTGTCAACTGGTACCAGCAGAAACCAGGCCA
GGCCCCTGTGTTGGTCATTAGTGAGGATGGCTACCGGCCCTCAGGGATCCCTGAACGATTC
TCTGGCTCCAACTCTGGGAACACGGCCACCCTTACCATCTCCAGGGTCGAGGCCGGGGATG
AGGCCGACTATTACTGTCAGTTCTGGGATGTTGACAGTGATCATCCGGTTTTCGGCGGAGG
GACCCAGCTCACCGTCCTA

> C8 Light chain variable domain amino acid sequence (SEQ ID NO: 24)

SYVLTQPPSVSVAPGQTARITCEGDDTDIGTVNWYQQKPGQAPVLVISEDGYRPSGIPERF
SGSNSGNTATLTISRVEAGDEADYYCQFWDVDSDHPVFGGGTQLTVL

The amino acids encompassing the complementarity determining regions (CDR) are as defined by Chothia et al. and E.A. Kabat et al. *(See* Chothia, C, *et al.,* Nature 342:877-883 (1989); Kabat, EA, *et al,* Sequences of Protein of immunological interest, Fifth Edition, US Department of Health and Human Services, US Government Printing Office (1991)). The heavy chain CDRs of the C8 antibody have the following sequences: SYAMH (SEQ ID NO:28), encoded by the nucleic acid sequence AGCTATGCTATGCAC (SEQ ID NO: 25); VISYDGSNKYYADSVKG (SEQ ID NO:29), encoded by the nucleic acid sequence GTTATATCATATGATGGAAGTAATAAATACTACGCAGACTCCGTGAAGGGC (SEQ ED NO: 26); and ETFPHYYYYYMDV (SEQ ID NO:30), encoded by the nucleic acid sequence GAAACTTTCCCCCACTACTACTACTACTAC ATGGACGTC (SEQ ID NO: 27). The light chain CDRs of the C8 antibody have the following sequences:

EGDDTDIGTVN (SEQ ID NO:34), encoded by the nucleic acid sequence GAGGGAGACGACACTGACATTGGTACTGTCAAC (SEQ ID NO:31); EDGYRPS (SEQ ID NO:35), encoded by the nucleic acid sequence GAGGATGGCTACCGGCCCTCA (SEQ ID NO: 32); and QFWDVDSDHPV (SEQ ID NO:36), encoded by the nucleic acid sequence CAGTTCTGGGATGTTGACAGTGATCATCCGGTT (SEQ ID NO: 33).

An exemplary huRANTES monoclonal antibody is the 3E7 antibody described herein. As shown below, the 3E7 antibody includes a heavy chain variable region (SEQ ID NO:38) encoded by the nucleic acid sequence shown in SEQ ID NO: 37, and a light chain variable region (SEQ ID NO:40) encoded by the nucleic acid sequence shown in SEQ ID NO: 39. The CDR sequences are shown in boxes.

> 3E7 Heavy chain variable domain nucleic acid sequence (SEQ ID NO: 37)

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTT
TCCTGCAAGGTTTCCGGATACACCCTCAATGACTTCGCCATGCACTGGGTGCGACAGGCT
CCTGGAAAAGGGCTTGAGTGGATGGGAGGTTATGTTCCTGAAGATGGTGACACAATCTAC
GCGCAGAAGTTCCAGGGCAGAGTCACCATGACCGAGGACACATCTACAGACACAGCCTAC
ATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCAACAGATCCC
CTGTATCCGCCTGGGCTGTCTCCTTGGGGGCAGGGGACCACGGTCACCGTCTCGAGT

> 3E7 Heavy chain variable domain amino acid sequence (SEQ ID NO: 38)

QVQLVQSGAEVKKPGASVKVSCKVSGYTLNDFAMHWVRQAPGKGLEWMGGYVPEDGDTIY
AQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATDPLYPPGLSPWGQGTTVTVSS

> 3E7 Light chain variable domain nucleic acid sequence (SEQ ID NO: 39):

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGACAGACGGCCAGGATT
ACCTGTGGGGGAAACAACATTGAAAGTAAAAGTGTGCACTGGTACCAGCAGAAGCCAGGC
CAGGCCCCTGTGCTGGTGGTCTATGATGATAGCGACCGGCCCTCAGGGATCCCTGAGCGA
TTCTCTGGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAAGCCGGG
GATGAGGCCGACTATTACTGTCAGGTGTGGGATAGTAATACTGATCATTGGGTGTTCGGC
GGAGGGACCAAGGTCACCGTCCTA

> 3E7 Light chain variable domain amino acid sequence (SEQ ID NO: 40)

SYVLTQPPSVSVAPGQTARITCGGNNIESKSVHWYQQKPGQAPVLVVYDDSDRPSGIPER
FSGSNSGNTATLTISRVEAGDEADYYCQVWDSNTDHWVFGGGTKVTVL

The amino acids encompassing the complementarity determining regions (CDR) are as defined by Chothia et al. and E.A. Kabat et al. *(See* Chothia, C, *et al.,* Nature 342:877-883 (1989); Kabat, EA, *et al,* Sequences of Protein of immunological interest, Fifth Edition, US Department of Health and Human Services, US Government Printing Office (1991)). The heavy chain CDRs of the 3E7 antibody have the following sequences:

DFAMH (SEQ ID NO:44), encoded by the nucleic acid sequence GACTTCGCCATGCAC (SEQ ID NO: 41); GYVPEDGDTIYAQKFQG (SEQ ID NO:45), encoded by the nucleic acid sequence GGTTATGTTCCTGAAGATGGTGACACAATCTACGCGCAGAAGTTCCAGGGC (SEQ ID NO: 42); and DPLYPPGLSP (SEQ ID NO:46), encoded by the nucleic acid sequence GATCCCCTGTATCCGCCTGGGCTGTCTCCT (SEQ ID NO: 43). The light chain CDRs of the 3E7 antibody have the following sequences: GGNNIESKSVH (SEQ ID NO: 14), encoded by the nucleic acid sequence GGGGGAAACAACATTGAAAGTAAAAGTGTGCAC (SEQ ID NO: 11); DDSDRPS (SEQ ID NO: 15), encoded by the nucleic acid sequence GATGATAGCGACCGGCCCTCA (SEQ ID NO: 12); and QVWDSNTDHWV (SEQ ID NO: 16), encoded by the nucleic acid sequence CAGGTGTGGGATAGTAATACTGATCATTGGGTG (SEQ ID NO: 13).

An exemplary huRANTES monoclonal antibody is the 4D8 antibody described herein. As shown below, the 4D8 antibody includes a heavy chain variable region (SEQ ID NO:48) encoded by the nucleic acid sequence shown in SEQ ID NO: 47, and a light chain variable region (SEQ ID NO:40) encoded by the nucleic acid sequence shown in SEQ ID NO: 39. The CDR sequences are shown in boxes.

> 4D8 Heavy chain variable domain nucleic acid sequence (SEQ ID NO: 47)

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTT
TCCTGCAAGGTTTCCGGATACACCCTCAATGACTTCGCCATGCACTGGGTGCGACAGGCT
CCTGGAAAAGGGCTTGAGTGGATGGGAGGTTATGTTCCTGAAGATGGTGACACAATCTAC
GCGCAGAAGTTCCAGGGCAGAGTCACCATGACCGAGGACACATCTACAGACACAGCCTAC
ATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCAACAGATCCC
CTGTATACGCCTGGTCTGTATGTGTGGGGGCAGGGGACCACGGTCACCGTCTCGAGT

> 4D8 Heavy chain variable domain amino acid sequence (SEQ ID NO: 48)

QVQLVQSGAEVKKPGASVKVSCKVSGYTLNDFAMHWVRQAPGKGLEWMGGYVPEDGDTIY
AQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATDPLYTPGLYVWGQGTTVTVSS

> 4D8 Light chain variable domain nucleic acid sequence (SEQ ID NO: 39):

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGACAGACGGCCAGGATT
ACCTGTGGGGGAAACAACATTGAAAGTAAAAGTGTGCACTGGTACCAGCAGAAGCCAGGC
CAGGCCCCTGTGCTGGTGGTCTATGATGATAGCGACCGGCCCTCAGGGATCCCTGAGCGA
TTCTCTGGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAAGCCGGG
GATGAGGCCGACTATTACTGTCAGGTGTGGGATAGTAATACTGATCATTGGGTGTTCGGC
GGAGGGACCAAGGTCACCGTCCTA

> 4D8 Light chain variable domain amino acid sequence (SEQ ID NO: 40)

SYVLTQPPSVSVAPGQTARITCGGNNIESKSVHWYQQKPGQAPVLVVYDDSDRPSGIPER
FSGSNSGNTATLTISRVEAGDEADYYCQVWDSNTDHWVFGGGTKVTVL

The amino acids encompassing the complementarity determining regions (CDR) are as defined by Chothia et al. and E.A. Kabat et al. *(See* Chothia, C, *et al,* Nature 342:877-883 (1989); Kabat, EA, *et al,* Sequences of Protein of immunological interest, Fifth Edition, US Department of Health and Human Services, US Government Printing Office (1991)). The heavy chain CDRs of the 4D8 antibody have the following sequences: DFAMH (SEQ ID NO:44), encoded by the nucleic acid sequence GACTTCGCCATGCAC (SEQ ID NO: 41); GYVPEDGDTIYAQKFQG (SEQ ID NO:45), encoded by the nucleic acid sequence GGTTATGTTCCTGAAGATGGTGACACAATCTACGCGCAGAAGTTCCAGGGC (SEQ ID NO: 42); and DPLYTPGLYV (SEQ ID NO:50), encoded by the nucleic acid sequence GATCCCCTGTATACGCCTGGTCTGTATGTG (SEQ ID NO: 49). The light chain CDRs of the 4D8 antibody have the following sequences: GGNNIESKSVH (SEQ ID NO: 14), encoded by the nucleic acid sequence GGGGGAAACAACATTGAAAGTAAAAGTGTGCAC (SEQ ID NO: 11); DDSDRPS (SEQ ID NO: 15), encoded by the nucleic acid sequence GATGATAGCGACCGGCCCTCA (SEQ ID NO: 12); and QVWDSNTDHWV (SEQ ID NO: 16), encoded by the nucleic acid sequence CAGGTGTGGGATAGTAATACTGATCATTGGGTG (SEQ ID NO: 13).

An exemplary huRANTES monoclonal antibody is the 5El antibody described herein. As shown below, the 5El antibody includes a heavy chain variable region (SEQ ID NO:52) encoded by the nucleic acid sequence shown in SEQ ED NO: 51, and a light chain variable region (SEQ ID NO:40) encoded by the nucleic acid sequence shown in SEQ ID NO: 39. The CDR sequences are shown in boxes.

> 5E1 Heavy chain variable domain nucleic acid sequence (SEQ ID NO: 51)

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTT
TCCTGCAAGGTTTCCGGATACACCCTCAATGACTTCGCCATGCACTGGGTGCGACAGGCT
CCTGGAAAAGGGCTTGAGTGGATGGGAGGTTATGTTCCTGAAGATGGTGACACAATCTAC
GCGCAGAAGTTCCAGGGCAGAGTCACCATGACCGAGGACACATCTACAGACACAGCCTAC
ATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCAACAGATTAT
TTGTATATTCCTAGCTTATCCTACTGGGGGCAGGGGACCACGGTCACCGTCTCGAGT

> 5E1 Heavy chain variable domain amino acid sequence (SEQ ID NO: 52)

QVQLVQSGAEVKKPGASVKVSCKVSGYTLNDFAMHWVRQAPGKGLEWMGGYVPEDGDTIY
AQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATDYLYIPSLSYWGQGTTVTVSS

> 5E1 Light chain variable domain nucleic acid sequence (SEQ ID NO: 39):

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGACAGACGGCCAGGATT
ACCTGTGGGGGAAACAACATTGAAAGTAAAAGTGTGCACTGGTACCAGCAGAAGCCAGGC
CAGGCCCCTGTGCTGGTGGTCTATGATGATAGCGACCGGCCCTCAGGGATCCCTGAGCGA
TTCTCTGGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAAGCCGGG
GATGAGGCCGACTATTACTGTCAGGTGTGGGATAGTAATACTGATCATTGGGTGTTCGGC
GGAGGGACCAAGGTCACCGTCCTA

> 5E1 Light chain variable domain amino acid sequence (SEQ ID NO: 40)

SYVLTQPPSVSVAPGQTARITCGGNNIESKSVHWYQQKPGQAPVLVVYDDSDRPSGIPER
FSGSNSGNTATLTISRVEAGDEADYYCQVWDSNTDHWVFGGGTKVTVL

The amino acids encompassing the complementarity determining regions (CDR) are as defined by Chothia et al. and E.A. Kabat et al. *(See* Chothia, C, *et al.,* Nature 342:877-883 (1989); Kabat, EA, *et al.,* Sequences of Protein of immunological interest, Fifth Edition, US Department of Health and Human Services, US Government Printing Office (1991)). The heavy chain CDRs of the 5El antibody have the following sequences: DFAMH (SEQ ID NO:44), encoded by the nucleic acid sequence GACTTCGCCATGCAC (SEQ ID NO: 41); GYVPEDGDTIYAQKFQG (SEQ ID NO:45), encoded by the nucleic acid sequence GGTTATGTTCCTGAAGATGGTGACACAATCTACGCGCAGAAGTTCCAGGGC (SEQ ID NO: 42); and DYLYTPSLSY (SEQ ID NO:54), encoded by the nucleic acid sequence GATTATTTGTATATTCCTAGCTTATCCTAC (SEQ ID NO: 53). The light chain CDRs of the 5El antibody have the following sequences: GGNNIESKSVH (SEQ ID NO: 14), encoded by the nucleic acid sequence GGGGGAAACAACATTGAAAGTAAAAGTGTGCAC (SEQ ID NO: 11); DDSDRPS (SEQ ED NO: 15), encoded by the nucleic acid sequence GATGATAGCGACCGGCCCTCA (SEQ ID NO: 12); and QVWDSNTDHWV (SEQ ID NO: 16), encoded by the nucleic acid sequence CAGGTGTGGGATAGTAATACTGATCATTGGGTG (SEQ ID NO: 13).

An exemplary huRANTES monoclonal antibody is the 6A8 antibody described herein. As shown below, the 6A8 antibody includes a heavy chain variable region (SEQ ED NO:56) encoded by the nucleic acid sequence shown in SEQ ED NO: 55, and a light chain

variable region (SEQ ID NO:40) encoded by the nucleic acid sequence shown in SEQ ID NO: 39. The CDR sequences are shown in boxes.

> 6A8 Heavy chain variable domain nucleic acid sequence (SEQ ID NO: 55)

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTT
TCCTGCAAGGTTTCCGGATACACCCTCAATGACTTCGCCATGCACTGGGTGCGACAGGCT
CCTGGAAAAGGGCTTGAGTGGATGGGAGGTTATGTTCCTGAAGATGGTGACACAATCTAC
GCGCAGAAGTTCCAGGGCAGAGTCACCATGACCGAGGACACATCTACAGACACAGCCTAC
ATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCAACAGATCCC
CTGTATCCTCCGGGGCTGCAGCCTTGGGGGCAGGGGACCACGGTCACCGTCTCGAGT

> 6A8 Heavy chain variable domain amino acid sequence (SEQ ID NO: 56)

QVQLVQSGAEVKKPGASVKVSCKVSGYTLNDFAMHWVRQAPGKGLEWMGGYVPEDGDTIY
AQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATDPLYPPGLQPWGQGTTVTVSS

> 6A8 Light chain variable domain nucleic acid sequence (SEQ ID NO: 39):

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGACAGACGGCCAGGATT
ACCTGTGGGGGAAACAACATTGAAAGTAAAAGTGTGCACTGGTACCAGCAGAAGCCAGGC
CAGGCCCCTGTGCTGGTGGTCTATGATGATAGCGACCGGCCCTCAGGGATCCCTGAGCGA
TTCTCTGGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAAGCCGGG
GATGAGGCCGACTATTACTGTCAGGTGTGGGATAGTAATACTGATCATTGGGTGTTCGGC
GGAGGGACCAAGGTCACCGTCCTA

> 6A8 Light chain variable domain amino acid sequence (SEQ ID NO: 40)

SYVLTQPPSVSVAPGQTARITCGGNNIESKSVHWYQQKPGQAPVLVVYDDSDRPSGIPER
FSGSNSGNTATLTISRVEAGDEADYYCQVWDSNTDHWVFGGGTKVTVL

The amino acids encompassing the complementarity determining regions (CDR) are as defined by Chothia et al. and E.A. Kabat et al. {*See* Chothia, C, *et al,* Nature 342:877-883 (1989); Kabat, EA, *et al,* Sequences of Protein of immunological interest, Fifth Edition, US Department of Health and Human Services, US Government Printing Office (1991)). The heavy chain CDRs of the 6A8 antibody have the following sequences: DFAMH (SEQ ID NO:44), encoded by the nucleic acid sequence GACTTCGCCATGCAC (SEQ ID NO: 41); GYVPEDGDTIYAQKFQG (SEQ ID NO:45), encoded by the nucleic acid sequence GGTTATGTTCCTGAAGATGGTGACACAATCTACGCGCAGAAGTTCCAGGGC (SEQ ID NO: 42); and DPLYPPGLQP (SEQ ID NO:58), encoded by the nucleic acid sequence GATCCCCTGTATCCTCCGGGGCTGCAGCCT (SEQ ID NO: 57). The light chain CDRs of the 6A8 antibody have the following sequences: GGNNIESKSVH (SEQ ID NO: 14), encoded by the nucleic acid sequence GGGGGAAACAACATTGAAAGTAAAAGTGTGCAC (SEQ ID NO: 11); DDSDRPS

(SEQ ID NO: 15), encoded by the nucleic acid sequence GATGATAGCGACCGGCCCTCA (SEQ ID NO: 12); and QVWDSNTDHWV (SEQ ID NO: 16), encoded by the nucleic acid sequence CAGGTGTGGGATAGTAATACTGATCATTGGGTG (SEQ ID NO: 13).

An exemplary huRANTES monoclonal antibody is the 7B5 antibody described herein. As shown below, the 7B5 antibody includes a heavy chain variable region (SEQ ID NO:60) encoded by the nucleic acid sequence shown in SEQ ID NO: 59, and a light chain variable region (SEQ ID NO:62) encoded by the nucleic acid sequence shown in SEQ ID NO: 61. The CDR sequences are shown in boxes.

> 7B5 Heavy chain variable domain nucleic acid sequence (SEQ ID NO: 59)

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTT
TCCTGCAAGGTTTCCGGATACACCCTCAATGACTTCGCCATGCACTGGGTGCGACAGGCT
CCTGGAAAAGGGCTTGAGTGGATGGGAGGTTATGTTCCTGAAGATGGTGACACAATCTAC
GCGCAGAAGTTCCAGGGCAGAGTCACCATGACCGAGGACACATCTACAGACACAGCCTAC
ATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCAACAGATCCC
CTGTATAGTGGGAGCTTATCCTACTGGGGGCAGGGGACCACGGTCACCGTCTCGAGT

> 7B5 Heavy chain variable domain amino acid sequence (SEQ ID NO: 60)

QVQLVQSGAEVKKPGASVKVSCKVSGYTLNDFAMHWVRQAPGKGLEWMGGYVPEDGDTIY
AQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATDPLYSGSLSYWGQGTTVTVSS

> 7B5 Light chain variable domain nucleic acid sequence (SEQ ID NO: 61):

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGACAGACGGCCAGGATT
ACCTGTGGGGGAAACAACATTGAAAGTAAAAGTGTGCACTGGTACCAGCAGAAGCCAGGC
CAGGCCCCTGTGCTGGCCGTCTATGATGATAGCGACCGGCCCTCAGGGATCCCTGAGCGA
TTCTCTGGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAAGCCGGG
GATGAGGCCGACTATTACTGTCAGGTGTGGGATAGTGGTCCTGTGTGGTGGATTTTCGGC
GGAGGGACCAAGGTCACCGTCCTA

> 7B5 Light chain variable domain amino acid sequence (SEQ ID NO: 62)

SYVLTQPPSVSVAPGQTARITCGGNNIESKSVHWYQQKPGQAPVLVVYDDSDRPSGIPER
FSGSNSGNTATLTISRVEAGDEADYYCQVWDSGPVWWIFGGGTKLTVL

The amino acids encompassing the complementarity determining regions (CDR) are as defined by Chothia et al. and E.A. Kabat et al. *(See* Chothia, C, *et al,* Nature 342:877-883 (1989); Kabat, EA, *et al.,* Sequences of Protein of immunological interest, Fifth Edition, US Department of Health and Human Services, US Government Printing Office (1991)). The heavy chain CDRs of the 7B5 antibody have the following sequences: DFAMH (SEQ ED NO:44), encoded by the nucleic acid sequence GACTTCGCCATGCAC (SEQ ID NO: 41); GYVPEDGDTIY AQKFQG (SEQ ID NO:45), encoded by the nucleic

acid sequence GGTTATGTTCCTGAAGATGGTGACACAATCTACGCGCAGAAGTTCCAGGGC (SEQ ID NO: 42); and DPLYSGSLSY (SEQ ID NO:64), encoded by the nucleic acid sequence GATCCCCTGTATAGTGGGAGCTTATCCTAC (SEQ ID NO: 53). The light chain CDRs of the 7B5 antibody have the following sequences: GGNNIESKSVH (SEQ ID NO: 14), encoded by the nucleic acid sequence GGGGGAAACAACATTGAAAGTAAAAGTGTGCAC (SEQ ID NO: 11); DDSDRPS (SEQ ID NO: 15), encoded by the nucleic acid sequence GATGATAGCGACCGGCCCTCA (SEQ ID NO: 12); and QVWDSGPV WWI (SEQ ID NO:66), encoded by the nucleic acid sequence TCAGGTGTGGGATAGTGGTCCTGTGTGGTGGATT (SEQ ID NO: 65).

An exemplary huRANTES monoclonal antibody is the CG 11 antibody described herein. As shown below, the CGl 1 antibody includes a heavy chain variable region (SEQ ID NO:68) encoded by the nucleic acid sequence shown in SEQ ID NO: 67, and a light chain variable region (SEQ ID NO: 70) encoded by the nucleic acid sequence shown in SEQ ID NO: 69. The CDR sequences are shown in boxes.

> CG11 Heavy chain variable domain nucleic acid sequence (SEQ ID NO: 67)

CAGGTGCAGCTGGTGCAGTCTGGGACTGAGGTGAAGAAGCCTGGGGCTACAGTGAATGTT
TCCTGCAAGATTTCCGGACACCTCTTCACCGACTACTACATACACTGGGTGCAACAGGCC
CCTGGAAAAGGGCTTGAGTGGGTGGGACTTATTGATCCTAAAGATGGTGAAATCCAATAC
GCAGAGAAATTCCAGGCCAGAGTCACCATTACAGCGGACACGTCCACAGACACAGTTTAC
ATGGAATTGAACAGCCTGAGATCTGAAGACACGGCCGTGTATTACTGTGCAACAGAGGTT
TTAAGCGGTATTAGGGTTTTCCCATTCGACCCCTGGGGCCAGGGCACCCTGGTCACCGTC
TCGAGT

> CG11 Heavy chain variable domain amino acid sequence (SEQ ID NO: 68)

QVQLVQSGTEVKKPGATVNVSCKISGHLFTDYYIHWVQQAPGKGLEWVGLIDPKDGEIQY
AEKFQARVTITADTSTDTVYMELNSLRSEDTAVYYCATEVLSGIRVFPFDPWGQGTLVTV
SS

> CG11 Light chain variable domain nucleic acid sequence (SEQ ID NO: 69):

CAGTCTGTGCTGACTCAGCCACCCTCAGTGTCTGGGGCCCCAGGGCAGAGGGTCACCATC
TCTTGCACTGGGAGCAGCTCCAACATCGGGGCAGGTTATGATGTATATTGGTACCAACAG
TTTCCAGGGAAAGCCCCCAAACTCCTCATCTATGATACCAACAATCGACCCCCAGGGGGTC
CCTGATCGATTCTCTGGCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCATCAGTGGGCTC
CAGACTGAAGATGAGGCTGATTATTACTGCCAGTCTTATGACATCGCCCTGAGTAACTCG
AATGTGGTTTTCGGCGGAGGGACCAAGCTGACCGTCCTA

> CG11 Light chain variable domain amino acid sequence (SEQ ID NO: 70)

QSVLTQPPSVSGAPGQRVTISCTGSSSNIGAGYDVYWYQQFPGKAPKLLIYDTNNRPPGV
PDRFSGSKSGTSASLAISGLQTEDEADYYCQSYDIALSNSNVVFGGGTKLTVL

The amino acids encompassing the complementarity determining regions (CDR) are as defined by Chothia et al. and E.A. Rabat et al. *{See* Chothia, C, *et al.,* Nature 342:877-883 (1989); Kabat, EA, *et al,* Sequences of Protein of immunological interest, Fifth Edition, US Department of Health and Human Services, US Government Printing Office (1991)). The heavy chain CDRs of the CGl 1 antibody have the following sequences: DYYIH (SEQ ID NO:74), encoded by the nucleic acid sequence GACTACTACATACAC (SEQ ID NO: 71); LIDPKDGEIQYAEKFQA (SEQ ID NO:75), encoded by the nucleic acid sequence GGTTATGTTCCTGAAGATGGTGACACAATCTACGCGCAGAAGTTCCAGGGC (SEQ ID NO: 72); and EVLSGIRVFPFDP (SEQ ID NO:76), encoded by the nucleic acid sequence GAGGTTTTAAGCGGTATTAGGGTTTTCCCATTCGACCCC (SEQ ID NO: 73). The light chain CDRs of the CGl 1 antibody have the following sequences: TGSSSNIGAGYDVY (SEQ ID NO: 77), encoded by the nucleic acid sequence ACTGGGAGCAGCTCCAACATCGGGGCAGGTTATGATGTATAT (SEQ ID NO:80); DTNNRPP (SEQ ID NO:81), encoded by the nucleic acid sequence GATACCAACAATCGACCCCCA (SEQ ID NO: 78); and QSYDIALSNSNVV (SEQ ID NO:82), encoded by the nucleic acid sequence CAGTCTTATGACATCGCCCTGAGTAACTCGAATGTGGTT (SEQ ID NO: 79).

An exemplary huRANTES monoclonal antibody is the BGl 1 antibody described herein. As shown below, the BGl 1 antibody includes a heavy chain variable region (SEQ ID NO:84) encoded by the nucleic acid sequence shown in SEQ ID NO: 83, and a light chain variable region (SEQ ID NO: 86) encoded by the nucleic acid sequence shown in SEQ ED NO: 85. The CDR sequences are shown in boxes.

> BG11 Heavy chain variable domain nucleic acid sequence (SEQ ID NO: 83)

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTC
TCCTGCAAGGTTTCCGGATACACCCTCACTGAATTATCCATGCACTGGGTGCGACAGGCT
CCTGGAAAAGGGCTTGAGTGGATGGGAGGTTTTGATCCTGAAGATGGTGAAACAATCTAC
GCACAGAAGTTCCAGGGCAGAGTCACCATGACCGAGGACACATCTACAGACACAGCCTAC
ATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCAACTTATTCT
GGTAGTAGTGGTTGGTGGGCTTTTGATATCTGGGGCCAAGGGACAATGGTCACCGTCTCG
AGT

> BG11 Heavy chain variable domain amino acid sequence (SEQ ID NO: 84)

QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIY
AQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATYSGSSGWWAFDIWGQGTMVTVS
S

> BG11 Light chain variable domain nucleic acid sequence (SEQ ID NO: 85):

TCTTCTGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATC
ACATGCCAAGGAGACAGCCTCAGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGA
CAGGCCCCTGTACTTGTCATCTATGGTAAAAACAACCGGCCCTCAGGGATCCCAGACCGA
TTCTCTGGCTCCAGCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCGGAA
GATGAGGCTGACTATTACTGTCAGACCTGGGGCACTGGCATTTGGGTGTTCGGCGGAGGG
ACCAAGCTGACCGTCCTA

> BG11 Light chain variable domain amino acid sequence (SEQ ID NO: 86)

SSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPSGIPDR
FSGSSSGNTASLTITGAQAEDEADYYCQTWGTGIWVFGGGTKLTVL

The amino acids encompassing the complementarity determining regions (CDR) are as defined by Chothia et al. and E.A. Kabat et al. *{See* Chothia, C, *et al,* Nature 342:877-883 (1989); Kabat, EA, *et al.,* Sequences of Protein of immunological interest, Fifth Edition, US Department of Health and Human Services, US Government Printing Office (1991)). The heavy chain CDRs of the BGl l_antibody have the following sequences: ELSMH (SEQ ID NO:90), encoded by the nucleic acid sequence GAATTATCCATGCAC (SEQ ID NO: 87); GFDPEDGETIY AQKFQG (SEQ ID NO:91), encoded by the nucleic acid sequence GGTTTTGATCCTGAAGATGGTGAAACAATCTACGCACAGAAGTTCCAGGGC (SEQ ID NO: 88); and YSGSSGWW AFDI (SEQ ID NO:92), encoded by the nucleic acid sequence TATTCTGGTAGTAGTGGTTGGTGGGCTTTTGATATC (SEQ ID NO: 89). The light chain CDRs of the BGl l.antibody have the following sequences: QGDSLRSYYAS (SEQ ID NO:96), encoded by the nucleic acid sequence CAAGGAGACAGCCTCAGAAGCTATTATGCAAGC (SEQ ID NO:93); GKNNRPS (SEQ ID NO:97), encoded by the nucleic acid sequence GGTAAAAACAACCGGCCCTCA (SEQ ID NO: 94); and QTWGTGIWV (SEQ ID NO:98), encoded by the nucleic acid sequence CAGACCTGGGGCACTGGCATTTGGGTG (SEQ ID NO: 95).

An exemplary huRANTES monoclonal antibody is the A9 antibody described herein. As shown below, the A9 antibody includes a heavy chain variable region (SEQ ID NO: 100) encoded by the nucleic acid sequence shown in SEQ ID NO: 99, and a light chain

variable region (SEQ ID NO: 102) encoded by the nucleic acid sequence shown in SEQ ID NO: 101. The CDR sequences are shown in boxes.

> A9 Heavy chain variable domain nucleic acid sequence (SEQ ID NO: 99)

GAGGTGCAGCTGGTGGAGTCCGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTC
TCCTGTGCAGCCTCTGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCT
CCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGGTAGTGGTGGTAGCACATACTAC
GCAGACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTAT
CTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCGAGAGATTTA
GGATATTGTACTAATGGTGTATGCTGGGGTATTGACTACTGGGGCCAGGGGACAATGGTC
ACCGTCTCGAGT

> A9 Heavy chain variable domain amino acid sequence (SEQ ID NO: 100)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYY
ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDLGYCTNGVCWGIDYWGQGTMV
TVSS

> A9 Light chain variable domain nucleic acid sequence (SEQ ID NO: 101):

AATTTTATGCTGACTCAGCCCCACTCTGTGTCGGAGTCTCCGGGGAAGACGGTAACCATC
TCCTGCACCCGCAGCAGTGGCAGCATTGCCGACAACTATGTGCAGTGGTACCAGCAGCGC
CCGGGCAGTGCCCCCACCACTATCATCTATGACGATGACCAAAGACTCTCTGGGGTCCCT
GATCGATTCTCTGGCTCCATTGACACTTCCTCCAACTCTGCCTCCCTCTCCATCTCTGGA
CTGAGGACTGAGGACGAGGCTGATTACTACTGTCAGTCTTATGATGACTCCAATGATGTG
TTCGGCGGAGGGACCAAGCTGACCGTCCTA

> A9 Light chain variable domain amino acid sequence (SEQ ID NO: 102)

NFMLTQPHSVSESPGKTVTISCTRSSGSIADNYVQWYQQRPGSAPTTIIYDDDQRLSGVP
DRFSGSIDTSSNSASLSISGLRTEDEADYYCQSYDDSNDVFGGGTKLTVL

The amino acids encompassing the complementarity determining regions (CDR) are as defined by Chothia et al. and E.A. Kabat et al. *(See* Chothia, C, *et al.,* Nature 342:877-883 (1989); Kabat, EA, *et al.,* Sequences of Protein of immunological interest, Fifth Edition, US Department of Health and Human Services, US Government Printing Office (1991)). The heavy chain CDRs of the A9 antibody have the following sequences: SYAMS (SEQ ID NO: 106), encoded by the nucleic acid sequence AGCTATGCCATGAGC (SEQ ID NO: 103); AISGSGGSTYYADSVKG (SEQ ID NO: 107), encoded by the nucleic acid sequence GCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGGC (SEQ ID NO: 104); and DLGYCTNGVCWGIDY (SEQ ID NO: 108), encoded by the nucleic acid sequence GATTTAGGATATTGTACTAATGGTGTATGCTGGGGTATTGACTAC (SEQ ID NO:

105). The light chain CDRs of the A9 antibody have the following sequences: TRSSGSIADNYVQ (SEQ ID NO: 112), encoded by the nucleic acid sequence ACCCGCAGCAGTGGCAGCATTGCCGACAACTATGTGCAG (SEQ ID NO: 109); DDDQRLS (SEQ ID NO:1 13), encoded by the nucleic acid sequence GACGATGACCAAAGACTCTCT (SEQ ID NO: 110); and QSYDDSNDV (SEQ ID NO:1 14), encoded by the nucleic acid sequence CAGTCTTATGATGACTCCAATGATGTG (SEQ ID NO: 111).

An exemplary huRANTES monoclonal antibody is the E6 antibody described herein. As shown below, the E6 antibody includes a heavy chain variable region (SEQ ID NO:1 16) encoded by the nucleic acid sequence shown in SEQ ID NO: 115, and a light chain variable region (SEQ ID NO: 118) encoded by the nucleic acid sequence shown in SEQ ID NO: 117. The CDR sequences are shown in boxes.

> E6 Heavy chain variable domain nucleic acid sequence (SEQ ID NO: 115):

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGGAGAAGCCTGGGGCCTCAGTGAAGGTC
TCCTGCAGGGTTTCGGGATACCCCCTCACTGAAATAGCCATACACTGGGTGCGACAGGCT
CCTGGAAAAGGGCTTGAGTGGATGGGAAGTTTTGAGCCTGAAGATGCTGAAGCAATCTAC
GCACAGAGGTTCCAGGGCAGAGTCACAATGACCGAGGAAACATCTGCAAACACTGCCTAC
ATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTTCTGTGCAACAGATCCC
TACTATGCTAGCAGTGGTTCTAACTACATGGAGGTCTGGGGCCGAGGAACCCTGGTCACC
GTCTCGAGT

> E6 Heavy chain variable domain amino acid sequence (SEQ ID NO: 116):

QVQLVQSGAEVEKPGASVKVSCRVSGYPLTEIAIHWVRQAPGKGLEWMGSFEPEDAEAIY
AQRFQGRVTMTEETSANTAYMELSSLRSEDTAVYFCATDPYYASSGSNYMEVWGRGTLVT
VSS

> E6 Light chain variable domain nucleic acid sequence (SEQ ID NO: 117):

AATTTTATGCTGACTCAGCCCCACTCTGTGTCGGAGTCTCCGGGGAAGACGGTAACCATT
TCCTGCACCGGCAGCGGCGGCAGCATTTCCAGCAACTATGTCCAGTGGTACCGACAGCGC
CCGGGCAGCGCCCCCAGCACTGTGATCTATGAGGATGACCAAAGACCCTCTGGGGTCCCT
GATCGGATCTCTGGCTCCATCGACAGTTCCTCCAACTCTGCCTCCCTCACCATCTCTGGA
CTGACAACTGAGGACGAGGCTGACTACTATTGTCACTCTTATGATGGCAACAATCGGTGG
GTCTTCGGCGGAGGGACCAAGCTGACCGTCCTA

> E6 Light chain variable domain amino acid sequence (SEQ ID NO: 118)

NFMLTQPHSVSESPGKTVTISCTGSGGSISSNYVQWYRQRPGSAPSTVIYEDDQRPSGVP
DRISGSIDSSSNSASLTISGLTTEDEADYYCHSYDGNNRWVFGGGTKLTVL

The amino acids encompassing the complementarity determining regions (CDR) are as defined by Chothia et al. and E.A. Kabat et al. *(See* Chothia, C, *et al.,* Nature 342:877-

883 (1989); Kabat, EA, *et al*, Sequences of Protein of immunological interest, Fifth Edition, US Department of Health and Human Services, US Government Printing Office (1991)). The heavy chain CDRs of the E6_antibody have the following sequences: EIAIH (SEQ ID NO: 122), encoded by the nucleic acid sequence GAAATAGCCATACAC (SEQ ID NO: 119); SFEPEDAEAIY AQRFQG (SEQ ID NO: 123), encoded by the nucleic acid sequence AGTTTTGAGCCTGAAGATGCTGAAGCAATCTACGCACAGAGGTTCCAGGGC (SEQ ID NO: 120); and DPYYASSGSNYMEV (SEQ ID NO: 124), encoded by the nucleic acid sequence GATCCCTACTATGCTAGCAGTGGTTCTAACTACATGGAGGTC (SEQ ID NO: 124). The light chain CDRs of the E6 antibody have the following sequences: TGSGGSISSNYVQ (SEQ ID NO: 128), encoded by the nucleic acid sequence ACCGGCAGCGGCGGCAGCATTTCCAGCAACTATGTCCAG (SEQ ID NO: 125); EDDQRPS (SEQ ID NO: 129), encoded by the nucleic acid sequence GAGGATGACCAAAGACCCTCT (SEQ ID NO: 126); and HSYDGNNR WV (SEQ ID NO: 130), encoded by the nucleic acid sequence CACTCTTATGATGGCAACAATCGGTGGGTC (SEQ ID NO: 127).

An exemplary huRANTES monoclonal antibody is the H6 antibody described herein. As shown below, the H6 antibody includes a heavy chain variable region (SEQ ID NO: 132) encoded by the nucleic acid sequence shown in SEQ ID NO: 131, and a light chain variable region (SEQ ID NO: 133) encoded by the nucleic acid sequence shown in SEQ ID NO: 132. The CDR sequences are shown in boxes.

> H6 Heavy chain variable domain nucleic acid sequence (SEQ ID NO: 131):

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAGGCCTGGGGCCTCAGTGAAGGTC
TCCTGCAAAGTTTCCGGAAACACCCTCAGTAAACAATCCATGCACTGGGTGCGACAGGCT
CCTGGAAAAGGGTTTGAGTGGATGGGAAGTTCTAATCCTGAAGATGATGAAACACTCTAC
GCAAAGAAGTTCCAGGGCAGAGTCACCATGACCGAGGACACATCCACAGACACAGCCTAT
TTGGAGTTGAGCAGTCTGAGGTCTGAGGACACGGCCGTGTATTATTGTGCAACAGACTCC
CAGGGTTTTTACTATTACTACGGTATGGACGTCTGGGGCCAGGGCACCCTGGTCACCGTC
TCGAGT

> H6 Heavy chain variable domain amino acid sequence (SEQ ID NO: 132):

QVQLVQSGAEVKRPGASVKVSCKVSGNTLSKQSMHWVRQAPGKGFEWMGSSNPEDDETLY
AKKFQGRVTMTEDTSTDTAYLELSSLRSEDTAVYYCATDSQGFYYYYGMDVWGQGTLVTV
SS

> H6 Light chain variable domain nucleic acid sequence (SEQ ID NO: 133):

CAGTCTGTGCTGACTCAGCCGCCCTCAGTGTCTGGGGCCCCAGGGCAGAGGGTCACCATC

TCCTGCACTGGGAGCAGCTCCAACATCGGGGCAGATTATGATGTACACTGGTACCAGCAA
CTTCCAGGAACAGTCCCCAAACTCCTCATCTATGATAACATCAATCGGCCCTCAGGGGTC
CCTGACCGATTCTCTGGCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCATCACTGGGCTC
CAGGCTGAGGATGAGGCTGATTATTACTGCCAGTCCTATGACAGCAGCCTGAGTGGTGTG
CTATTCGGCGGAGGGACCAAGGTCACCGTCCTA

> H6 Light chain variable domain amino acid sequence (SEQ ID NO: 134)

QSVLTQPPSVSGAPGQRVTISCTGSSSNIGADYDVHWYQQLPGTVPKLLIYDNINRPSGV
PDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLSGVLFGGGTKVTVL

The amino acids encompassing the complementarity determining regions (CDR) are as defined by Chothia et al. and E.A. Kabat et al. *(See* Chothia, C, *et al,* Nature 342:877-883 (1989); Kabat, EA, *et al.,* Sequences of Protein of immunological interest, Fifth Edition, US Department of Health and Human Services, US Government Printing Office (1991)). The heavy chain CDRs of the Hó.antibody have the following sequences: KQSMH (SEQ ID NO: 138), encoded by the nucleic acid sequence AAACAATCCATGCAC (SEQ ID NO: 135); SSNPEDDETLYAKKFQG (SEQ ID NO: 139), encoded by the nucleic acid sequence AGTTCTAATCCTGAAGATGATGAAACACTCTACGCAAAGAAGTTCCAGGGC (SEQ ID NO: 136); and DSQGFYYYYGMDV (SEQ ID NO: 140), encoded by the nucleic acid sequence GACTCCCAGGGTTTTTACTATTACTACGGTATGGACGTC (SEQ ID NO: 137). The light chain CDRs of the H6 antibody have the following sequences: TGSSSNIGADYDVH (SEQ ID NO: 144), encoded by the nucleic acid sequence ACTGGGAGCAGCTCCAACATCGGGGCAGATTATGATGTACAC (SEQ ID NO: 141); DNINRPS (SEQ ID NO: 145), encoded by the nucleic acid sequence GATAACATCAATCGGCCCTCA (SEQ ID NO: 142); and QSYDSSLSGVL (SEQ ID NO: 146), encoded by the nucleic acid sequence CAGTCCTATGACAGCAGCCTGAGTGGTGTGCTA (SEQ ID NO: 143).

An exemplary huRANTES monoclonal antibody is the G2 antibody described herein. As shown below, the G2 antibody includes a heavy chain variable region (SEQ ID NO: 148) encoded by the nucleic acid sequence shown in SEQ ID NO: 147, and a light chain variable region (SEQ ID NO: 150) encoded by the nucleic acid sequence shown in SEQ ID NO: 149. The CDR sequences are shown in boxes.

> G2 Heavy chain variable domain nucleic acid sequence (SEQ ID NO: 147):

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTC
TCCTGCAGGGCTTCGGGATACGCCCTCACTGAATTATCCATTCACTGGGTGCGACAGGCT
CCTGGAAAAGGGCTTGAGTGGATGGGAGGTTTTGATCCTGAAGATGGTGAAACAATCTAC

GCACAGAATTTCCAGGGCAGAGTCATCATGACCGAGGACACATCTACAGACACAGCCTAC
ATGGAGCTGAGCAGCCTGAAATCTGAGGACACGGCCGTGTATTATTGTGCGACAGATCTA
ACTGGAAGTAGGGACTCCTGGGGCCAAGGCACCCTGGTCACCGTCTCGAGT

> G2 Heavy chain variable domain amino acid sequence (SEQ ID NO: 148):

QVQLVQSGAEVKKPGASVKVSCRASGYALTELSIHWVRQAPGKGLEWMGGFDPEDGETIY
AQNFQGRVIMTEDTSTDTAYMELSSLKSEDTAVYYCATDLTGSRDSWGQGTLVTVSS

> G2 Light chain variable domain nucleic acid sequence (SEQ ID NO: 149):

CAGTCTGTGCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCGATCACCATC
TCCTGCACTGGAAGCAGGAGTGACATTGGTTACTATAACTATGTCTCCTGGTACCAACAA
CACCCAGGGAAAGTCCCCAAACTCATAATTTATGATGTCACTGAGCGACCCTCAGGGGTT
TCTGATCGCTTCTCTGGCTCCAAGTCTGCCAACACGGCCTCCCTGACCATCTCTGGGCTC
CAGGCTGAGGACGAGGCTGATTATTACTGCAGCTCATTTTCAAGTGGCGACACCTTCGTG
GTTTTCGGCGGAGGGACCAAGCTGACCGTCCTA

> G2 Light chain variable domain amino acid sequence (SEQ ID NO: 150)

QSVLTQPASVSGSPGQSITISCTGSRSDIGYYNYVSWYQQHPGKVPKLIIYDVTERPSGV
SDRFSGSKSANTASLTISGLQAEDEADYYCSSFSSGDTFVVFGGGTKLTVL

The amino acids encompassing the complementarity determining regions (CDR) are as defined by Chothia et al. and E.A. Kabat et al. {*See* Chothia, C, *et al,* Nature 342:877-883 (1989); Kabat, EA, *et al,* Sequences of Protein of immunological interest, Fifth Edition, US Department of Health and Human Services, US Government Printing Office (1991)). The heavy chain CDRs of the G2_antibody have the following sequences: ELSIH (SEQ ID NO: 154), encoded by the nucleic acid sequence GAATTATCCATTCAC (SEQ ID NO: 151); GFDPEDGETIY AQNFQG (SEQ ID NO: 155), encoded by the nucleic acid sequence GGTTTTGATCCTGAAGATGGTGAAACAATCTACGCACAGAATTTCCAGGGC (SEQ ID NO: 152); and DLTGSRDS (SEQ ID NO: 156), encoded by the nucleic acid sequence GATCTAACTGGAAGTAGGGACTCC (SEQ ID NO: 153). The light chain CDRs of the G2 antibody have the following sequences: TGSRSDIGYYNYVS (SEQ ID NO: 160), encoded by the nucleic acid sequence ACTGGAAGCAGGAGTGACATTGGTTACTATAACTATGTCTCC (SEQ ID NO: 157); DVTERPS (SEQ ID NO: 161), encoded by the nucleic acid sequence GATGTCACTGAGCGACCCTCA (SEQ ID NO: 158); and SSFSSGDTFW (SEQ ID NO: 162), encoded by the nucleic acid sequence AGCTCATTTTCAAGTGGCGACACCTTCGTGGTT (SEQ ID NO: 159).

An exemplary huRANTES monoclonal antibody is the ElO antibody described herein. As shown below, the ElO antibody includes a heavy chain variable region (SEQ ID NO: 164) encoded by the nucleic acid sequence shown in SEQ ID NO: 163, and a light chain variable region (SEQ ID NO: 166) encoded by the nucleic acid sequence shown in SEQ ID NO: 165. The CDR sequences are shown in boxes.

> E10 Heavy chain variable domain nucleic acid sequence (SEQ ID NO: 163):

CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTC
TCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATGCTATGCACTGGGTCCGCCAGGCT
CCAGGCAAGGGGCTAGAGTGGGTGGCAGTTATATCATATGATGGAAGTAATAAATACTAC
GCAGACTCCGTGAAGGGCCGATTCTCCATCTCCAGAGACAATTCCAAGAACACGCTGTAT
CTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGAGAAACT
TTCCCCCACTACTACTACTACATGGACGTCTGGGGCAAGGGGACAATGGTCACCGTC
TCGAGT

> E10 Heavy chain variable domain amino acid sequence (SEQ ID NO: 164):

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYY
ADSVKGRFSISRDNSKNTLYLQMNSLRAEDTAVYYCARETFPHYYYYYMDVWGKGTMVTV
SS

> E10 Light chain variable domain nucleic acid sequence (SEQ ID NO: 165):

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCCGTGGCCCCAGGGCAGACGGCCAGAATT
TCCTGTGGGGGAGGCAACTTTGACGATGAAGGTGTTCACTGGTACCAGCAGACCCCAGGC
CAGGCCCCTGTACTGGTCGTCTATGATGATACCGGCCGGCCCTCAGGGATCCCTGAGCGA
TTCTCTGGCTCCAGTTCTGGGAATACGGCCACCCTGACCATCAGCCGGGTCGAAGCCGGG
GATGAGGCCGACTATTACTGTCAGGCGTGGGATAGTAGTAATGATCATCCCGTGTTCGGC
GGAGGGACCCAGCTCACCGTCCTA

> E10 Light chain variable domain amino acid sequence (SEQ ID NO: 166)

SYVLTQPPSVSVAPGQTARISCGGGNFDDEGVHWYQQTPGQAPVLVVYDDTGRPSGIPER
FSGSSSGNTATLTISRVEAGDEADYYCQAWDSSNDHPVFGGGTQLTVL

The amino acids encompassing the complementarity determining regions (CDR) are as defined by Chothia et al. and E.A. Kabat et al. *(See* Chothia, C, *et al,* Nature 342:877-883 (1989); Kabat, EA, *et al,* Sequences of Protein of immunological interest, Fifth Edition, US Department of Health and Human Services, US Government Printing Office (1991)). The heavy chain CDRs of the ElO antibody have the following sequences: SYAMH (SEQ ID NO:28), encoded by the nucleic acid sequence AGCTATGCTATGCAC (SEQ ID NO: 167); VISYDGSNKYY ADSVKG (SEQ ID NO:29), encoded by the nucleic acid sequence

GTTATATCATATGATGGAAGTAATAAATACTACGCAGACTCCGTGAAGGGC

(SEQ ID NO: 168); and ETFPHYYYYYMDV (SEQ ID NO:30), encoded by the nucleic acid sequence GAAACTTTCCCCCACTACTACTACTACATGGACGTC (SEQ ID NO: 169). The light chain CDRs of the ElO antibody have the following sequences: GGGNFDDEGVH (SEQ ID NO: 176), encoded by the nucleic acid sequence GGGGGAGGCAACTTTGACGATGAAGGTGTTCAC (SEQ ID NO: 173); DDTGRPS (SEQ ID NO: 177), encoded by the nucleic acid sequence GATGATACCGGCCGGCCCTCA (SEQ ID NO: 174); and QAWDSSNDHPV (SEQ ID NO: 178), encoded by the nucleic acid sequence CAGGCGTGGGATAGTAGTAATGATCATCCCGTG (SEQ ID NO: 175).

An exemplary huRANTES monoclonal antibody is the ClO antibody described herein. As shown below, the ClO antibody includes a heavy chain variable region (SEQ ID NO: 180) encoded by the nucleic acid sequence shown in SEQ ID NO: 179, and a light chain variable region (SEQ ID NO: 182) encoded by the nucleic acid sequence shown in SEQ ID NO: 181. The CDR sequences are shown in boxes.

> C10 Heavy chain variable domain nucleic acid sequence (SEQ ID NO: 179):

GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTC
TCCTGTGCAGCCTCTGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCT
CCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGGTAGTGGTGGTAGCACATACTAC
GCAGACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAAAAACACGCTGTAT
CTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCAAGAGTAAGG
GGGAGTTCCCAGTACGATTTTTGGAGTGGGTCCGAGTTTGACTACTGGGGCCAGGGGACA
ATGGTCACCGTCTCGAGT

> C10 Heavy chain variable domain amino acid sequence (SEQ ID NO: 180):

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYY
ADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARVRGSSQYDFWSGSEFDYWGQGT
MVTVSS

> C10 Light chain variable domain nucleic acid sequence (SEQ ID NO: 181):

TCCTATGTGCTGACTCAGCCACCCTCAGTGTCAGTGGCCCCAGGAAAGACGGCCAGCATT
TCCTGTGGGGGAGACAACATTGGAGGTCAAAATGTTCACTGGTATCAGCAGAAGCCAGGC
CAGGCCCCTGTGCTCGTCATCTATTATGATACCGACCGGCCCTCAGGGATCCCTGAGCGA
TTCTCTGGCTCCAACTCTGGGAACACGGCCACCCTGTCCATCAGCAGGGTCGAAGCCGCG
GATGAGGCCGACTATTACTGTCAGGTGTGGGATGTTGATAGTGATCATCCTTGGGTGTTC
GGCGGAGGGACCAAGCTGACCGTCCTA

> C10 Light chain variable domain amino acid sequence (SEQ ID NO: 182)

SYVLTQPPSVSVAPGKTASISCGGDNIGGQNVHWYQQKPGQAPVLVIYYDTDRPSGIPER
FSGSNSGNTATLSISRVEAADEADYYCQVWDVDSDHPWVFGGGTKLTVL

The amino acids encompassing the complementarity determining regions (CDR) are as defined by Chothia et al. and E.A. Kabat et al. {*See* Chothia, C, *et al*, Nature 342:877-883 (1989); Kabat, EA, *et al.*, Sequences of Protein of immunological interest, Fifth Edition, US Department of Health and Human Services, US Government Printing Office (1991)). The heavy chain CDRs of the ClO antibody have the following sequences: SYAMS (SEQ ID NO: 106), encoded by the nucleic acid sequence AGCTATGCCATGAGC (SEQ ID NO: 183); AISGSGGSTYY ADSVKG (SEQ ID NO: 107), encoded by the nucleic acid sequence GCTATTAGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGGC (SEQ ID NO: 184); and VRGSSQ YDFWSGSEFDY (SEQ ID NO: 188), encoded by the nucleic acid sequence GTAAGGGGGAGTTCCCAGTACGATTTTTGGAGTGGGTCCGAGTTTGACTAC (SEQ ID NO: 185). The light chain CDRs of the ClO antibody have the following sequences: GGDNIGGQNVH (SEQ ID NO: 192), encoded by the nucleic acid sequence GGGGGAGACAACATTGGAGGTCAAAATGTTCAC (SEQ ID NO: 189); YDTDRPS (SEQ ID NO: 193), encoded by the nucleic acid sequence TATGATACCGACCGGCCCTCA (SEQ ID NO: 190); and QVWD VDSDHPWV (SEQ ID NO: 194), encoded by the nucleic acid sequence CAGGTGTGGGATGTTGATAGTGATCATCCTTGGGTG (SEQ ID NO: 191).

An exemplary huRANTES monoclonal antibody is the 2Dl antibody described herein. As shown below, the 2Dl antibody includes a heavy chain variable region (SEQ ID NO:60) encoded by the nucleic acid sequence shown in SEQ ID NO: 59, and a light chain variable region (SEQ ID NO: 196) encoded by the nucleic acid sequence shown in SEQ ID NO: 195. The CDR sequences are shown in boxes.

> 2D1 Heavy chain variable domain nucleic acid sequence (SEQ ID NO: 59)

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTT
TCCTGCAAGGTTTCCGGATACACCCTCAATGACTTCGCCATGCACTGGGTGCGACAGGCT
CCTGGAAAAGGGCTTGAGTGGATGGGAGGTTATGTTCCTGAAGATGGTGACACAATCTAC
GCGCAGAAGTTCCAGGGCAGAGTCACCATGACCGAGGACACATCTACAGACACAGCCTAC
ATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCAACAGATCCC
CTGTATAGTGGGAGCTTATCCTACTGGGGGCAGGGGACCACGGTCACCGTCTCGAGT

> 2D1 Heavy chain variable domain amino acid sequence (SEQ ID NO: 60)

QVQLVQSGAEVKKPGASVKVSCKVSGYTLNDFAMHWVRQAPGKGLEWMGGYVPEDGDTIY
AQKFQGRVTMTEDTSTDTAYMELSSLRSEDTAVYYCATDPLYSGSLSYWGQGTTVTVSS

> 2D1 Light chain variable domain nucleic acid sequence (SEQ ID NO: 195):

TCCTATGTGCTGACTCAGCCACCCTCGGTGTCAGTGGCCCCAGGACAGACGGCCAGGATT
ACCTGTGGGGGAAACAACATTGAAAGTAAAAGTGTGCACTGGTACCAGCAGAAGCCAGGC
CAGGCCCCTGTGCTGGTGGTCTATGATGATAGCGACCGGCCCTCAGGGATCCCTGAGCGA
TTCTCTGGCTCCAACTCTGGGAACACGGCCACCCTGACCATCAGCAGGGTCGAAGCCGGG
GATGAGGCCGACTATTACTGTCAGGTGTGGGATAGTAATACTGATCATTGGGTGTTCGGC
GGAGGGACCAAGGTCACCGTCCTA

> 2D1 Light chain variable domain amino acid sequence (SEQ ID NO: 196)

SYVLTQPPSVSVAPGQTARITCGGNNIESKSVHWYQQKPGQAPVLVVYDDSDRPSGIPER
FSGSNSGNTATLTISRVEAGDEADYYCQVWDSNTDHWVFGGGTKVTVL

The amino acids encompassing the complementarity determining regions (CDR) are as defined by Chothia et al. and E.A. Kabat et al. *(See* Chothia, C, *et al,* Nature 342:877-883 (1989); Kabat, EA, *et al,* Sequences of Protein of immunological interest, Fifth Edition, US Department of Health and Human Services, US Government Printing Office (1991)). The heavy chain CDRs of the 2Dl antibody have the following sequences: DFAMH (SEQ ID NO:44), encoded by the nucleic acid sequence GACTTCGCCATGCAC (SEQ ID NO: 41); GYVPEDGDTIYAQKFQG (SEQ ID NO:45), encoded by the nucleic acid sequence GGTTATGTTCCTGAAGATGGTGACACAATCTACGCGCAGAAGTTCCAGGGC (SEQ ID NO: 42); and DPLYSGSLSY (SEQ ID NO:64), encoded by the nucleic acid sequence GATCCCCTGTATAGTGGGAGCTTATCCTAC (SEQ ID NO: 53). The light chain CDRs of the 2Dl antibody have the following sequences: GGNNIESKS VH (SEQ ID NO: 14), encoded by the nucleic acid sequence GGGGGAAACAACATTGAAAGTAAAAGTGTGCAC (SEQ ID NO: 11); DDSDRPS (SEQ ID NO: 15), encoded by the nucleic acid sequence GATGATAGCGACCGGCCCTCA (SEQ ID NO: 12); and QVWDSNTDHWV (SEQ ID NO: 198), encoded by the nucleic acid sequence CAGGTGTGGGATAGTAATACTGATCATTGGGTG (SEQ ID NO: 197).

An exemplary huRANTES monoclonal antibody is the A5 antibody described herein. As shown below, the A5 antibody includes a heavy chain variable region (SEQ ID NO:200) encoded by the nucleic acid sequence shown in SEQ ID NO: 199, and a light chain variable region (SEQ ID NO:202) encoded by the nucleic acid sequence shown in SEQ ID NO: 201 . The CDR sequences are shown in boxes.

≥ A5 Heavy chain variable domain nucleic acid sequence (SEQ ID NO: 199)

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTC
TCCTGCAAGGTTTCCGGATACGCCCTCAGTGAATTATCCATACACTGGGTGCGACAGGCT
CCTGGCAAAGGCCTTGAGTGGATGTCGTATATTGATCCTGAAGATGGTGAACCAATTTAC
GCACAGAAGTTCCAGGGCAGAGCCACCATGACCGAGGACTCATCTACAGACACAGCCTAC
ATGGAGATGGGCAGCCTGACATCTGACGACACGGCCGTTTATTACTGTGCAGGTGTCACT
GGAAGTACTTCGGATGCCTTTGATCTCTGGGGCCGGGGAACCCTGGTCACCGTCTCGAGT

≥ A5 Heavy chain variable domain amino acid sequence (SEQ ID NO: 200)

QVQLVQSGAEVKKPGASVKVSCKVSGYALSELSIHWVRQAPGKGLEWMSYIDPEDGEPIY
AQKFQGRATMTEDSSTDTAYMEMGSLTSDDTAVYYCAGVTGSTSDAFDLWGRGTLVTVSS

≥ A5 Light chain variable domain nucleic acid sequence (SEQ ID NO: 201):

TCCTATGTGCTGACTCAGGACCCCTCGGTGTCAGTGGCCCCAGGACAGACGGCCAGGATC
ACCTGTGGGGGAGCCAATCTTTGGGGTCTAGGTGTCCATTGGTATCAACAAAAGTCAGGC
CAGGCCCCTGTGTTGGTCGTCTCTGATAATAGCGACCGGGCCTCAGGGATCCCTGAGCGA
TTCTCTGGCTCCAATTCTGGGACCACGGCCACCCTGACCCTCAGCAGGGTCGAAGTCGGC
GATGAGGCCGACTATTACTGTCAGGTGTGGGATAGTAGTAGTGATCACTGGGTGTTCGGC
GGCAGGACCAAGCTGACCGTCCTA

≥ A5 Light chain variable domain amino acid sequence (SEQ ID NO: 202)

SYVLTQDPSVSVAPGQTARITCGGANLWGLGVHWYQQKSGQAPVLVVSDNSDRASGIPER
FSGSNSGTTATLTLSRVEVGDEADYYCQVWDSSSDHWVFGGRTKLTVL

The amino acids encompassing the complementarity determining regions (CDR) are as defined by Chothia et al. and E.A. Kabat et al. {*See* Chothia, C, *et al.,* Nature 342:877-883 (1989); Kabat, EA, *et al.,* Sequences of Protein of immunological interest, Fifth Edition, US Department of Health and Human Services, US Government Printing Office (1991)). The heavy chain CDRs of the A5 antibody have the following sequences: ELSIH (SEQ ID NO: 154), encoded by the nucleic acid sequence GAATTATCCATACAC (SEQ ID NO: 203); YIDPEDGEPIYAQKFQG (SEQ ID NO:207), encoded by the nucleic acid sequence

TATATTGATCCTGAAGATGGTGAACCAATTTACGCACAGAAGTTCCAGGGC

(SEQ ID NO: 204); and VTGSTSDAFDL (SEQ ID NO:208), encoded by the nucleic acid sequence GTCACTGGAAGTACTTCGGATGCCTTTGATCTC (SEQ ID NO: 205). The light chain CDRs of the A5 antibody have the following sequences: GGANLWGLGVH (SEQ ED NO:212), encoded by the nucleic acid sequence

GGGGGAGCCAATCTTTGGGGTCTAGGTGTCCAT (SEQ ED NO:209); DNSDRAS (SEQ ED NO:213), encoded by the nucleic acid sequence

GATAATAGCGACCGGGCCTCA (SEQ ID NO: 210); and QVWDSSSDHWV (SEQ ID

NO:214), encoded by the nucleic acid sequence CAGGTGTGGGATAGTAGTAGTGATCACTGGGTG (SEQ ID NO: 211).

An exemplary huRANTES monoclonal antibody is the H11 antibody described herein. As shown below, the H11 antibody includes a heavy chain variable region (SEQ ID NO:216) encoded by the nucleic acid sequence shown in SEQ ID NO: 215, and a light chain variable region (SEQ ID NO:218) encoded by the nucleic acid sequence shown in SEQ ID NO: 217. The CDR sequences are shown in boxes.

> H11 Heavy chain variable domain nucleic acid sequence (SEQ ID NO: 215)

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCGTCGGTGAAGGTC
TCCTGCAAGGCCTCTGGAGGCATCTCCGACAACTATGCTCTCAGCTGGGTGCGACAGGCC
CCTGGCCAAGGACTTGAGTGGATGGGAGGGTTCATCCCTCTCGTCGATACTACGAACTAC
GCACAGAGGTTTCAGGGCAGACTCACGATTACCGCGGACGACTCCATGAGTACAGTCTAC
ATGGAACTAAGAAGCCTGCGATCTGACGACACGGCCATGTATTATTGTGCGAGAGAGCAG
GTGGCGGTGGGACCTGGACCCACCTCAGACCGGGGGCCCGATGGTCTTGATGTCTGGGGC
CAAGGGACAATGGTCACCGTCTCGAGT

> H11 Heavy chain variable domain amino acid sequence (SEQ ID NO: 216)

QVQLVQSGAEVKKPGSSVKVSCKASGGISDNYALSWVRQAPGQGLEWMGGFIPLVDTTNY
AQRFQGRLTITADDSMSTVYMELRSLRSDDTAMYYCAREQVAVGPGPTSDRGPDGLDVWG
QGTMVTVSS

> H11 Light chain variable domain nucleic acid sequence (SEQ ID NO: 217):

CAGTCTGTGCTGACTCAGCCGTCCTCAGTGTCTGGGGCCCCAGGGCACAGGGTCACCATT
TCCTGCACTGGGAGCAACTCCAACCTCGGGGCGGATTATGATGTACACTGGTATCAGCAG
CTTCCAGGGTCAGCCCCCAAACTCCTCATCTATGATAACAACATTCGTCCCTCAGGGGTC
CCTGCCCGATTCTCTGGCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCATCACTGGGCTC
CAGGCTGAAGATGAGGCTGATTATTACTGCCAGTCGTATGACACCGGCCTGACTTCTTCG
GATGTGATATTCGGCGGAGGGACCAAGCTGACCGTCCTA

> H11 Light chain variable domain amino acid sequence (SEQ ID NO: 218)

QSVLTQPSSVSGAPGHRVTISCTGSNSNLGADYDVHWYQQLPGSAPKLLIYDNNIRPSGV
PARFSGSKSGTSASLAITGLQAEDEADYYCQSYDTGLTSSDVIFGGGTKLTVL

The amino acids encompassing the complementarity determining regions (CDR) are as defined by Chothia et al. and E.A. Kabat et al. *(See* Chothia, C, *et al,* Nature 342:877-883 (1989); Kabat, EA, *et al,* Sequences of Protein of immunological interest, Fifth Edition, US Department of Health and Human Services, US Government Printing Office (1991)). The heavy chain CDRs of the H11 antibody have the following sequences: NYALS (SEQ ID NO:222), encoded by the nucleic acid sequence AACTATGCTCTCAGC (SEQ ID NO: 219); GFIPLVDTTNY AQRFQG (SEQ ID NO:223), encoded by the nucleic

acid sequence GGGTTCATCCCTCTCGTCGATACTACGAACTACGCACAGAGGTTTCAGGGC (SEQ ID NO: 220); and EQVAVGPGPTSDRGPDGLDV (SEQ ID NO:224), encoded by the nucleic acid sequence GAGCAGGTGGCGGTGGGACCTGGACCCACCTCAGACCGGGGGCCCGATGGTCT TGATGTC (SEQ ID NO: 221). The light chain CDRs of the H1 1 antibody have the following sequences: TGSNSNLGADYDVH (SEQ ID NO:228), encoded by the nucleic acid sequence ACTGGGAGCAACTCCAACCTCGGGGCGGATTATGATGTACAC (SEQ ID NO:225); DNNIRPS (SEQ ID NO:229), encoded by the nucleic acid sequence GATAACAACATTCGTCCCTCA (SEQ ID NO: 226); and QSYDTGLTSSDVI (SEQ ID NO:230), encoded by the nucleic acid sequence CAGTCGTATGACACCGGCCTGACTTCTTCGGATGTGATA (SEQ ID NO: 227).

An exemplary huRANTES monoclonal antibody is the D1 antibody described herein. As shown below, the D1 antibody includes a heavy chain variable region (SEQ ID NO:232) encoded by the nucleic acid sequence shown in SEQ ID NO: 23 1, and a light chain variable region (SEQ ID NO:234) encoded by the nucleic acid sequence shown in SEQ ID NO: 233. The CDR sequences are shown in boxes.

> D1 Heavy chain variable domain nucleic acid sequence (SEQ ID NO: 231)

GAGGTGCAGCTGGTGCAGTCTGGGCCTGAGGTGAAGAAGCCTGGGGCCACAGTGAAAATTT
CCTGCAACGTCTCTGCAGAAACCTTCACCGACTACTACATACACTGGGTCAAACAGGCCCC
TGGA
AGAGGGCTGGAGTGGATGGGGCTTGTTGATTCTGAAGAAGATGGTGAAACATTATTCGCAG
AGACTTTCAGGGGCAGAGTCGCCCTAACCGCGGACAGGTCCACAAACACCGCCTACATGGA
GTTGCGCAGCCTGAGACATGACGACACGGCCGTCTATTATTGTGCAGCAGAATATGGTGAA
TATGGGTTCTTCCAATCGTGGGGCCAGGGAACCCTGGTCACCGTCTCGAGT

> D1 Heavy chain variable domain amino acid sequence (SEQ ID NO: 232)

EVQLVQSGPEVKKPGATVKISCNVSAETFTDYYIHWVKQAPGRGLEWMGLVDSEEDGETLF
A
ETFRGRVALTADRSTNTAYMELRSLRHDDTAVYYCAAEYGEYGFFQSWGQGTLVTVSS

> D1 Light chain variable domain nucleic acid sequence (SEQ ID NO: 233):

CAGTCTGTGCTGACTCAGCCACCCTCAGTGTCTGGGGCCCCAGGGCAGAGGGTCACCATC
TCCTGCACTGGGAGCAGCTCCAACATCGGGGCAGATTATGATGTAAACTGGTACCAGCAG
CTTCCAGGAACTTCCCCCAAACTCCTCATCTATGGTGACATCAATCGGCCCTCAGGGGTC
CCTGACCGATTCTCTGCCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCATCACTGGGCTC
CAGGCTGAGGATGAGGCTGATTATTACTGCCAGTCGTTTGACAACAGCCTGAGTGGGTCT
GTGATTTTCGGCGGAGGGACCAAGCTGACCGTCCTA

> D1 Light chain variable domain amino acid sequence (SEQ ID NO: 234)
QSVLTQPPSVSGAPGQRVTISCTGSSSNIGADYDVNWYQQLPGTSPKLLIYGDINRPSGV
PDRFSASKSGTSASLAITGLQAEDEADYYCQSFDNSLSGSVIFGGGTKLTVL

The amino acids encompassing the complementarity determining regions (CDR) are as defined by Chothia et al. and E.A. Kabat et al. *(See* Chothia, C, *et al.,* Nature 342:877-883 (1989); Kabat, EA, *et al.,* Sequences of Protein of immunological interest, Fifth Edition, US Department of Health and Human Services, US Government Printing Office (1991)). The heavy chain CDRs of the D1 antibody have the following sequences: DYYlH (SEQ ID NO:74), encoded by the nucleic acid sequence GACTACTACATACAC (SEQ ID NO: 235); LVDSEEDGETLFAETFRG(SEQ ID NO:239), encoded by the nucleic acid sequence CTTGTTGATTCTGAAGAAGATGGTGAAACATTATTCGCAGAGACTTTCAGGGGC (SEQ ID NO: 236); and EYGEYGFFQS (SEQ ID NO:240), encoded by the nucleic acid sequence GAATATGGTGAATATGGGTTCTTCCAATCG (SEQ ID NO: 237). The light chain CDRs of the D1 antibody have the following sequences: TGSSSNIGADYDVN (SEQ ID NO:244), encoded by the nucleic acid sequence ACTGGGAGCAGCTCCAACATCGGGGCAGATTATGATGTAAAC (SEQ ID NO:241); GDINRPS (SEQ ID NO: 245), encoded by the nucleic acid sequence GGTGACATCAATCGGCCCTCA (SEQ ID NO: 242); and QSFDNSLSGSVI (SEQ ID NO:246), encoded by the nucleic acid sequence CAGTCGTTTGACAACAGCCTGAGTGGGTCTGTGATT (SEQ ID NO: 243).

An exemplary huRANTES monoclonal antibody is the E7 antibody described herein. As shown below, the E7 antibody includes a heavy chain variable region (SEQ ID NO:248) encoded by the nucleic acid sequence shown in SEQ ID NO: 247, and a light chain variable region (SEQ ID NO:250) encoded by the nucleic acid sequence shown in SEQ ID NO: 249. The CDR sequences are shown in boxes.

> E7 Heavy chain variable domain nucleic acid sequence (SEQ ID NO: 247)
CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCGGGGTCGTCGGTGAAGGTC
TCCTGCAAGATTTCTGGAGGCATCTCCGACAACTACGCTCTGAGCTGGGTGCGACAGGCC
CCTGGGCAAGGACTTGAGTGGATGGGAGCGGTCATCCCTCTCGTCGAGACTACGAGCTAC
GCACAGAGGTTCCAGGGCAGACTCACAATTACCGCGGACGACTCCTTGAATACACTGTAC
ATGGAATTGGGAAGCCTGCGATCTGACGACACGGCCATGTATTACTGTGCGAGAGAGCAG
GTGGCGGTGGGACCTGGACCCACTTCAAATCGGGGGCCCGATGGCCTAGATGTCTGGGGC
AGAGGGACAATGGTCACCGTCTCGAGT

> E7 Heavy chain variable domain amino acid sequence (SEQ ID NO: 248)

QVQLVQSGAEVKKPGSSVKVSCKISGGISDNYALSWVRQAPGQGLEWMGAVIPLVETTSY
AQRFQGRLTITADDSLNTLYMELGSLRSDDTAMYYCAREQVAVGPGPTSNRGPDGLDVWG
RGTMVTVSS

> E7 Light chain variable domain nucleic acid sequence (SEQ ID NO: 249):

CAGTCTGTGCTGACTCAGCCACCCTCAGTGTCTGGGGCCCCAGGGCAGAGGGTCACCATC
TCCTGCACTGGGAGCAGCTCCAACATCGGGGACGGTTATGATGTACACTGGTATCAGCAG
CTTCCAGGAACAGCCCCCAAACTCCTCATCTATGGTAACAGTAATCGGCCCTCAGGGGTC
CCTGACCGATTCTCTGGCTCCACCTCTGGCACCTCCGCCTCCCTGGCCATCCGTGGGCTC
CAGTCTGAGGATGAGGCTGATTACTACTGTGGAACATGGGATGACATCCTGAATGGTTGG
GTGTTCGGCGGAGGGACCAAGCTGACCGTCCTA

> E7 Light chain variable domain amino acid sequence (SEQ ID NO: 250)

QSVLTQPPSVSGAPGQRVTISCTGSSSNIGDGYDVHWYQQLPGTAPKLLIYGNSNRPSGV
PDRFSGSTSGTSASLAIRGLQSEDEADYYCGTWDDILNGWVFGGGTKLTVL

The amino acids encompassing the complementarity determining regions (CDR) are as defined by Chothia et al. and E.A. Kabat et al. *(See* Chothia, C, *et al,* Nature 342:877-883 (1989); Kabat, EA, *et al.,* Sequences of Protein of immunological interest, Fifth Edition, US Department of Health and Human Services, US Government Printing Office (1991)). The heavy chain CDRs of the E7 antibody have the following sequences: NYALS (SEQ ID NO:222), encoded by the nucleic acid sequence AACTACGCTCTGAGC (SEQ ID NO: 249); AVIPLVETTSY AQRFQG (SEQ ID NO:255), encoded by the nucleic acid sequence

GCGGTCATCCCTCTCGTCGAGACTACGAGCTACGCACAGAGGTTCCAGGGC

(SEQ ID NO: 252); and EQVAVGPGPTSNRGPDGLDV (SEQ ID NO:256), encoded by the nucleic acid sequence

GAGCAGGTGGCGGTGGGACCTGGACCCACTTCAAATCGGGGGCCCGATGGCCT AGATGTC (SEQ ID NO: 253). The light chain CDRs of the E7 antibody have the following sequences: TGSSSN1GDGYDVH (SEQ ID NO:260), encoded by the nucleic acid sequence ACTGGGAGCAGCTCCAACATCGGGGACGGTTATGATGTACAC (SEQ ID NO:257); GNSNRPS (SEQ ID NO:261), encoded by the nucleic acid sequence GGTAACAGTAATCGGCCCTCA (SEQ ID NO: 258); and GTWDDILNGWV (SEQ ID NO:262), encoded by the nucleic acid sequence GGAACATGGGATGACATCCTGAATGGTTGGGTG (SEQ ID NO: 259).

huRANTES antibodies of the invention also include antibodies that include a heavy chain variable amino acid sequence that is at least 90%, 92%, 95%, 97%, 98%, 99% or

more identical the amino acid sequence of SEQ ID NO: 2, 18, 22, 38, 48, 52, 56, 60, 68, 84, 100, 116, 132, 148, 164, 180, 200, 216, 232, or 248 and/or a light chain variable amino acid that is at least 90%, 92%, 95%, 97%, 98%, 99% or more identical the amino acid sequence of SEQ ID NO: 4, 24, 40, 62, 70, 86, 102, 118, 134, 150, 166, 182, 196, 202, 218, 234, or 250.

Alternatively, the monoclonal antibody is an antibody that binds to the same epitope as 1D9, 1E4, C8, 3E7, 4D8, 5El, 6A8, 7B5, CGl 1, BGl 1, A9, E6, H6, G2, ElO, ClO, 2Dl, A5, HIl, Dl and/or E7.

Unless otherwise defined, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures utilized in connection with, and techniques of, cell and tissue culture, molecular biology, and protein and oligo- or polynucleotide chemistry and hybridization described herein are those well known and commonly used in the art. Standard techniques are used for recombinant DNA and oligonucleotide synthesis, as well as tissue culture and transformation *(e.g.*, electroporation, lipofection). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures 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 *See e.g.*, Sambrook *et al.* Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)). The nomenclatures utilized 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. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, delivery and treatment of patients.

As utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

As used herein, the term "antibody" refers to immunoglobulin molecules and immunologically active portions of immunoglobulin (Ig) molecules, *i.e.*, molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen.

Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F_{ab}, F_{a^b}, and $F_{(ab')2}$ fragments, and antibodies in an F_{ab} expression library. By "specifically bind" or "immunoreacts with" is meant that the antibody reacts with one or more antigenic determinants of the desired antigen and does not react (i.e., bind) with other polypeptides or binds at much lower affinity ($IQ > 10^{-6}$) with other polypeptides.

The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. *See* generally, Fundamental Immunology Ch. 7 (Paul, W., ea., 2nd ed. Raven Press, N.Y. (1989)). The variable regions of each light/heavy chain pair form the antibody binding site.

The term "monoclonal antibody" (MAb) or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one molecular species of antibody molecule consisting of a unique light chain gene product and a unique heavy chain gene product. In particular, the complementarity determining regions (CDRs) of the monoclonal antibody are identical in all the molecules of the population. MAbs contain an antigen binding site capable of immunoreacting with a particular epitope of the antigen characterized by a unique binding affinity for it.

In general, antibody molecules obtained from humans relate to any of the classes IgG, IgM, IgA, IgE and IgD, which differ from one another by the nature of the heavy chain present in the molecule. Certain classes have subclasses as well, such as IgGi, IgG_2, and others. Furthermore, in humans, the light chain may be a kappa chain or a lambda chain.

The term "antigen-binding site," or "binding portion" refers to the part of the immunoglobulin molecule that participates in antigen binding. The antigen binding site is formed by amino acid residues of the N-terminal variable ("V") regions of the heavy ("H") and light ("L") chains. Three highly divergent stretches within the V regions of the heavy

and light chains, referred to as "hypervariable regions," are interposed between more conserved flanking stretches known as "framework regions," or "FRs". Thus, the term "FR" refers to amino acid sequences which are naturally found between, and adjacent to, hypervariable regions in immunoglobulins. In an antibody molecule, the three hypervariable regions of a light chain and the three hypervariable regions of a heavy chain are disposed relative to each other in three dimensional space to form an antigen-binding surface. The antigen-binding surface is complementary to the three-dimensional surface of a bound antigen, and the three hypervariable regions of each of the heavy and light chains are referred to as "complementarity-determining regions," or "CDRs." The assignment of amino acids to each domain is in accordance with the definitions of Kabat Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk J. MoI. Biol. 196:901-917 (1987), Chothia *et al.* Nature 342:878-883 (1989).

As used herein, the term "epitope" includes any protein determinant capable of specific binding to an immunoglobulin or fragment thereof, or a T-cell receptor. The term "epitope" includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. An antibody is said to specifically bind an antigen when the dissociation constant is $\leq 1\,\mu M$; *e.g.*, ≤ 100 nM, preferably ≤ 10 nM and more preferably ≤ 1 nM.

As used herein, the terms "immunological binding," and "immunological binding properties" refer to the non-covalent interactions of the type which occur between an immunoglobulin molecule and an antigen for which the immunoglobulin is specific. The strength, or affinity of immunological binding interactions can be expressed in terms of the dissociation constant (IQ) of the interaction, wherein a smaller K_d represents a greater affinity. Immunological binding properties of selected polypeptides are quantified using methods well known in the art. One such method entails measuring the rates of antigen-binding site/antigen complex formation and dissociation, wherein those rates depend on the concentrations of the complex partners, the affinity of the interaction, and geometric parameters that equally influence the rate in both directions. Thus, both the "on rate constant" (K_{0n}) and the "off rate constant" (K_{off}) can be determined by calculation of the concentrations and the actual rates of association and dissociation. *(See* Nature 361 :186-87

(1993)). The ratio of K_{off}/K_{on} enables the cancellation of all parameters not related to affinity, and is equal to the dissociation constant K_d. *{See, generally,* Davies et al. (1990) Annual Rev Biochem 59:439-473). An antibody of the present invention is said to specifically bind to a RANTES epitope when the equilibrium binding constant (IQ) is ≤1 μM, *e.g.,* ≤ 100 nM, preferably ≤ 10 nM, and more preferably ≤ 1 nM, as measured by assays such as radioligand binding assays or surface plasmon resonance (SPR) or similar assays known to those skilled in the art. For example, the huRANTES antibodies provided herein exhibit a K_d in the range approximately between ≤ 10 nM to about 100 pM.

Those skilled in the art will recognize that it is possible to determine, without undue experimentation, if a human monoclonal antibody has the same specificity as a human monoclonal antibody of the invention *(e.g.,* monoclonal antibody D9, E4 or C8) by ascertaining whether the former prevents the latter from binding to a RANTES antigen polypeptide. If the human monoclonal antibody being tested competes with a human monoclonal antibody of the invention, as shown by a decrease in binding by the human monoclonal antibody of the invention, then the two monoclonal antibodies bind to the same, or a closely related, epitope. Another way to determine whether a human monoclonal antibody has the specificity of a human monoclonal antibody of the invention is to pre-incubate the human monoclonal antibody of the invention with the RANTES antigen polypeptide with which it is normally reactive, and then add the human monoclonal antibody being tested to determine if the human monoclonal antibody being tested is inhibited in its ability to bind the RANTES antigen polypeptide. If the human monoclonal antibody being tested is inhibited then, in all likelihood, it has the same, or functionally equivalent, epitopic specificity as the monoclonal antibody of the invention.

Various procedures known within the art are used for the production of the monoclonal antibodies directed against a protein such as a RANTES protein, or against derivatives, fragments, analogs homologs or orthologs thereof. *(See, e.g.,* Antibodies: A Laboratory Manual, Harlow E, and Lane D, 1988, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, incorporated herein by reference). Fully human antibodies are antibody molecules in which the entire sequence of both the light chain and the heavy chain, including the CDRs, arise from human genes. Such antibodies are termed "human antibodies", or "fully human antibodies" herein. Human monoclonal antibodies are prepared, for example, using the procedures described in the Examples provided below. Human monoclonal antibodies can be also prepared by using the trioma technique; the

human B-cell hybridoma technique *(see* Kozbor, et al., 1983 Immunol Today 4: 72); and the EBV hybridoma technique to produce human monoclonal antibodies *(see* Cole, et al., 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized and may be produced by using human hybridomas *(see* Cote, et al., 1983. Proc Natl Acad Sci USA 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus *in vitro* (see Cole, et al., 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96).

Antibodies are purified by well-known techniques, such as affinity chromatography using protein A or protein G. Subsequently, or alternatively, the specific antigen which is the target of the immunoglobulin sought, or an epitope thereof, may be immobilized on a column to purify the immune specific antibody by immunoaffinity chromatography. Purification of immunoglobulins is discussed, for example, by D. Wilkinson (The Scientist, published by The Scientist, Inc., Philadelphia PA, Vol. 14, No. 8 (April 17, 2000), pp. 25-28).

In some instances, it may be desirable to modify the antibody of the invention with respect to effector function, so as to enhance, *e.g.*, the effectiveness of the antibody in treating immune-related diseases. For example, cysteine residue(s) can be introduced into the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated can have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). *(See* Caron et al., J. Exp Med., 176: 1191-1 195 (1992) and Shopes, J. Immunol., 148: 2918-2922 (1992)). Alternatively, an antibody can be engineered that has dual Fc regions and can thereby have enhanced complement lysis and ADCC capabilities. *(See* Stevenson et al., Anti-Cancer Drug Design, 3: 219-230 (1989)). In a preferred embodiment, the huRANTES antibodies of the invention are not modified with respect to effector function.

The invention also includes F_v, F_{ab}, $F_{ab'}$, and $F(_{ab'})_2$ huRANTES antibody fragments, single chain huRANTES antibodies, bispecific huRANTES antibodies and heteroconjugate huRANTES antibodies.

Bispecific antibodies are antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for RANTES. The second binding target is any other antigen, and in some embodiments, the second binding target is an extracellular target such as a cell-surface protein or receptor or receptor subunit.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, Nature, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture often different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker et al., EMBO J., 10:3655-3659 (1991).

Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CHl) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. For further details of generating bispecific antibodies see, for example, Suresh et al., Methods in Enzymology, 121 :210 (1986).

Other approaches for generating bispecific antibodies are described, *e.g.,* in WO 96/2701 1, which is hereby incorporated by reference in its entirety. Bispecific antibodies can be prepared as full length antibodies or antibody fragments *(e.g.* $F(ab')_2$ bispecific antibodies). Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. See e.g., Brennan et al., Science 229:81 (1985) , which is hereby incorporated by reference in its entirety.

Additionally, Fab' fragments can be directly recovered from E. coli and chemically coupled to form bispecific antibodies. See e.g., Shalaby et al., J. Exp. Med. 175:217-225 (1992) , which is hereby incorporated by reference in its entirety.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. See e.g., Kostelny et al., J. Immunol. 148(5): 1547- 1553 (1992) , which is hereby incorporated by reference in its entirety. The

"diabody" technology described by Hollinger et al., Proc. Natl. Acad. Sci. USA 90:6444-6448 (1993), which is hereby incorporated by reference in its entirety, has provided an alternative mechanism for making bispecific antibody fragments. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See, Gruber et al., J. Immunol. 152:5368 (1994), which is hereby incorporated by reference in its entirety.

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. See, Tutt et al., J. Immunol. 147:60 (1991).

Exemplary bispecific antibodies can bind to two different epitopes, at least one of which originates in the protein antigen of the invention. Alternatively, an anti-antigenic arm of an immunoglobulin molecule can be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule *(e.g.* CD2, IFNγ, CD28, or B7), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the cell expressing the particular antigen. Bispecific antibodies can also be used to direct cytotoxic agents to cells which express a particular antigen. These antibodies possess an antigen-binding arm and an arm which binds a cytotoxic agent or a radionuclide chelator, such as EOTUBE, DPTA, DOTA, or TETA. Another bispecific antibody of interest binds the protein antigen described herein and further binds tissue factor (TF).

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360; WO 92/200373; EP 03089). It is contemplated that the antibodies can be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins can be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a toxin *(e.g.*, an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope *(i.e.*, a radioconjugate).

Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charanria inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include ^{212}Bi, ^{131}I, ^{131}In, ^{90}Y, and ^{186}Re.

Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutareldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as l,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science 238: 1098 (1987). Carbon- 14-labeled l-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. *(See* WO94/ 11026).

Those of ordinary skill in the art will recognize that a large variety of possible moieties can be coupled to the resultant antibodies or to other molecules of the invention. *{See, for example,* "Conjugate Vaccines", Contributions to Microbiology and Immunology, J. M. Cruse and R. E. Lewis, Jr (eds), Carger Press, New York, (1989), the entire contents of which are incorporated herein by reference).

Coupling is accomplished by any chemical reaction that will bind the two molecules so long as the antibody and the other moiety retain their respective activities. This linkage can include many chemical mechanisms, for instance covalent binding, affinity binding, intercalation, coordinate binding and complexation. The preferred binding is, however, covalent binding. Covalent binding is achieved either by direct condensation of existing side chains or by the incorporation of external bridging molecules. Many bivalent or polyvalent linking agents are useful in coupling protein molecules, such as the antibodies of the present invention, to other molecules. For example, representative coupling agents can include

organic compounds such as thioesters, carbodiimides, succinimide esters, diisocyanates, glutaraldehyde, diazobenzenes and hexamethylene diamines. This listing is not intended to be exhaustive of the various classes of coupling agents known in the art but, rather, is exemplary of the more common coupling agents. *(See* Killen and Lindstrom, Jour. Immun. 133:1335-2549 (1984); Jansen et al., Immunological Reviews 62:185-216 (1982); and Vitetta et al., Science 238:1098 (1987). Preferred linkers are described in the literature. *(See, for example,* Ramakrishnan, S. et al., Cancer Res. 44:201-208 (1984) describing use of MBS (M-maleimidobenzoyl-N-hydroxysuccinimide ester). *See also,* U.S. Patent No. 5,030,719, describing use of halogenated acetyl hydrazide derivative coupled to an antibody by way of an oligopeptide linker. Particularly preferred linkers include: (i) EDC (l-ethyl-3-(3-dimethylamino-propyl) carbodiimide hydrochloride; (ii) SMPT (4-succinimidyloxycarbonyl-alpha-methyl-alpha-(2-pridyl-dithio)-toluene (Pierce Chem. Co., Cat. (21558G); (iii) SPDP (succinimidyl-6 [3-(2-pyridyldithio) propionamido] hexanoate (Pierce Chem. Co., Cat #2165IG); (iv) Sulfo-LC-SPDP (sulfosuccinimidyl 6 [3-(2-pyridyldithio)-propianamide] hexanoate (Pierce Chem. Co. Cat. #2165-G); and (v) sulfo-NHS (N-hydroxysulfo-succinimide: Pierce Chem. Co., Cat. #24510) conjugated to EDC.

The term "isolated polynucleotide" as used herein shall mean a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, which by virtue of its origin the "isolated polynucleotide" (1) is not associated with all or a portion of a polynucleotide in which the "isolated polynucleotide" is found in nature, (2) is operably linked to a polynucleotide which it is not linked to in nature, or (3) does not occur in nature as part of a larger sequence.

The term "isolated protein" referred to herein means a protein of cDNA, recombinant RNA, or synthetic origin or some combination thereof, which by virtue of its origin, or source of derivation, the "isolated protein" (1) is not associated with proteins found in nature, (2) is free of other proteins from the same source, *e.g.,* free of murine proteins, (3) is expressed by a cell from a different species, or (4) does not occur in nature.

The term "polypeptide" is used herein as a generic term to refer to native protein, fragments, or analogs of a polypeptide sequence. Hence, native protein fragments, and analogs are species of the polypeptide genus. Preferred polypeptides in accordance with the invention comprise the human heavy chain immunoglobulin molecules presented herein and the human light chain immunoglobulin molecules presented herein, as well as antibody molecules formed by combinations comprising the heavy chain immunoglobulin molecules

with light chain immunoglobulin molecules, such as kappa light chain immunoglobulin molecules, and vice versa, as well as fragments and analogs thereof.

The term "naturally-occurring" as used herein as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory or otherwise is naturally-occurring.

The term "operably linked" as used herein refers to positions of components so described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

The term "control sequence" as used herein refers to polynucleotide sequences which are necessary to effect the expression and processing of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term "control sequences" is intended to include, at a minimum, all components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences. The term "polynucleotide" as referred to herein means a polymeric boron 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 of DNA.

The term oligonucleotide referred to herein includes naturally occurring, and modified nucleotides linked together by naturally occurring, and non-naturally occurring oligonucleotide linkages. Oligonucleotides are a polynucleotide subset generally comprising a length of 200 bases or fewer. Preferably oligonucleotides are 10 to 60 bases in length and most preferably 12, 13, 14, 15, 16, 17, 18, 19, or 20 to 40 bases in length. Oligonucleotides are usually single stranded, *e.g.,* for probes, although oligonucleotides may be double stranded, *e.g.,* for use in the construction of a gene mutant. Oligonucleotides of the invention are either sense or antisense oligonucleotides.

The term "naturally occurring nucleotides" referred to herein includes deoxyribonucleotides and ribonucleotides. The term "modified nucleotides" referred to herein includes nucleotides with modified or substituted sugar groups and the like. The term "oligonucleotide linkages" referred to herein includes Oligonucleotides linkages such as phosphorothioate, phosphorodithioate, phosphoroselerloate, phosphorodiselenoate, phosphoroanilothioate, phoshoraniladate, phosphoronmidate, and the like. *See e.g.,* LaPlanche *et al.* Nucl. Acids Res. 14:9081 (1986); Stec *et al.* J. Am. Chem. Soc. 106:6077 (1984), Stein *et al.* Nucl. Acids Res. 16:3209 (1988), Zon *et al.* Anti Cancer Drug Design 6:539 (1991); Zon *et al.* Oligonucleotides and Analogues: A Practical Approach, pp. 87-108 (F. Eckstein, Ed., Oxford University Press, Oxford England (1991)); Stec *et al.* U.S. Patent No. 5,151,510; Uhlmann and Peyman Chemical Reviews 90:543 (1990). An oligonucleotide can include a label for detection, if desired.

The term "selectively hybridize" referred to herein means to detectably and specifically bind. Polynucleotides, oligonucleotides and fragments thereof in accordance with the invention selectively hybridize to nucleic acid strands under hybridization and wash conditions that minimize appreciable amounts of detectable binding to nonspecific nucleic acids. High stringency conditions can be used to achieve selective hybridization conditions as known in the art and discussed herein. Generally, the nucleic acid sequence homology between the polynucleotides, oligonucleotides, and fragments of the invention and a nucleic acid sequence of interest will be at least 80%, and more typically with preferably increasing homologies of at least 85%, 90%, 95%, 99%, and 100%. Two amino acid sequences are homologous if there is a partial or complete identity between their sequences. For example, 85% homology means that 85% of the amino acids are identical when the two sequences are aligned for maximum matching. Gaps (in either of the two sequences being matched) are allowed in maximizing matching gap lengths of 5 or less are preferred with 2 or less being more preferred. Alternatively and preferably, two protein sequences (or polypeptide sequences derived from them of at least 30 amino acids in length) are homologous, as this term is used herein, if they have an alignment score of at more than 5 (in standard deviation units) using the program ALIGN with the mutation data matrix and a gap penalty of 6 or greater. *See* Dayhoff, M.O., in Atlas of Protein Sequence and Structure, pp. 101-1 10 (Volume 5, National Biomedical Research Foundation (1972)) and Supplement 2 to this volume, pp. 1-10. The two sequences or parts thereof are more preferably homologous if their amino acids are greater than or equal to 50% identical when

optimally aligned using the ALIGN program. The term "corresponds to" is used herein to mean that a polynucleotide sequence is homologous *(i.e.,* is identical, not strictly evolutionarily related) to all or a portion of a reference polynucleotide sequence, or that a polypeptide sequence is identical to a reference polypeptide sequence. In contradistinction, the term "complementary to" is used herein to mean that the complementary sequence is homologous to all or a portion of a reference polynucleotide sequence. For illustration, the nucleotide sequence "TATAC" corresponds to a reference sequence "TATAC" and is complementary to a reference sequence "GTATA".

The following terms are used to describe the sequence relationships between two or more polynucleotide or amino acid sequences: "reference sequence", "comparison window", "sequence identity", "percentage of sequence identity", and "substantial identity". A "reference sequence" is a defined sequence used as a basis for a sequence comparison a reference sequence may be a subset of a larger sequence, for example, as a segment of a full-length cDNA or gene sequence given in a sequence listing or may comprise a complete cDNA or gene sequence. Generally, a reference sequence is at least 18 nucleotides or 6 amino acids in length, frequently at least 24 nucleotides or 8 amino acids in length, and often at least 48 nucleotides or 16 amino acids in length. Since two polynucleotides or amino acid sequences may each (1) comprise a sequence *(i.e.,* a portion of the complete polynucleotide or amino acid sequence) that is similar between the two molecules, and (2) may further comprise a sequence that is divergent between the two polynucleotides or amino acid sequences, sequence comparisons between two (or more) molecules are typically performed by comparing sequences of the two molecules over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window", as used herein, refers to a conceptual segment of at least 18 contiguous nucleotide positions or 6 amino acids wherein a polynucleotide sequence or amino acid sequence may be compared to a reference sequence of at least 18 contiguous nucleotides or 6 amino acid sequences and wherein the portion of the polynucleotide sequence in the comparison window may comprise additions, deletions, substitutions, and the like *(i.e.,* gaps) of 20 percent or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by the local homology algorithm of Smith and Waterman Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman and Wunsch J. MoI. Biol. 48:443 (1970), by the search for similarity method of

Pearson and Lipman Proc. Natl. Acad. Sci. (U.S.A.) 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, (Genetics Computer Group, 575 Science Dr., Madison, Wis.), Geneworks, or MacVector software packages), or by inspection, and the best alignment *(i.e.,* resulting in the highest percentage of homology over the comparison window) generated by the various methods is selected.

The term "sequence identity" means that two polynucleotide or amino acid sequences are identical *(i.e.,* on a nucleotide-by-nucleotide or residue-by-residue basis) over the comparison window. The term "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base *(e.g.,* A, T, C, G, U or I) or residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the comparison window *(i.e.,* the window size), and multiplying the result by 100 to yield the percentage of sequence identity. The terms "substantial identity" as used herein denotes a characteristic of a polynucleotide or amino acid sequence, wherein the polynucleotide or amino acid comprises a sequence that has at least 85 percent sequence identity, preferably at least 90 to 95 percent sequence identity, more usually at least 99 percent sequence identity as compared to a reference sequence over a comparison window of at least 18 nucleotide (6 amino acid) positions, frequently over a window of at least 24-48 nucleotide (8-16 amino acid) positions, wherein the percentage of sequence identity is calculated by comparing the reference sequence to the sequence which may include deletions or additions which total 20 percent or less of the reference sequence over the comparison window. The reference sequence may be a subset of a larger sequence.

As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. *See* Immunology - A Synthesis (2nd Edition, E.S. Golub and D.R. Gren, Eds., Sinauer Associates, Sunderland Mass. (1991)). Stereoisomers *(e.g.,* D- amino acids) of the twenty conventional amino acids, unnatural amino acids such as α-, α-disubstituted amino acids, N-alkyl amino acids, lactic acid, and other unconventional amino acids may also be suitable components for polypeptides of the present invention. Examples of unconventional amino acids include: 4 hydroxyproline, γ-carboxyglutamate, ε-N,N,N-trimethyllysine, ε -N-acetyllysine, O-phosphoserine, N- acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, σ-N-methylarginine, and other similar amino acids and

imino acids *(e.g.,* 4- hydroxyproline). In the polypeptide notation used herein, the lefthand direction is the amino terminal direction and the righthand direction is the carboxy-terminal direction, in accordance with standard usage and convention.

Similarly, unless specified otherwise, the lefthand end of single- stranded polynucleotide sequences is the 5' end the lefthand direction of double-stranded polynucleotide sequences is referred to as the 5' direction. The direction of 5' to 3' addition of nascent RNA transcripts is referred to as the transcription direction sequence regions on the DNA strand having the same sequence as the RNA and which are 5' to the 5' end of the RNA transcript are referred to as "upstream sequences", sequence regions on the DNA strand having the same sequence as the RNA and which are 3' to the 3' end of the RNA transcript are referred to as "downstream sequences".

As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80 percent sequence identity, preferably at least 90 percent sequence identity, more preferably at least 95 percent sequence identity, and most preferably at least 99 percent sequence identity.

Preferably, residue positions which are not identical differ by conservative amino acid substitutions.

Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine valine, glutamic- aspartic, and asparagine-glutamine.

As discussed herein, minor variations in the amino acid sequences of antibodies or immunoglobulin molecules are contemplated as being encompassed by the present invention, providing that the variations in the amino acid sequence maintain at least 75%, more preferably at least 80%, 90%, 95%, and most preferably 99%. In particular, conservative amino acid replacements are contemplated. Conservative replacements are

those that take place within a family of amino acids that are related in their side chains. Genetically encoded amino acids are generally divided into families: (1) acidic amino acids are aspartate, glutamate; (2) basic amino acids are lysine, arginine, histidine; (3) non-polar amino acids are alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan, and (4) uncharged polar amino acids are glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. The hydrophilic amino acids include arginine, asparagine, aspartate, glutamine, glutamate, histidine, lysine, serine, and threonine. The hydrophobic amino acids include alanine, cysteine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine and valine. Other families of amino acids include (i) serine and threonine, which are the aliphatic-hydroxy family; (ii) asparagine and glutamine, which are the amide containing family; (iii) alanine, valine, leucine and isoleucine, which are the aliphatic family; and (iv) phenylalanine, tryptophan, and tyrosine, which are the aromatic family. For example, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the binding or properties of the resulting molecule, especially if the replacement does not involve an amino acid within a framework site. Whether an amino acid change results in a functional peptide can readily be determined by assaying the specific activity of the polypeptide derivative. Assays are described in detail herein. Fragments or analogs of antibodies or immunoglobulin molecules can be readily prepared by those of ordinary skill in the art. Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. Preferably, computerized comparison methods are used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known. Bowie *et al.* Science 253:164 (1991). Thus, the foregoing examples demonstrate that those of skill in the art can recognize sequence motifs and structural conformations that may be used to define structural and functional domains in accordance with the invention.

Preferred amino acid substitutions are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming

protein complexes, (4) alter binding affinities, and (4) confer or modify other physicochemical or functional properties of such analogs. Analogs can include various muteins of a sequence other than the naturally-occurring peptide sequence. For example, single or multiple amino acid substitutions (preferably conservative amino acid substitutions) may be made in the naturally- occurring sequence (preferably in the portion of the polypeptide outside the domain(s) forming intermolecular contacts. A conservative amino acid substitution should not substantially change the structural characteristics of the parent sequence *(e.g.,* a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in Proteins, Structures and Molecular Principles (Creighton, Ed., W. H. Freeman and Company, New York (1984)); Introduction to Protein Structure (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton et at. Nature 354:105 (1991).

The term "polypeptide fragment" as used herein refers to a polypeptide that has an amino terminal and/or carboxy-terminal deletion, but where the remaining amino acid sequence is identical to the corresponding positions in the naturally-occurring sequence deduced, for example, from a full length cDNA sequence. Fragments typically are at least 5, 6, 8 or 10 amino acids long, preferably at least 14 amino acids long' more preferably at least 20 amino acids long, usually at least 50 amino acids long, and even more preferably at least 70 amino acids long. The term "analog" as used herein refers to polypeptides which are comprised of a segment of at least 25 amino acids that has substantial identity to a portion of a deduced amino acid sequence and which has at least one of the following properties: (1) specific binding to RANTES, under suitable binding conditions or (2) ability to block appropriate RANTES binding. Typically, polypeptide analogs comprise a conservative amino acid substitution (or addition or deletion) with respect to the naturally-occurring sequence. Analogs typically are at least 20 amino acids long, preferably at least 50 amino acids long or longer, and can often be as long as a full-length naturally-occurring polypeptide.

Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. These types of non-peptide compound are termed "peptide mimetics" or "peptidomimetics". Fauchere, J. Adv. Drug Res. 15:29 (1986), Veber and Freidinger TIBS p.392 (1985); and Evans *et al.* J. Med.

Chem. 30:1229 (1987). Such compounds are often developed with the aid of computerized molecular modeling. Peptide mimetics that are structurally similar to therapeutically useful peptides may be used to produce an equivalent therapeutic or prophylactic effect. Generally, peptidomimetics are structurally similar to a paradigm polypeptide *(i.e.,* a polypeptide that has a biochemical property or pharmacological activity), such as human antibody, but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of: - CH_2NH--, -CH_2S-, -CH_2-CH_2--, -CH=CH-(cis and trans), -$COCH_2$- , $CH(OH)CH_2$- , and -CH_2SO-, by methods well known in the art. Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type *(e.g.,* D-lysine in place of L-lysine) may be used to generate more stable peptides. In addition, constrained peptides comprising a consensus sequence or a substantially identical consensus sequence variation may be generated by methods known in the art (Rizo and Gierasch Ann. Rev. Biochem. 61:387 (1992)); for example, by adding internal cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide.

The term "agent" is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials.

As used herein, the terms "label" or "labeled" refers to incorporation of a detectable marker, *e.g.,* by incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin *(e.g.,* streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or calorimetric methods). In certain situations, the label or marker can also be therapeutic. Various methods of labeling polypeptides and glycoproteins are known in the art and may be used. Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides *(e.g.,* ^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I), fluorescent labels *(e.g.,* FITC, rhodamine, lanthanide phosphors), enzymatic labels *(e.g.,* horseradish peroxidase, p-galactosidase, luciferase, alkaline phosphatase), chemiluminescent, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter *(e.g.,* leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance. The term "pharmaceutical agent or drug" as

used herein refers to a chemical compound or composition capable of inducing a desired therapeutic effect when properly administered to a patient.

Other chemistry terms herein are used according to conventional usage in the art, as exemplified by The McGraw-Hill Dictionary of Chemical Terms (Parker, S., Ed., McGraw-Hill, San Francisco (1985)).

As used herein, "substantially pure" means an object species is the predominant species present *(i.e.,* on a molar basis it is more abundant than any other individual species in the composition), and preferably a substantially purified fraction is a composition wherein the object species comprises at least about 50 percent (on a molar basis) of all macromolecular species present.

Generally, a substantially pure composition will comprise more than about 80 percent of all macromolecular species present in the composition, more preferably more than about 85%, 90%, 95%, and 99%. Most preferably, the object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) wherein the composition consists essentially of a single macromolecular species.

The term patient includes human and veterinary subjects. The term subject includes humans and other mammals.

Human Antibodies and Humanization of Antibodies

A huRANTES antibody is generated, for example, using the procedures described in the Examples provided below.

In other, alternative methods, a huRANTES antibody is developed, for example, using phage-display methods using antibodies containing only human sequences. Such approaches are well-known in the art, *e.g.,* in WO92/01047 and U.S. Pat. No. 6,521,404, which are hereby incorporated by reference. In this approach, a combinatorial library of phage carrying random pairs of light and heavy chains are screened using natural or recombinant source of RANTES or fragments thereof. In another approach, a huRANTES antibody can be produced by a process wherein at least one step of the process includes immunizing a transgenic, non-human animal with human RANTES protein. In this approach, some of the endogenous heavy and/or kappa light chain loci of this xenogenic non-human animal have been disabled and are incapable of the rearrangement required to generate genes encoding immunoglobulins in response to an antigen. In addition, at least one human heavy chain locus and at least one human light chain locus have been stably

transfected into the animal. Thus, in response to an administered antigen, the human loci rearrange to provide genes encoding human variable regions immunospecific for the antigen. Upon immunization, therefore, the xenomouse produces B-cells that secrete fully human immunoglobulins.

A variety of techniques are well-known in the art for producing xenogenic non-human animals. For example, see U.S. Pat. No. 6,075,181 and No. 6,150,584, which is hereby incorporated by reference in its entirety. This general strategy was demonstrated in connection with generation of the first XenoMouse™ strains as published in 1994. *See* Green *et al.* Nature Genetics 7:13-21 (1994), which is hereby incorporated by reference in its entirety. *See also*, U.S. Patent Nos. 6,162,963, 6,150,584, 6, 114,598, 6,075,181, and 5,939,598 and Japanese Patent Nos. 3 068 180 B2, 3 068 506 B2, and 3 068 507 B2 and European Patent No., EP 0 463 151 B1 and International Patent Applications No. WO 94/02602, WO 96/34096, WO 98/24893, WO 00/76310 and related family members.

In an alternative approach, others have utilized a "minilocus" approach in which an exogenous Ig locus is mimicked through the inclusion of pieces (individual genes) from the Ig locus. Thus, one or more VH genes, one or more D_H genes, one or more J_H genes, a mu constant region, and a second constant region (preferably a gamma constant region) are formed into a construct for insertion into an animal. See e.g., U.S. Patent Nos. 5,545,806; 5,545,807; 5,591,669; 5,612,205;5,625,825; 5,625,126; 5,633,425; 5,643,763; 5,661,016; 5,721,367; 5,770,429; 5,789,215; 5,789,650; 5,814,318; 5,877; 397; 5,874,299; 6,023,010; and 6,255,458; and European Patent No. 0 546 073 Bl; and International Patent Application Nos. WO 92/03918, WO 92/22645, WO 92/22647, WO 92/22670, WO 93/12227, WO 94/00569, WO 94/25585, WO 96/14436, WO 97/13852, and WO 98/24884 and related family members.

Generation of human antibodies from mice in which, through microcell fusion, large pieces of chromosomes, or entire chromosomes, have been introduced, has also been demonstrated. *See* European Patent Application Nos. 773 288 and 843 961 .

Human anti-mouse antibody (HAMA) responses have led the industry to prepare chimeric or otherwise humanized antibodies. While chimeric antibodies have a human constant region and a immune variable region, it is expected that certain human anti-chimeric antibody (HACA) responses will be observed, particularly in chronic or multi-dose utilizations of the antibody. Thus, it would be desirable to provide fully human antibodies

against RANTES in order to vitiate or otherwise mitigate concerns and/or effects of HAMA or HACA response.

The production of antibodies with reduced immunogenicity is also accomplished via humanization, chimerization and display techniques using appropriate libraries. It will be appreciated that murine antibodies or antibodies from other species can be humanized or primatized using techniques well known in the art *See e.g.,* Winter and Harris Immunol Today 14:43 46 (1993) and Wright *et al.* Crit, Reviews in Immunol. 12125-168 (1992). The antibody of interest may be engineered by recombinant DNA techniques to substitute the CHl, CH2, CH3, hinge domains, and/or the framework domain with the corresponding human sequence *{See* WO 92102190 and U.S. Patent Nos. 5,530,101, 5,585,089, 5, 693,761, 5,693,792, 5,714,350, and 5,777,085). Also, the use of Ig cDNA for construction of chimeric immunoglobulin genes is known in the art (Liu *et al.* P.N.A.S. 84:3439 (1987) and J. Immunol. 139:3521 (1987)). mRNA is isolated from a hybridoma or other cell producing the antibody and used to produce cDNA. The cDNA of interest may be amplified by the polymerase chain reaction using specific primers (U.S. Pat. Nos. 4,683, 195 and 4,683,202). Alternatively, a library is made and screened to isolate the sequence of interest. The DNA sequence encoding the variable region of the antibody is then fused to human constant region sequences. The sequences of human constant regions genes may be found in Kabat *et al.* (1991) Sequences of Proteins of immunological Interest, N.I.H. publication no. 91-3242. Human C region genes are readily available from known clones. The choice of isotype will be guided by the desired effecter functions, such as complement fixation, or activity in antibody-dependent cellular cytotoxicity. Preferred isotypes are IgGl, IgG3 and IgG4. Either of the human light chain constant regions, kappa or lambda, may be used. The chimeric, humanized antibody is then expressed by conventional methods.

Antibody fragments, such as Fv, $F(ab')_2$ and Fab may be prepared by cleavage of the intact protein, *e.g.,* by protease or chemical cleavage. Alternatively, a truncated gene is designed. For example, a chimeric gene encoding a portion of the $F(ab')_2$ fragment would include DNA sequences encoding the CHl domain and hinge region of the H chain, followed by a translational stop codon to yield the truncated molecule.

Consensus sequences of H and L J regions may be used to design oligonucleotides for use as primers to introduce useful restriction sites into the J region for subsequent linkage of V region segments to human C region segments. C region cDNA can be

modified by site directed mutagenesis to place a restriction site at the analogous position in the human sequence.

Expression vectors include plasmids, retroviruses, YACs, EBV derived episomes, and the like. A convenient vector is one that encodes a functionally complete human CH or CL immunoglobulin sequence, with appropriate restriction sites engineered so that any VH or VL sequence can be easily inserted and expressed. In such vectors, splicing usually occurs between the splice donor site in the inserted J region and the splice acceptor site preceding the human C region, and also at the splice regions that occur within the human CH exons. Polyadenylation and transcription termination occur at native chromosomal sites downstream of the coding regions. The resulting chimeric antibody may be joined to any strong promoter, including retroviral LTRs, *e.g.,* SV-40 early promoter, (Okayama *et al.* MoI. Cell. Bio. 3:280 (1983)), Rous sarcoma virus LTR (Gorman *et al.* P.N.A.S. 79:6777 (1982)), and moloney murine leukemia virus LTR (Grosschedl *et al.* Cell 41:885 (1985)). Also, as will be appreciated, native Ig promoters and the like may be used.

Further, human antibodies or antibodies from other species can be generated through display type technologies, including, without limitation, phage display, retroviral display, ribosomal display, and other techniques, using techniques well known in the art and the resulting molecules can be subjected to additional maturation, such as affinity maturation, as such techniques are well known in the art. Wright *et al.* Crit, Reviews in Immunol. 12125-168 (1992), Hanes and Plückthun PNAS USA 94:4937-4942 (1997) (ribosomal display), Parmley and Smith Gene 73:305-318 (1988) (phage display), Scott, TIBS, vol. 17:241-245 (1992), Cwirla *et al.* PNAS USA 87:6378-6382 (1990), Russel *et al.* Nucl. Acids Research 21:1081-1085 (1993), Hoganboom *et al.* Immunol. Reviews 130:43-68 (1992), Chiswell and McCafferty TIBTECH; 1O:8O-8A (1992), and U.S. Patent No. 5,733,743. If display technologies are utilized to produce antibodies that are not human, such antibodies can be humanized as described above.

Using these techniques, antibodies can be generated to RANTES expressing cells, RANTES itself, forms of RANTES, epitopes or peptides thereof, and expression libraries thereto *{See e.g.,* U.S. Patent No. 5,703,057) which can thereafter be screened as described above for the activities described above.

Design and Generation of Other Therapeutics

In accordance with the present invention and based on the activity of the antibodies that are produced and characterized herein with respect to RANTES, the design of other

therapeutic modalities beyond antibody moieties is facilitated. Such modalities include, without limitation, advanced antibody therapeutics, such as bispecifïc antibodies, immunotoxins, and radiolabeled therapeutics, generation of peptide therapeutics, gene therapies, particularly intrabodies, antisense therapeutics, and small molecules.

For example, in connection with bispecific antibodies, bispecifïc antibodies can be generated that comprise (i) two antibodies one with a specificity to RANTES and another to a second molecule that are conjugated together, (ii) a single antibody that has one chain specific to RANTES and a second chain specific to a second molecule, or (iii) a single chain antibody that has specificity to RANTES and a second molecule. Such bispecific antibodies are generated using techniques that are well known for example, in connection with (i) and (ii) *See e.g.,* Fanger *et al.* Immunol Methods 4:72-81 (1994) and Wright *et al.* Crit, Reviews in Immunol. 12125-168 (1992), and in connection with (iii) *See e.g.,* Traunecker *et al.* Int. J. Cancer (Suppl.) 7:51-52 (1992).

In connection with immunotoxins, antibodies can be modified to act as immunotoxins utilizing techniques that are well known in the art *See e.g.,* Vitetta Immunol Today 14:252 (1993). *See* also U.S. Patent No. 5,194,594. In connection with the preparation of radiolabeled antibodies, such modified antibodies can also be readily prepared utilizing techniques that are well known in the art. *See e.g.,* Junghans *et al.* in Cancer Chemotherapy and Biotherapy 655-686 (2d edition, Chafher and Longo, eds., Lippincott Raven (1996)). *See* also U.S. Patent Nos. 4,681,581, 4,735,210, 5,101,827, 5,102,990 (RE 35,500), 5,648,471, and 5,697,902. Each of immunotoxins and radiolabeled molecules would be likely to kill cells expressing RANTES.

In connection with the generation of therapeutic peptides, through the utilization of structural information related to RANTES and antibodies thereto, such as the antibodies of the invention or screening of peptide libraries, therapeutic peptides can be generated that are directed against RANTES. Design and screening of peptide therapeutics is discussed in connection with Houghten *et al.* Biotechniques 13:412-421 (1992), Houghten PNAS USA 82:5131-5135 (1985), Pinalla *et al.* Biotechniques 13:901-905 (1992), Blake and Litzi-Davis BioConjugate Chem. 3:510-513 (1992). Immunotoxins and radiolabeled molecules can also be prepared, and in a similar manner, in connection with peptidic moieties as discussed above in connection with antibodies. Assuming that the RANTES molecule (or a form, such as a splice variant or alternate form) is functionally active in a disease process, it will also be possible to design gene and antisense therapeutics thereto through conventional

techniques. Such modalities can be utilized for modulating the function of RANTES. In connection therewith the antibodies of the present invention facilitate design and use of functional assays related thereto. A design and strategy for antisense therapeutics is discussed in detail in International Patent Application No. WO 94/29444. Design and strategies for gene therapy are well known. However, in particular, the use of gene therapeutic techniques involving intrabodies could prove to be particularly advantageous. *See e.g.,* Chen *et al.* Human Gene Therapy 5:595-601 (1994) and Marasco Gene Therapy 4:11-15 (1997). General design of and considerations related to gene therapeutics is also discussed in International Patent Application No. WO 97/38137.

Knowledge gleaned from the structure of the RANTES molecule and its interactions with other molecules in accordance with the present invention, such as the antibodies of the invention, and others can be utilized to rationally design additional therapeutic modalities. In this regard, rational drug design techniques such as X-ray crystallography, computer-aided (or assisted) molecular modeling (CAMM), quantitative or qualitative structure-activity relationship (QSAR), and similar technologies can be utilized to focus drug discovery efforts. Rational design allows prediction of protein or synthetic structures which can interact with the molecule or specific forms thereof which can be used to modify or modulate the activity of RANTES. Such structures can be synthesized chemically or expressed in biological systems. This approach has been reviewed in Capsey *et al.* Genetically Engineered Human Therapeutic Drugs (Stockton Press, NY (1988)). Further, combinatorial libraries can be designed and synthesized and used in screening programs, such as high throughput screening efforts.

Therapeutic Administration and Formulations

It will be appreciated that administration of therapeutic entities in accordance with the invention will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences (15th ed, Mack Publishing Company, Easton, PA (1975)), particularly Chapter 87 by Blaug, Seymour, therein. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as Lipofectin™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid

mixtures containing carbowax. Any of the foregoing mixtures may be appropriate in treatments and therapies in accordance with the present invention, provided that the active ingredient in the formulation is not inactivated by the formulation and the formulation is physiologically compatible and tolerable with the route of administration *See* also Baldrick P. "Pharmaceutical excipient development: the need for preclinical guidance." Regul. Toxicol Pharmacol. 32(2):210-8 (2000), Wang W. "Lyophilization and development of solid protein pharmaceuticals." Int. J. Pharm. 203(1-2): 1-60 (2000), Charman WN "Lipids, lipophilic drugs, and oral drug delivery-some emerging concepts." J Pharm Sci. 89(8):967-78 (2000), Powell *et al.* "Compendium of excipients for parenteral formulations" PDA J Pharm Sci Technol. 52:238-31 1 (1998) and the citations therein for additional information related to formulations, excipients and carriers well known to pharmaceutical chemists.

The RANTES antagonists, huRANTES antibodies and therapeutic formulations of the invention, which include a RANTES antagonist, such as a huRANTES antibody of the invention, are used to treat or alleviate ischemia, a clinical indication associated with ischemia, reperfusion injury, a symptom associated with an immune-related disorder, such as, for example, an autoimmune disease or an inflammatory disorder.

Autoimmune diseases include, for example, Acquired Immunodeficiency Syndrome (AIDS, which is a viral disease with an autoimmune component), alopecia areata, ankylosing spondylitis, antiphospholipid syndrome, autoimmune Addison's disease, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune inner ear disease (AIED), autoimmune lymphoproliferative syndrome (ALPS), autoimmune thrombocytopenic purpura (ATP), Behcet's disease, cardiomyopathy, celiac sprue-dermatitis hepetiformis; chronic fatigue immune dysfunction syndrome (CFIDS), chronic inflammatory demyelinating polyneuropathy (CIPD), cicatricial pemphigoid, cold agglutinin disease, crest syndrome, Crohn's disease, Degos' disease, dermatomyositis-juvenile, discoid lupus, essential mixed cryoglobulinemia, fibromyalgia-fibromyositis, Graves' disease, Guillain-Barre syndrome, Hashimoto's thyroiditis, idiopathic pulmonary fibrosis, idiopathic thrombocytopenia purpura (ITP), IgA nephropathy, insulin-dependent diabetes mellitus, juvenile chronic arthritis (Still's disease), juvenile rheumatoid arthritis, Meniere's disease, mixed connective tissue disease, multiple sclerosis, myasthenia gravis, pemacious anemia, polyarteritis nodosa, polychondritis, polyglandular syndromes, polymyalgia rheumatica, polymyositis and dermatomyositis, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, psoriatic arthritis, Raynaud's phenomena, Reiter's

syndrome, rheumatic fever, rheumatoid arthritis, sarcoidosis, scleroderma (progressive systemic sclerosis (PSS), also known as systemic sclerosis (SS)), Sjogren's syndrome, stiff-man syndrome, systemic lupus erythematosus, Takayasu arteritis, temporal arteritis/giant cell arteritis, ulcerative colitis, uveitis, vitiligo and Wegener's granulomatosis.

Inflammatory disorders include, for example, chronic and acute inflammatory disorders. Examples of inflammatory disorders include Alzheimer's disease, asthma, atopic allergy, allergy, atherosclerosis, bronchial asthma, eczema, glomerulonephritis, graft vs. host disease, hemolytic anemias, osteoarthritis, sepsis, stroke, transplantation of tissue and organs, vasculitis, diabetic retinopathy and ventilator induced lung injury.

The huRANTES antibodies modulate an immune response in a subject, *e.g.,* in a human subject and transplanted organ. In one embodiment, the RANTES antagonist, huRANTES antibody, fragment thereof or therapeutic formulation thereof is used to treat ischemia, a clinical indication associated with ischemia, reperfusion injury, and/or another immune-related disorder in conjunction with a surgical treatment or other interventional therapy used in the art to treat a given disorder. For example, interventional therapies used in the treatment of ischemia, a clinical indication associated with ischemia, and/or reperfusion injury include surgical intervention or angioplasty. The RANTES antagonist, huRANTES antibody, fragment thereof or therapeutic formulation thereof is administered simultaneously *(i.e.,* during) the interventional therapy, or the RANTES antagonist, huRANTES antibody, fragment thereof or therapeutic formulation thereof is administered at a different time than the interventional therapy. For example, the RANTES antagonist, huRANTES antibody, fragment thereof or therapeutic formulation thereof is administered in some embodiments after an interventional therapy.

In one embodiment, the RANTES antagonist, huRANTES antibody, fragment thereof or therapeutic formulation thereof used to treat ischemia, a clinical indication associated with ischemia, reperfusion injury, and/or another immune-related disorder are administered in combination with any of a variety of anti-cytokine agents or anti-chemokine agents. Suitable anti-cytokine or anti-chemokine reagents recognize, for example, cytokines such as interleukin 1 (IL-I), IL-2, IL-4, IL-6, IL-12, IL-13, IL-15, IL-17, IL-18, IL-20, IL-21, IL-22, IL-23, IL-27 and IL-31, and/or chemokines such as MIP 1 alpha, MIP 1 beta, RANTES, MCPl, RANTES, ITAC, MIG, SDF and fractalkine.

In one embodiment, the RANTES antagonist, huRANTES antibody, fragment thereof or therapeutic formulation thereof used to treat ischemia, a clinical indication

associated with ischemia, reperfusion injury, and/or another immune-related disorder are administered in conjunction with one or more additional agents, or a combination of additional agents. For example, the RANTES antagonist *(e.g.,* huRANTES antibody) and additional agent are formulated into a single therapeutic composition, and the RANTES antagonist and additional agent are administered simultaneously. Alternatively, the RANTES antagonist and additional agent are separate from each other, *e.g.,* each is formulated into a separate therapeutic composition, and the RANTES antagonist and the additional agent are administered simultaneously, or the RANTES antagonist and the additional agent are administered at different times during a treatment regimen. For example, the RANTES antagonist *(e.g.,* huRANTES antibody) is administered prior to the administration of the additional agent, the RANTES antagonist is administered subsequent to the administration of the additional agent, or the RANTES antagonist and the additional agent are administered in an alternating fashion. As described herein, the RANTES antagonist and additional agent are administered in single doses or in multiple doses.

For example, in the treatment of coronary artery disease, the RANTES antagonist, huRANTES antibody, fragment thereof or therapeutic formulation thereof, is administered in conjunction with one or more additional agents such as cholesterol-lowering medicines, such as statins; anticoagulants, such as heparin and/or oral anticoagulants such as warfarin and dicumarol; aspirin, and other antiplatelet medicines; ACE (angiotensin-converting enzyme) inhibitors, such as sulfhydryl-containing ACE inhibitors *(e.g.,* Captopril), dicarboxylate-containing ACE inhibitors (e.g., Enalapril, Ramipril, Quinapril, Perindopril, Lisinopril, Benazepril); phosphonate-containing ACE inhibitors *(e.g.,* Fosinopril); beta blockers; calcium channel blockers; nitroglycerin; long-acting nitrates; glycoprotein Ilb-UIa inhibitors; and thrombolytic agents. The RANTES antagonist and the additional agent are administered simultaneously, or RANTES antagonist and the additional agent are administered at different times during a treatment regimen.

For example, in the treatment of cerebral vascular disease, the RANTES antagonist, huRANTES antibody, fragment thereof or therapeutic formulation thereof, is administered in conjunction with one or more additional agents such as cholesterol-lowering medicines, such as statins, aspirin, and other antiplatelet medicines. The RANTES antagonist and the additional agent are administered simultaneously, or RANTES antagonist and the additional agent are administered at different times during a treatment regimen.

For example, in the treatment of cardiac ischemia, the RANTES antagonist, huRANTES antibody, fragment thereof or therapeutic formulation thereof, is administered in conjunction with one or more additional agents such as aspirin, and other antiplatelet medicines; ACE (angiotensin-converting enzyme) inhibitors, such as sulfhydryl-containing ACE inhibitors *(e.g.,* Captopril), dicarboxylate-containing ACE inhibitors *(e.g.,* Enalapril, Ramipril, Quinapril, Perindopril, Lisinopril, Benazepril); phosphonate-containing ACE inhibitors *(e.g.,* Fosinopril); beta blockers; calcium channel blockers; nitroglycerin; and long-acting nitrates. The RANTES antagonist and the additional agent are administered simultaneously, or RANTES antagonist and the additional agent are administered at different times during a treatment regimen.

For example, in the treatment of myocardial ischemia, the RANTES antagonist, huRANTES antibody, fragment thereof or therapeutic formulation thereof is administered in conjunction with one or more additional agents such as beta blockers; calcium channel blockers; nitroglycerin; and long-acting nitrates. The RANTES antagonist and the additional agent are administered simultaneously, or RANTES antagonist and the additional agent are administered at different times during a treatment regimen.

For example, in the treatment of renal ischemia, the RANTES antagonist, huRANTES antibody, fragment thereof or therapeutic formulation thereof is administered in conjunction with one or more additional agents such as cholesterol-lowering medicines, such as aspirin, and other antiplatelet medicines. The RANTES antagonist and the additional agent are administered simultaneously, or RANTES antagonist and the additional agent are administered at different times during a treatment regimen.

For example, in the treatment of peripheral vascular disease, the RANTES antagonist, huRANTES antibody, fragment thereof or therapeutic formulation thereof is administered in conjunction with one or more additional agents such as anticoagulants, such as heparin and/or oral anticoagulants such as warfarin and dicumarol; aspirin, and other antiplatelet medicines; pentoxifylline; cilostazol ;and thrombolytic agents. The RANTES antagonist and the additional agent are administered simultaneously, or RANTES antagonist and the additional agent are administered at different times during a treatment regimen.

For example, in the treatment of multiple sclerosis, the huRANTES antibody, or therapeutic formulation thereof, is administered in conjunction with one or more additional agents such as interferon beta Ia, interferon beta Ib, glatiramer acetate, natalizumab, Copaxone, and combinations thereof. The huRANTES antibody and the additional agent are

administered simultaneously, or the huRANTES antibody and the additional agent are administered at different times during a treatment regimen.

In the treatment of Crohn's disease, the huRANTES antibody, or therapeutic formulation thereof, is administered in conjunction with one or more additional agents such as an antibiotic, an aminosalicylate, Infliximab, Adalimumab, and combinations thereof. Suitable antibiotics include, *e.g.,* metronidazole and/or ciprofloxacin. Suitable aminosalicylates include, for example, mesalamine and/or sulfasalazine. The huRANTES antibody and the additional agent are administered simultaneously, or the huRANTES antibody and the additional agent are administered at different times during a treatment regimen.

In the treatment of ulcerative colitis, the huRANTES antibody, or therapeutic formulation thereof, is administered in conjunction with one or more additional agents such as 6-mercaptopurine, azathioprine, Infliximab and combinations thereof. The huRANTES antibody and the additional agent are administered simultaneously, or the huRANTES antibody and the additional agent are administered at different times during a treatment regimen.

In the treatment of psoriasis, the huRANTES antibody, or therapeutic formulation thereof, is administered in conjunction with one or more additional agents such as alefacept, efalizumab, Adalimumab, Infliximab, cyclosporine, Methotrexate, and combinations thereof. The huRANTES antibody and the additional agent are administered simultaneously, or the huRANTES antibody and the additional agent are administered at different times during a treatment regimen.

In the treatment of atherosclerosis, the huRANTES antibody, or therapeutic formulation thereof, is administered in conjunction with one or more additional agents such as warfarin, a cholesterol lowering drug, and combinations thereof. Suitable cholesterol lowering drugs include, for example, statins and fibrates. The huRANTES antibody and the additional agent are administered simultaneously, or the huRANTES antibody and the additional agent are administered at different times during a treatment regimen.

The huRANTES antibodies and therapeutic formulations thereof are used in methods of treating or alleviating a symptom associated with an immune-related disorder. For example, the compositions of the invention are used to treat or alleviate a symptom of any of the autoimmune diseases and inflammatory disorders described herein. Symptoms associated with immune-related disorders include, for example, inflammation, fever, loss of

appetite, weight loss, abdominal symptoms such as, for example, abdominal pain, diarrhea or constipation, joint pain or aches (arthralgia), fatigue, rash, anemia, extreme sensitivity to cold (Raynaud's phenomenon), muscle weakness, muscle fatigue, changes in skin or tissue tone, shortness of breath or other abnormal breathing patterns, chest pain or constriction of the chest muscles, abnormal heart rate *(e.g.,* elevated or lowered), light sensitivity, blurry or otherwise abnormal vision, and reduced organ function.

The RANTES antagonists, such as a huRANTES antibody, and therapeutic formulations thereof are administered to a subject suffering from ischemia, a clinical indication associated with ischemia, reperfusion injury, and/or an immune-related disorder, such as an autoimmune disease or an inflammatory disorder. A subject or organ suffering from ischemia, a clinical indication associated with ischemia, reperfusion injury, an autoimmune disease or an inflammatory disorder is identified by methods known in the art. For example, subjects are identified using any of a variety of clinical and/or laboratory tests such as, physical examination, radiologic examination and blood, urine and stool analysis to evaluate immune status. For example, patients suffering from lupus are identified, *e.g.,* by using the anti-nuclear antibody test (ANA) to determine if auto-antibodies to cell nuclei are present in the blood. Patients suffering from Crohn's are identified, *e.g.,* using an upper gastrointestinal (GI) series and/or a colonoscopy to evaluate the small and large intestines, respectively. Patients suffering from psoriasis are identified, *e.g.,* using microscopic examination of tissue taken from the affected skin patch, while patients suffering from rheumatoid arthritis are identified using, *e.g.,* blood tests and/or x-ray or other imaging evaluation. Patients suffering from atherosclerosis are identified, *e.g.,* using blood tests, electrocardiograms (ECG), stress testing, coronary angiography, ultrasound, and computed tomography (CT).

Administration of a RANTES antagonist, such as a huRANTES antibody, to a patient suffering from ischemia, a clinical indication associated with ischemia, reperfusion injury, or an immune-related disorder such as an autoimmune disease or an inflammatory disorder is considered successful if any of a variety of laboratory or clinical results is achieved. For example, administration of a huRANTES antibody to a patient suffering from ischemia, a clinical indication associated with ischemia, reperfusion injury, an immune-related disorder such as an autoimmune disease or an inflammatory disorder is considered successful one or more of the symptoms associated with the disorder is alleviated, reduced, inhibited or does not progress to a further, *i.e.,* worse, state. Administration of a

huRANTES antibody to a patient suffering from ischemia, a clinical indication associated with ischemia, reperfusion injury, an immune-related disorder such as an autoimmune disease or an inflammatory disorder is considered successful if the disorder, *e.g.*, an autoimmune disorder, enters remission or does not progress to a further, *i.e.*, worse, state.

Diagnostic and Prophylactic Formulations

The fully human anti-RANTES MAbs of the invention are used in diagnostic and prophylactic formulations. In one embodiment, a RANTES antagonist, such as a huRANTES MAb of the invention, is administered to patients that are at risk of developing ischemia, a clinical indication associated with ischemia, reperfusion injury, and/or one of the aforementioned autoimmune diseases. A patient's or organ's predisposition to ischemia, a clinical indication associated with ischemia, reperfusion injury, and/or one or more of the aforementioned autoimmune diseases can be determined using genotypic, serological or biochemical markers.

In another embodiment of the invention, a RANTES antagonist, such as a huRANTES antibody is administered to human individuals diagnosed with a clinical indication associated with ischemia, reperfusion injury, one or more of the aforementioned autoimmune diseases. Upon diagnosis, a RANTES antagonist, such as a huRANTES antibody is administered to mitigate or reverse the effects of the clinical indication associated with ischemia, reperfusion injury, or autoimmunity.

Antibodies of the invention are also useful in the detection of RANTES in patient samples and accordingly are useful as diagnostics. For example, the huRANTES antibodies of the invention are used in *in vitro* assays, *e.g.*, ELISA, to detect RANTES levels in a patient sample.

In one embodiment, a huRANTES antibody of the invention is immobilized on a solid support *(e.g.*, the well(s) of a microliter plate). The immobilized antibody serves as a capture antibody for any RANTES that may be present in a test sample. Prior to contacting the immobilized antibody with a patient sample, the solid support is rinsed and treated with a blocking agent such as milk protein or albumin to prevent nonspecific adsorption of the analyte.

Subsequently the wells are treated with a test sample suspected of containing the antigen, or with a solution containing a standard amount of the antigen. Such a sample is, *e.g.*, a serum sample from a subject suspected of having levels of circulating antigen considered to be diagnostic of a pathology. After rinsing away the test sample or standard,

the solid support is treated with a second antibody that is detectably labeled. The labeled second antibody serves as a detecting antibody. The level of detectable label is measured, and the concentration of RANTES antigen in the test sample is determined by comparison with a standard curve developed from the standard samples.

It will be appreciated that based on the results obtained using the huRANTES antibodies of the invention in an *in vitro* diagnostic assay, it is possible to stage a disease *(e.g.*, a clinical indication associated with ischemia, an autoimmune or inflammatory disorder) in a subject based on expression levels of the RANTES antigen. For a given disease, samples of blood are taken from subjects diagnosed as being at various stages in the progression of the disease, and/or at various points in the therapeutic treatment of the disease. Using a population of samples that provides statistically significant results for each stage of progression or therapy, a range of concentrations of the antigen that may be considered characteristic of each stage is designated.

All publications and patent documents cited herein are incorporated herein by reference as if each such publication or document was specifically and individually indicated to be incorporated herein by reference. Citation of publications and patent documents is not intended as an admission that any is pertinent prior art, nor does it constitute any admission as to the contents or date of the same. The invention having now been described by way of written description, those of skill in the art will recognize that the invention can be practiced in a variety of embodiments and that the foregoing description and examples below are for purposes of illustration and not limitation of the claims that follow.

EXAMPLES

The following examples, including the experiments conducted and results achieved are provided for illustrative purposes only and are not to be construed as limiting upon the present invention.

EXAMPLE 1: Cloning, Expression and Purification of Human RANTES

Cloning

The gene encoding the mature protein human RANTES (GenBank Accession No. M21121) or other chemokines were cloned in an expression plasmid pET43 (Novagen Madison, WI) by PCR amplification. The sequence for the Factor X protease cleavage site

was introduced at the C-terminus of NusA. The sequence for the AviTag (Avidity, Denver CO) biotinylation site was introduced at the C-terminus of the chemokine coding sequence. The pET-derived plasmids were used for the co-transformation of bacterial strain Origami B with pACYC184-BirA plasmid that encodes the biotin ligase gene. For expression in mammalian cells, the gene encoding relevant chemokines were cloned from cDNA in the pEAK8 expression vector (Edge Biosystems, Gaithersburg, MD). An AviTag biotinylation site was introduced at the C-terminus of the protein followed by an internal ribosome entry site (IRES) allowing for the expression of the BirA gene encoding a biotin ligase. This construct allows for the secreted expression of chemokines biotinylated *in vivo* at a single site.

Expression of NusA-huRANTESfusion protein in E.coli

An overnight culture of bacteria harboring the expression construct was diluted 1:30 into Terrific broth (InvitroGen) containing 50 μg/mL Ampicillin, 10 μg /mL Kanamicin, 5 μg/mL Tetracycline, 20 μg/mL Chloramphenicol and 50 μM Biotin. The culture was incubated at 37°C with shaking until OD 600=0.7 was reached. IPTG was then added to a final concentration of 1 mM, incubated for 15 min. at 37 °C and overnight at 25°C.

Expression of huRANTES in mammalian cells

PEAK cells were cultures in DMEM (Sigma) supplemented with 10 % FCS, 2 mM L-Glutamine (Sigma), 25 μg/ml gentamycin (Sigma) and incubated at 37°C, 5% CO_2. PEAK cells were transfected with the modified pEAK8 vectors using Minis transfection reagent. Puromycin (Sigma) was added at 1 μg/ml after cell adherence in order to select and maintain transfected cell populations. Biotin (Sigma) was added to production batches at 50 μM. Biotinylated chemokines from the transfected PEAK cell supernatants were shown to be active in chemotaxis assays.

Purification and cleavage affusion proteins

Bacterial pellets were resuspended in Bugbuster (Novagen) containing Benzonase Nuclease and protease inhibitor Complete EDTA-free (Roche) and incubated for 1 hour at 4°C. The soluble and insoluble fractions were separated by centrifugation at 10,000 g for 15 min at 4°C. Soluble and insoluble protein fractions were analyzed by SDS-PAGE (Novex gels, InvitroGen). The soluble fraction was diluted 1/2 with Buffer A (Tris-HCl 100 mM pH 8.0, NaCl 600 mM, $CaCl_2$ 10 mM, Imidazole 40 mM), mixed with 50% (v/v) Ni-NTA

agarose (Qiagen) previously equilibrated in Buffer B (Tris-HCl 50 mM pH 8.0, NaCl 300 mM, CaCh 5 mM, Imidazole 20 mM). The mixture was incubated for 30 min at RT with gentle shaking. The beads obtained after centrifugation were loaded in Poly-Prep chromatography columns (Biorad), washed three times with 5 volumes of Buffer B and eluted with Buffer C (Tris-HCl 50 mM pH 8.0, NaCl 200 mM, $CaCl_2$ 5 mM, Imidazole 400 mM). Elution fractions containing the protein were pooled and desalted using PD-10 columns (Amersham). NusA-chemokine fusion proteins were cleaved by Factor X (Novagen, Madison, WI) by incubating 1 mg protein with 25 U Factor X at 30 °C for up to 24 h in cleavage buffer (Tris-HCl 50 mM pH 8.0 , NaCl 200 mM , $CaCl_2$ 5 mM). For some of the fusions proteins, the parameters for optimal cleavage were slightly different but were easily determined by varying incubation time (4-24h) and/or temperature (25-37°C). The cleaved protein was analyzed by SDS-PAGE and the activity tested by chemotaxis.

EXAMPLE 2: Screening of human scFv libraries

General procedures for construction and handling of human scFv libraries are described in Vaughan et al., (Nat. Biotech. 1996, 14:309-314), hereby incorporated by reference in its entirety. Libraries of human scFv were screened against huRANTES according to the following procedure.

Liquid phase selections.

Aliquots of scFv phage libraries (10^{12} Pfu) obtained from Cambridge Antibody Technology (Cambridge, UK) were blocked with PBS containing 3% (w/v) skimmed milk for one hour at room temperature on a rotary mixer. Blocked phage was then deselected on streptavidin magnetic beads (Dynal M-280) for one hour at room temperature on a rotary mixer. Deselected phage was then incubated with in vivo biotinylated huRANTES (100 nM) for two hours at room temperature on a rotary mixer. This selection step was performed either on NusA-huRANTES biotinylated fusion protein or on biotinylated-huRANTES released from the fusion by proteolytic cleavage. Beads were captured using a magnetic stand followed by four washes with PBS/0.1% Tween 20 and 3 washes with PBS. Beads were then directly added to 10 ml of exponentially growing TGl cells and incubated for one hour at 37 °C with slow shaking (100 rpm). An aliquot of the infected TGl was serial diluted to titer the selection output. The remaining infected TGl were spun at 3000 rpm for 15 minutes and re-suspended in 0.5 ml 2xTY-AG (2xTY media containing 100 μg/ml ampicilin and 2% glucose) and spread on 2xTYAG agar Bioassay plates. After

overnight incubation at 30 °C 10 ml of 2xTYAG was added to the plates and the cells were scraped form the surface and transferred to a 50 ml polypropylene tube. 2xTYAG containing 50% glycerol was added to the cell suspension to obtain a final concentration of 17% glycerol. Aliquots of the selection round were kept at -80 °C.

Phage rescue.

100 µl of cell suspension obtained from previous selection rounds were added to 20 ml of 2xTYAG and grown at 37 °C with agitation (240 rpm) until an OD_{600} of 0.3 to 0.5 was reached. The culture was then super-infected with 3.3 x 10^{10} MK13K07 helper phage and incubated for one hour at 37 °C (150 rpm). The medium was then changed by centrifugating the cells at 2000 rpm for 10 minutes, removing the medium and resuspending the pellet in 20 ml of 2xTY-AK (lOOµg/ml ampicilin; 50 µg/ml kanamycin). The culture was then grown overnight at 30 °C (240 rpm).

Monoclonal phage rescuefor ELISA.

Single clones were picked into a microtiter plate containing 150µl of 2xTYAG media (2% glucose) per well and grown at 37°C (100-120 rpm) for 5-6h. M13KO7 helper phage was added to each well to obtain a multiplicity of infection (MOI) of 10 *(i.e.,* 10 phage for each cell in the culture) and incubated at 37°C (100 rpm) for Ih. Following growth, plates were centrifuged at 3,200 rpm for 10 min. Supernatant was carefully removed, cells re-suspended in 150 µl 2xTYAK medium and grown overnight at 30 °C (120 rpm). For the ELISA, the phage are blocked by adding 150µl of 2x concentration PBS containing 5% skimmed milk powder followed by one hour incubation at room temperature. The plates were then centrifuged 10 minutes at 3000 rpm and the phage containing supernatant used for the ELISA.

Phage ELISA.

ELISA plates (Maxisorb, NUNC) were coated overnight with 2 µg/ml NusA-Rantes fusion protein in PBS. Control plates were coated with 2µg/ml NusA. Plates were then blocked with 3% skimmed milk / PBS at room temperature for Ih. Plates were washed 3 times with PBS 0.05% Tween 20 before transferring the pre-blocked phage supernatants and incubation for one hour at room temperature. Plates were then washed 3 times with PBS 0.05% Tween 20. 50µl of 3% skimmed milk / PBS containing (HRP)-conjugated anti-M13

antibody (Amersham, diluted 1:10,000) to each well. Following incubation at room temperature for 1 hr, the plates were washed 5 times with PBS 0.05% Tween 20. The ELISA was then revealed by adding 50µl of TMB (Sigma) and 50µl of 2N H_2SO_4 to stop the reaction. Absorption intensity was read at 450nm.

Phage clone sequencing

Single clones were placed in a microtiter plate containing 150µI of 2xTYAG media (2% glucose) per well and grown at 30 °C (120 rpm) overnight. The next day 5 µl of culture was transferred into another plate containing 45 µl of dH_2O and mixed. The plates was then frozen at -20 ºC. After thawing, 1 µl of this suspension was used for PCR amplification using standard PCR protocols with primer specific for pCANTAB6: mycseq, 5'-CTCTTCTGAGATGAGTTTTTG-S' (SEQ ID NO: 269) and gene3 leader, 5'-TTATTATTCGCAATTCCTTTAGTTGTTCCT-3' (SEQ ID NO: 270).

The PCR reactions were purified in 96 well format using the Montage PCRµ96 system (Millipore). 5 µl of the eluted DNA was sequencing using the mycseq and gene31eader primers.

ScFv periplasmic preparation for functional tests.

Individual clones were inoculated into a deep well microtiter plate containing 0.9 ml of 2xTYAG media (0.1% glucose) per well and grown at 37 °C for 5-6h (250 rpm). lOOµl per well of 0.2 mM IPTG in 2xTY medium were then added to give a final concentration of 0.02 mM IPTG. Plates were then incubated overnight at 30 °C with shaking at 250 rpm. The deep-well plates were centrifuged at 2,500 rpm for 10 min and the supernatant carefully removed. The pellets were re-suspended in 150µl TES buffer (50 mM Tris / HCl (pH 8), 1 mM EDTA (pH 8), 20% sucrose, complemented with Complete protease inhibitor, Roche). A hypotonic shock was produced by adding 150 µl of diluted TES buffer (1:5 TES:water dilution) and incubation on ice for 30 min. Plates were then centrifuged at 4000 rpm for 10 minutes to remove cells and debris. The supernatants were carefully transferred into another microtiter plate and kept on ice for immediate testing in functional assays or ELISAs.

Large scale scFv purification

A starter culture of 1 ml of 2xTYAG was inoculated with a single colony from a freshly streaked 2xTYAG agar plate and incubated with shaking (240 rpm) at 37 °C for 5

hours. 0.9 ml of this culture was used to inoculate a 400 ml culture of the same media and was grown overnight at 30 °C with vigorous shaking (300 rpm).

The next day the culture was induced by adding 400 µl of IM IPTG and incubation was continued for an additional 3 hours. The cells were collected by centrifugation at 5,000 rpm for 10 minutes at 4 ºC. Pelleted cells were resuspended in 10 ml of ice-cold TES buffer complemented with protease inhibitors as described above. Osmotic shock was achieved by adding 15 ml of 1:5 diluted TES buffer and incubation for 1 hour on ice. Cells were centrifuged at 10,000 rpm for 20 minutes at 4 °C to pellet cell debris. The supernatant was carefully transferred to a fresh tube. Imidazole was added to the supernatant to a final concentration of 10 mM. 1 ml of Ni-NTA resin (Qiagen), equilibrated in PBS was added to each tube and incubated on a rotary mixer at 4 °C (20 rpm) for 1 hour.

The tubes were centrifuged at 2,000 rpm for 5 minutes and the supernatant carefully removed. The pelleted resin was resuspended in 10 ml of cold (4 ºC) Wash buffer 1 (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH to 8.0). The suspension was added to a polyprep column (Biorad). 8 ml of cold Wash Buffer 2 (50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole, pH to 8.0) were used to wash the column by gravity flow. The scFv were eluted from the column with 2 ml of Elution buffer (50 mM NaH_2PO_4, 300 mM NaCl, 250 mM imidazole, pH to 8.0). Fractions were analyzed by absorption at 280 nm and protein containing fractions were pooled before buffer exchange on a PDlO desalting column (Amersham) equilibrated with PBS. The scFv in PBS were analyzed by SDS-PAGE and quantified by absorption at 280 nm. The purified scFv were aliquoted and stored at -20ºC and at 4ºC.

EXAMPLE 3: Inhibition of huRANTES induced Calcium Flux using scFv Extracts

Periplasmic extracts of various huRANTES scFv were produced as described above. L1.2 cells expressing hCCR5 were cultured in RPMI medium supplemented with 10% FCS. Extracts containing the scFv were incubated with 2-1OnM of huRANTES (Peprotech, Rocky Hill NJ) for 30 minutes at room temperature. Cells were washed in PBS and loaded with 2 µM Fura 2/AM. 100 µl of loaded cells were added to each well of a 96-well black, transparent flat-bottom plate and calcium flux kinetics were recorded by measuring the fluorescence at 514 nm upon excitation at 340 or 380nm on a Flex station II instrument (Molecular Devices) upon addition of the chemokine scFv mix. The inhibitory activity of each scFv extract was assessed by comparison to an extract containing an irrelevant scFv.

EXAMPLE 4: scFv Inhibition of huRANTES-Induced cell chemotaxis

Wild type Ll. 2 cells and Ll. 2 cells expressing hCCR5 were cultured in RPMI medium supplemented with 10% FCS. The day before the experiment cells were incubated with 0.6 mg/ml of butyric acid. Different concentrations of purified scFv were incubated with 0.2-1OnM huRANTES and placed in the bottom chamber of chemotaxis 96-well plate (Neuroprobe). The filter plate was placed on top of the chemotaxis plate and each well was overlaid with 20µl of a 10^6 cells/ml suspension. The plate was incubated for 2 hours at 37°C. Cells that migrated through the filter were stained with DRAQ5 (Alexis Corporation) and counted on an FMAT 8200 reader (Applied Biosystems, Foster City CA). The ICs_0 (where 50% of the huRANTES induced cell migration is inhibited, *i.e.*, 50% inhibitory concentration), for each candidate antibody was determined (Table 4).

Table 4. Potency of antibodies tested in scFv format in chemotaxis functional assays. Chemotaxis was performed using InM, of huRANTES,

Clone ID	Chemotaxis IC_{50} (nM)
CG11	3.6
BG11	7
A9	72
E6	72
H6	25
G2	62
E10	9.4
C10	41
2D1	1.3
A5	21
H11	4.3
D1	22
E7	3.8
C8	90
1D9	0.82
1E4	1.25
3E7	0.47

4D8	**0.08**
5E1	**0.2**
6A8	**0.94**
7B5	**1.6**

EXAMPLE 5: Reformatting scFv into IgG Format

The V_H and V_L sequence of selected scFv were amplified with specific oligonucleotides introducing a leader sequence and a HindIII restriction site at the 5' end. An Apal or an Avrll site was introduced at the 3' end of the heavy and light chain sequence, respectively. The amplified V_H sequences were digested HindIII/Apal and cloned into the pCon_gammal expression vector (LONZA, Basel, Switzerland). The amplified V_L sequences were digested HindIII/ Avrll and cloned into the pCon_lambda2 expression vector (LONZA). The constructions were verified by sequencing before transfection into mammalian cells.

The V_H and V_L CDNA sequences in their appropriate expression vectors were transfected into mammalian cells using the Fugene 6 Transfection Reagent (Roche, Basel, Switzerland). Briefly, Peak cells were cultured in 6-well plates at a concentration of 6×10^5 cells per well in 2 ml culture media containing fetal bovine serum. The expression vectors, encoding the candidate V_H and V_L sequences, were co-transfected into the cells using the Fugene 6 Transfection Reagent according to manufacturer's instructions. One day following transfection, the culture media was aspirated, and 3 ml of fresh serum-free media was added to cells and cultured for three days at 37 °C. Following three days culture period, the supernatant was harvested for IgG purified on protein G-Sepharose 4B fast flow columns (Sigma, St. Louis, MO) according to manufacturer's instructions. Briefly, supernatants from transfected cells were incubated overnight at 4 °C with ImmunoPure (G) IgG binding buffer (Pierce, Rockford IL). Samples were then passed over Protein G-Sepharose 4B fast flow columns and the IgG consequently purified using elution buffer. The eluted IgG fraction was then dialyzed against PBS and the IgG content quantified by absorption at 280 nm. Purity and IgG integrity were verified by SDS-PAGE.

EXAMPLE 6: **Production of native human huRANTES.**

THPl cells were cultured in 10ml media at a concentration at lxioVml with 10 μg/ml LPS. Following overnight incubation at 37ºC, cells were centrifuged, supernatant

was collected and the concentration of native huRANTES was estimated in a chemotaxis assay as described in Example 4. Not only native huRANTES but also other ligands of CCR5 are produced by THPl cells when stimulated with LPS as described above. Therefore, when using these supernatants in chemotaxis assays to determine the neutralization potential of anti-huRANTES antibodies, the other ligands of CCR5 were neutralized with a mixture of antibodies against hMIP-l α, hMIP-l β, hMCP-2, hMIP-1δ each at a concentration of 5 μg/ml (R&D Systems).

EXAMPLE 7: Inhibition of huRANTES-Induced Calcium Flux or Cell Chemotaxis using Reformatted scFv into IgGl Format

scFv were reformatted into an IgG format as described above in Example 5. The neutralizing potential of the IgG on huRANTES-induced calcium flux or cell chemotaxis was evaluated using the cell-based assays described in Example 3 and 4. As shown as examples in Figure 1 IgGs C8, 1D9 and 1E4 inhibit the activity of both recombinant and native huRANTES in a dose-dependent manner. The IC_{50} values in these assays for all antibodies are summarized in Tables 5 and 6.

Table 5. Potency of antibodies tested in IgGl format in chemotaxis and calcium flux functional assays. Chemotaxis was performed using either InM or 0.2 nM of recombinant huRANTES while calcium flux was induced with 1OnM of recombinant huRANTES.

Clone ID	Chemotaxis IC_{50} (nM) 1 nM huRANTES	Chemotaxis IC_{50} (nM) 0.2 nM huRANTES	Calcium Flux IC_{50} (nM) rhuRANTES
CG11	4.8	ND	9.5
BG11	29	ND	7.7
A9	1	ND	3.3
E6	14	ND	12.7
H6	8.7	ND	9
G2	18.4	ND	ND
E10	16	ND	ND
C10	17	ND	ND
2D1	1.7	1.3	ND
A5	13.2	ND	ND

H11	**1.3**	**ND**	**ND**
D1	**7**	**ND**	**ND**
E7	**2.2**	**ND**	**ND**
C8	**2.1**	**0.49**	**ND**
1D9	**0.35**	**0.038**	**ND**
1E4	**0.46**	**0.034**	**ND**
3E7	**0.68**	**0.25**	**ND**
4D8	**1.16**	**0.22**	**ND**
5E1	**0.82**	**0.25**	**ND**
6A8	**0.74**	**0.31**	**ND**
7B5	**1.1**	**0.31**	**ND**

Table 6. Potency of antibodies tested in IgGl format in chemotaxis functional assay performed using native human RANTES produced by THPl cells at a concentration of < 1 nM.

Clone ID	**Chemotaxis IC_{50} (nM) >1nM native huRANTES**
C8	**1.6**
1D9	**0.033**
1E4	**0.028**

EXAMPLE 8: Antibody **binding to huRANTES immobilized on glycosaminoglycan**

As with many chemokines, huRANTES is able to oligomerize and bind to glycosaminoglycans (GAG) expressed at surface of cells such as endothelial cells. In order to make sure that the antibodies were able to bind to huRANTES in this context, they were tested in the following assay. Streptavidin coated 96 well plates (Roche, Basel, Switzerland) were coated with biotin labeled heparin as a prototypic GAG (Sigma, St. Louis, MO). After washing the excess heparin huRANTES was added the wells for immobilization GAG. After incubation with the antibodies to be tested, the wells were washed and binding was revealed with an anti-human IgG Fcg specific antibody coupled to HRP (Jackson, West Grove, PA). As shown in Figure 2 some antibodies were able to bind huRANTES when

bound to GAG whereas others were unable to do so probably because their epitope on huRANTES was no longer accessible within the oligomeric structure. The capacity of the antibodies to bind huRANTES in the context of GAG is summarized in Table 7.

Table 7. Ability of antibodies to bind to huRANTES immobilized on GAG.

Clone ID	Binding to huRANTES immobilized on GAGs
CG11	No
BG11	Yes
A9	No
E6	Yes
H6	No
G2	Yes
E10	Yes
C10	Yes
2D1	Yes
A5	Yes
H11	No
D1	No
E7	No
C8	Yes
1E4	Yes
1D9	Yes

EXAMPLE 9: Affinity Maturation of antibody 2Dl

A selected lead candidate (2Dl) was subjected to affinity maturation in order to increase its affinity for huRANTES and its potency in huRANTES neutralization assays. Stretches of 5 residues in the CDR3 of the heavy or light chain were randomized in order to generate 6 libraries (Library size ranging from $5x10^7$ to 10^9). Three high stringency selection rounds were performed as described in Example 2. Screening for improved variant was performed using scFv periplasmic extracts in an epitope competition assay. Briefly, the parent antibody (2Dl) was coated on plates and diluted periplasmic scFv extracts were added to each well. Biotinylated huRANTES was then added and incubated for 2 hours at room temperature. After washing, huRANTES remaining bound to the coated parent

antibody was revealed using streptavidin coupled HRP (Jackson, West Grove PA). As a reference to identify improved variants 2Dl scFv was used to compete coated 2Dl in an IgG format.

EXAMPLE 10: Generation of a stable CHOKlSV cell line expressing 1E4

The CHOKlSV cell line, property of Lonza Biologies, pic, was used to generate pools through semi-stable transfection for the production of the antibody 1E4. Briefly, exponentially growing cells in the medium CD-CHO (Invitrogen) supplemented with 6mM of L-glutamine, were electroporated under the following conditions: in a 0.4 cm cuvette, 1.0×10^7 viable cells in 700 µL of fresh CD-CHO were gently mixed with 40 µg of DNA in 100 µL of Tris EDTA buffer solution, pH 7,4, immediately followed by delivering of a single pulse of 300 volts, 900 µF . The contents of 2 cuvettes were immediately transferred in 200 mL of fresh pre-warmed CD-CHO medium. This cell suspension was subsequently distributed in 4 tissue culture-treated T75 flasks and placed in a humidified incubator set at 10% CO_2 in air and a temperature of 37ºC to generate semi-stable pools. Around twenty-four hours after transfection, selective pressure (by MSX supplementation at 50 µM) was applied. Individual stably transfected clones were then selected using ClonePix technology (Genetix) and screened for 1E4 productivity.

EXAMPLE 11: Large scale purification of 1E4 from CHO **supernatant**

The process involves MabSelect SuRe affinity chromatography (GE Healthcare), retrovirus inactivation by low pH treatment, pH adjustment for SP Sepharose cation exchange chromatography, concentration/diafiltration before Capto Q (GE Healthcare) anion exchange chromatography and concentration/diafïltration into final formulation buffer.

Briefly, supernatant produced by 25L Wave Bag fermentation of clone was clarified and captured on MabSelect SuRe Protein A Affinity column with an overall recovery of 95 % at 80 % of the maximum loading capacity (32 mg of Antibody per mL of matrix). The eluate was proven to be stable at elution pH up to 48h. The stability of the 1E4 antibody was also evaluated at the low pH (3.7) used for viral inactivation and the Antibody was stable up to 48h.

The pool of Protein A eluates was then loaded onto an SP Sepharose cation exchange column after pH adjustment (pH 5).This step was optimised for efficient residual aggregate removal, the optimal elution buffer was found to be 107 mM Sodium Chloride (in

25 mM Sodium Acetate pH 5). A concentration/diafiltration step was then used to buffer exchange the 1E4 antibody into the appropriate buffer for Capto Q Chromatography (25 mM Sodium Acetate, 40 mM Sodium Chloride pH 5). A concentration of about 50 mg/mL was reached without any problems of degradation or aggregation. The Capto Q Chromatography in non-binding mode was optimised for efficient contaminant removal (Host Cell Proteins, Protein A and DNA). Antibody 1E4 was finally concentrated and diafiltered into the 25 mM Histidine, 125 mM NaCl, pH 6 formulation buffer to a final concentration of about 10 mg/mL.

Antibody 1E4 did not show a tendency to aggregate throughout the purification process, and presented good stability across the purification process. The final product reached all prerequisite specifications in terms of aggregates levels and residual contamination.

EXAMPLE 12: Functional characterization of antibody 1E4 purified form **CHO supernatant**

RANTES is a ligand for the receptors CCRl , CCR3 and CCR5. The capacity of 1E4 purified from CHO supernatants to block the interaction with each one of these receptors was assessed in chemotaxis and calcium flux assays.

Calcium Flux

L l.2 cells expressing either hCCRl , hCCR3 or hCCR5 were cultured in RPMI medium supplemented with 10% FCS. For optimal results, cells expressing hCCRl were starved overnight in medium containing 1% of FCS. The day before the experiment all cells were incubated with 0.3 mg/ml of butyric acid. Different concentrations of 1E4 were incubated with 4 to 25nM of huRANTES (Peprotech, Rocky Hill NJ) for 30 minutes at room temperature. Cells were washed in PBS and loaded with 2 μM Fura 2/AM. 100 μl of loaded cells were added to each well of a 96-well black, transparent flat-bottom plate and calcium flux kinetics were recorded by measuring the fluorescence at 514 nm upon excitation at 340 or 380nm on a Flex station II instrument (Molecular Devices) after addition of the chemokine-antibody mix. As shown in Figure 3, 1E4 was able to inhibit calcium flux in cells that express either hCCRl, hCCR3 or hCCR5 in a dose dependent manner. The IC50 (where 50% of the huRANTES induced calcium flux is inhibited, *i.e.,* 50% inhibitory concentration) was determined (Table 8).

Table 8. Potency of antibody 1E4 purified from CHO supernatant in calcium flux functional assay using cells expressing the one three cognate receptors of RANTES.

Cells and concentration of huRANTES used for calcium flux induction	**IC_{50} (nM)**
L1.2-hCCR1; 25nM huRANTES	4.9
L1.2-hCCR3; 25nM huRANTES	4.46
L1.2-hCCR5; 4nM huRANTES	0.54

Chemotaxis

Wild type Ll. 2 cells and Ll. 2 cells expressing either hCCRl, hCCR3 or hCCR5 were cultured in RPMI medium supplemented with 10% FCS. For optimal results, cells expressing hCCRl were starved overnight in medium containing 1% of FCS. The day before the experiment all cells were incubated with 0.3 mg/ml of butyric acid. For optimal results, cells expressing hCCRl were starved overnight in medium containing 1% of FCS. Different concentrations of 1E4 were incubated with 1-1OnM of recombinant huRANTES or InM of native huRANTES (generated as described in example 6) and placed in the bottom chamber of chemotaxis 96-well plate (Neuroprobe). The filter plate was placed on top of the chemotaxis plate and each well was overlaid with 20µl of a 10^6 cells/ml suspension. The plate was incubated for 2 hours at 37°C. Cells that migrated through the filter were stained with DRAQ5 (Alexis Corporation) and counted on an FMAT 8200 reader (Applied Biosystems, Foster City CA). As shown in Figure 4, 1E4 was able to inhibit calcium flux in cells that express either hCCRl, hCCR3 or hCCR5 in a dose dependent manner. The IC50 (where 50% of the huRANTES induced cell migration is inhibited, *i.e.*, 50% inhibitory concentration) was determined (Table 9).

Table 9. Potency of antibody 1E4 purified from CHO supernatant in chemotaxis functional assay using cells expressing the one three cognate receptors of RANTES.

Cells and concentration of huRANTES used for chemotaxis assays	**IC_{50} (nM)**
L1.2-hCCR1; 2nM huRANTES	0.46
L1.2-hCCR3; 10nM huRANTES	3.33
L1.2-hCCR5; 1nM huRANTES	0.2
L1.2-hCCR5; 1nM native huRANTES	0.09

EXAMPLE 13: Cross-Reactivity of 1E4 Antibody

1E4 was tested for its ability to bind to a panel of chemokines from different species in an ELISA. The panel included the following chemokines: human RANTES, *cynomolgus* monkey RANTES, rat RANTES, mouse RANTES, human ITAC, human IP-IO, *cynomolgus* monkey IP-10, human MIG, *cynomolgus* monkey MIG, human MIPl α, human MIPl β, human MCP-I, human MCP-2. Briefly, chemokines cloned from cDNA isolated from human, mouse, rat, and *cynomolgus* monkey were expressed as fusion proteins and purified as described in Example 1. The chemokines were coated at 5μg/ml in an maxisopb plate (Nunc, Denmark) and incubated with a concentration range of 1E4. The level of binding was revealed using an anti-human Fc-γ specific antibody coupled to horse radish peroxidase (Jackson) and a fluorescent substrate. As shown in Figure 5, the antibody 1E4 only binds to human and *cynomolgus* RANTES and not with RANTES from other species nor with any of the other human chemokines tested. Proper coating of all the chemokines was controlled using monoclonal antibodies directed against each chemokine and all the chemokines tested could be detected in this format.

EXAMPLE 14: Epitope mapping of 1E4 Antibody

In an ELISA, the antibody 1E4 binds with equivalent apparent affinity to both human and cynomolgus RANTES (Figure 5). In order to identify residues potentially required on huRANTES for binding to 1E4, the RANTES protein sequences from several species were aligned as shown in Figure 6. In the alignment, residues that are conserved between the human and *cynomolgus* sequences and that are different in mouse and rat RANTES to which 1E4 is unable to bind were analyzed to identify the following amino acids: A16, R17, P18, G32, P37, R59 and S64. Three mutants of mouse RANTES were generated by site directed mutagenesis in order to introduce the human residues at those positions: [S16A/L17R/A18P]; [S32G/L37P] and [Q59R/Y64S]. These mutant forms of mouse RANTES were expressed and biotinylated *in vivo* as described in Example 1. These variant of mouse RANTES were captured in streptavidin coated plates (Streptawell, Roche). The coating of the biotinylated chemokine was confirmed using a anti-mouse RANTES polyclonal antibody (R&D Systems). It was then tested whether the introduction of these residues could restore 1E4 binding to mouse RANTES. Briefly, mouse RANTES, human RANTES as well as three mutant forms of mouse RANTES and control supernatants were captured in Streptawell plates (Roche) for 30 minutes at room temperature. After washing,

antibody 1E4 was added at a concentration of 1μg/ml in 1%BSA-PBS and incubated for 1 hour at room temperature. The plate was washed and incubated with a goat anti-human IgG Fcγ-specific antibody coupled to horse radish peroxidase (Jackson). After washing the signal was revealed with TMB (Roche) and stopped with H_2SO_4. The plates were read at 450nm. As shown in Figure 7, the [S16A/L17R/A18P]mutant restores binding of 1E4 to mouse RANTES indicating that A16, R17 and P18 are critical for the 1E4 epitope integrity on human RANTES.

EXAMPLE 15: Affinity **and Binding Kinetics of 1E4**

The affinity and binding kinetics of 1E4 on human RANTES and *cynomolgus* RANTES were characterized on a Biacore 2000 instrument (Biacore AB, Uppsala, Sweden). 433 RU (response unit) of a donkey anti-human IgG polyclonal antibody were immobilized by EDC/NHS chemistry on a C1 Biacore chip. This surface was used to capture antibody 1E4. The surface was regenerated after each cycle by injection of 10mM glycine pH=2 at 30μL/min, for 60s followed by 1 min. of stabilization time in HBS-EP buffer.

Binding was measured by passing either huRANTES (Peprotech, Rocky Hill NJ) or a NusA- fusion proteins of human RANTES (NusA-huRANTES) and *Cynomolgus* RANTES (NusA-cynoRANTES) at various concentrations. All proteins were diluted in the running buffer HBS-EP buffer (Biacore AB, Uppsala, Sweden). Injection was performed at 75μl/min for 3 min. followed by 15 min. of dissociation time and the temperature was set at 25°C. The data was fitted according to 1:1 Langmuir model and the K_{0n}, K_{0f} and K_D values determined. Very similar values were obtained using huRANTES or the NusA-huRANTES fusion, but better response signals were obtained with the fusion protein due to its larger size that induces a better response on the Biacore. The affinity of antibody 1E4 for huRANTES and cynoRANTES are 0.45 nM and 2.24 nM, respectively. The Affinities and kinetic constants of both antibodies are summarized in Table 10.

Table 10. Kinetic and affinity constants of antibody 1E4 for human and *cynomolgus* RANTES measured by Biacore.

	huRANTES	NusA-huRANTES	NusA-cynoRANTES
Ka (1/Ms)	$5.36x10^{6}$	$1.87x10^{6}$	$5.46x10^{6}$
Kd (1/s)	$2.44x10^{-3}$	$8.35x10^{-4}$	$1.22x10^{-3}$
KD (M)	**$4.55x10^{-10}$**	**$4.47x10^{-10}$**	**$2.24x10^{-9}$**

EXAMPLE 16: Animal model of ischemia

Materials and Methods

Animals: Eight to 12 week old C57BL/6 mice are used for the experiments. All animal studies were approved by the local ethical Committee.

Antibodies and in vivo treatment: C57BL/6 mice were injected either in the peritoneal cavity (i.p.) or intraveneously (i.v.). For the ischemia followed by reperfusion model, monoclonal antibodies (mAb) were injected 5 minutes before the end of the occlusion period. For the permanent ligation model, mAbs were injected 5 minutes after the chronic ligature was put in place. The mAbs included: (1) the rat anti-mouse RANTES (mRANTES), mAb478 and (2) the rat anti-mouse isotype mAb control, mAb64. Hybridomas that produced mAb478 or mAb64 were obtained from R&D or the American Tissue Culture Collection, respectively, and all mAb were produced, purified and stored in-house.

For the i.p. treatment, 1 mg/mouse of the IgG control or anti-mRANTES mAbs was administered. For the i.v. treatment, either 0.1, 0.3, 0.5 or 1mg/mouse of the anti-mRANTES mAb or 1mg/mouse of the IgG control (i.e. highest dose of anti-mRANTES) was administered.

***In vivo* Ischemia-Reperfusion or Ligation Permanent Model**

Surgery: Mice were initially anesthetized with 4% isofluorane then intubated. Mechanical ventilation was performed with a tidal volume of 300 μL at 120 breaths using a rodent respirator (model 683; Harvard Apparatus). Anesthesia was maintained with 2%

isofluorane delivered 100% through the ventilator. A thoracotomy was performed in the left fourth intercostal space, and the pericardial sac was then removed. An 8-0 Prolene suture was passed under the left coronary artery at the inferior edge of the left atrium and tied with a slipknot to produce occlusion.

For the reperfusion model, a small piece of polyethylene tubing was used to secure the ligature without damaging the artery and after 30 minutes of ischemia, the left anterior descending (LAD) coronary artery occlusion was released and reperfusion permitted to occur.

For the permanent legation model, the LAD coronary artery was irreversibly occluded by using a double knot 8-0 Prolene suture. The chest was then closed and air was evacuated from the chest cavity. The endotracheal tube was then removed and normal respiration restored.

After 24 hours of reperfusion or after 24 hours of permanent occlusion, animals were euthanized to determine infarct size.

Evaluation of Risk Zone and Infarct Size: At the end of the reperfusion period, mice were re-anesthetized with 0.3 mL ketamine-xylazine and the LAD coronary artery was re-ligated. 3% Evans Blue dye (Sigma) was injected i.v. (retro-orbital administration) to delineate the in vivo risk zone (R). The heart was rapidly excised and rinsed in saline. After removal of the right ventricle and connective tissues, the heart was frozen and then sectioned into 3-mm transverse sections from apex to base (5 slices/heart). Following thawing, the sections were incubated at 37°C with 1% triphenyltetrazolium chloride in phosphate buffer (pH7.4) for 15 min, fixed in 10% formaldehyde solution and, after 24 hours, photographed with a digital camera to distinguish areas of stained viable versus unstained necrotic tissue. Left ventricular infarct zone (I) was determined using a computerized planimetric technique (MetaMorphó software, Zeiss) and expressed as a percentage of either the area at risk (AAR) or ventricular area (V).

EXAMPLE 17: Effect of Inhibiting RANTES in Ischemia Reperfusion Models

Model 1: Ischemia Reperfusion

A diagram illustrating the protocol of the murine ischemia reperfusion model is shown in Figure 8. In this protocol, B6 mice are divided into three groups and administered a vehicle control (PBS), an isotype control (mAb 64 described in Example 10) or a rat anti-mRANTES monoclonal antibody according to the following schedule:

Group 1: PBS administered i.p. or i.v. 5 minutes prior to reperfusion;

Group 2: rat IgG2a (mAb 64; isotype control) administered i.p. (lmg/mouse) or i.v. (1.0 mg/mouse) 5 minutes prior to repertusion;

Group 3: rat anti-mouse RANTES (mAb 478) administered i.p. (lmg/mouse) or i.v. (0.1, 0.3, 0.5, 1.0 mg/mouse) 5 minutes prior to repertusion;

All animals were killed 24 hours post-repertusion. Each group of mice was evaluated by assessing the following parameters:

- weight of mice;
- AAR/V = area at risk divided by the total area of heart (ischemic zone);
- I/AAR = infarcted area divided by the area at risk; and
- I/V = infarcted area divided by the total area of the ventricles.

Both I/AAR and I/V provide data on extent of infarcted tissue.

As shown in Figure 9, treatment with the anti-RANTES monoclonal antibody decreased infarct size in the murine model of ischemia repertusion provided herein. Injecting mAb 478 (1 mg/mouse i.p.) five minutes prior to repertusion significantly decreased the infarct size as compared to isotype control or PBS treated mice. Data represents 20 mice per group.

Figure 10 demonstrates that treatment with the anti-RANTES monoclonal antibody decreased infarct size in the murine model of ischemia repertusion in a dose-dependent manner. Injecting mAb 478 i.v. (at doses of 0.1, 0.3, 0.5, 1.0 mg/mouse) five minutes prior to repertusion significantly decreased the infarct size at higher doses as compared to isotype control (1 mg/mouse). Data represents 3 mice per group.

Model 2: Permanent Occlusion

A diagram illustrating the protocol of the murine permanent occlusion model is shown in Figure 11. In this protocol, B6 mice are divided into three groups and administered a vehicle control (PBS), an isotype control (mAb 64 described in Example 10) or a rat anti-mRANTES monoclonal antibody according to the following schedule:

Group 1: PBS administered i.p. or i.v.;

Group 2: rat IgG2a (mAb 64; isotype control) administered i.p. (lmg/mouse) or i.v. (1.0 mg/mouse);

Group 3: rat anti-mouse RANTES (mAb 478) administered i.p. (lmg/mouse) or i.v. (0.1, 0.3, 0.5, 1.0 mg/mouse).

Each group of mice was evaluated by assessing the following parameters:

- weight of mice;
- AAR/V = area at risk divided by the total area of heart (ischemic zone);
- I/AAR = infarcted area divided by the area at risk; and
- I/V = infarcted area divided by the total area of the ventricles .

All animals were killed at 24 hrs post occlusion.

As shown in Figure 12, treatment with the anti-RANTES monoclonal antibody decreased infarct size in the murine model of ischemia provided herein. Injecting mAb 478 (1 mg/mouse i.p.) significantly decreased the infarct size as compared to isotype control or PBS treated mice. Data represents 10 mice per group.

Figure 13 demonstrates that treatment with the anti-RANTES monoclonal antibody decreased infarct size in the murine model of ischemia in a dose-dependent manner. Injecting mAb 478 i.v. (at doses of 0.1, 0.3, 0.5, 1.0 mg/mouse) significantly decreased the infarct size at higher doses as compared to isotype control (1 mg/mouse). Data represents 3 mice per group.

Other Embodiments

While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

What is claimed is:

1. An isolated antagonist molecule of human RANTES, wherein said antagonist molecule binds a human RANTES polypeptide that comprises at least residues acid residues 16-18 of SEQ ID NO: 170 and reduces a biological activity of RANTES activity upon interaction between said antagonist and said human RANTES polypeptide.

2. The antagonist molecule of claim 1, wherein said antagonist molecule does not bind a human RANTES polypeptide that lacks amino acid residues 16-18 of SEQ ID NO: 170.

3. The antagonist molecule of claim 1, wherein said antagonist molecule is selected from a small molecule inhibitor; a polypeptide, a peptide, and a nucleic acid-based antagonist.

4. The antagonist molecule of claim 1, wherein said antagonists is an isolated monoclonal anti-human RANTES antibody or antigen-binding fragment thereof, wherein said antibody or antigen-binding fragment thereof binds to an epitope on a mature human RANTES polypeptide, said epitope comprising amino acid residues 16-18 of SEQ ID NO: 170.

5. The antagonist molecule of claim 4, wherein said monoclonal antibody is a fully human monoclonal anti-human RANTES antibody or antigen-binding fragment thereof.

6. The antagonist molecule of claim 5, wherein said antibody is an IgGl isotype.

7. An isolated fully human monoclonal anti-human RANTES antibody or fragment thereof, wherein said antibody comprises:

(a) a V_H CDRl region comprising the amino acid sequence of SEQ ID NO: 8, 28. 44. 74. 90, 106, 122, 138, 154, or 222;

(b) a V_H CDR2 region comprising the amino acid sequence of SEQ ID NO: 9, 29. 45. 75. 91, 107, 123, 139, 155, 207, 223, 239, or 255;

(c) a V_H CDR3 region comprising the amino acid sequence of SEQ ID NO: 10, 20, 30, 46, 50, 54, 58, 64, 76, 92, 108, 124, 140, 156, 188, 208, 224, 240 and 256;

(d) a V_L CDRl region comprising the amino acid sequence of SEQ ID NO: 14, 34, 80, 96, 112, 128, 144, 160, 176, 192, 212, 228, 244 or 260;

(e) a V_L CDR2 region comprising the amino acid sequence of SEQ ID NO: 15, 35, 97, 113, 129, 145, 161, 177, 193, 213, 229, 245 or 261; and

(f) a V_L CDR3 region comprising the amino acid sequence of SEQ ID NO: 16, 36, 66, 82, 98, 114, 130, 146, 162, 178, 194, 198, 214, 230, 246 or 262;

wherein said antibody binds RANTES.

8. The antibody of claim 7, wherein said antibody is an IgG isotype.

9. The antibody of claim 7, wherein said antibody is an IgGl isotype.

10. The antibody of claim 7, wherein said antibody comprises a heavy chain variable sequence comprising an amino acid sequence selected from SEQ ID NO: 2, 18, 22, 38, 48, 52, 56, 60, 68, 84, 100, 116, 132, 148, 164, 180, 200, 216, 232, or 248 and a light chain variable sequence comprising the amino acid sequence of SEQ ID NO: 4, 24, 40, 62, 70, 86, 102, 118, 134, 150, 166, 182, 196, 202, 218, 234, or 250.

11. An isolated fully human monoclonal antibody comprising a heavy chain variable sequence comprising the amino acid sequence of SEQ ID NO: 2, 18, 22, 38, 48, 52, 56, 60, 68, 84, 100, 116, 132, 148, 164, 180, 200, 216, 232, or 248 and a light chain variable sequence comprising the amino acid sequence of SEQ ID NO: 4, 24, 40, 62, 70, 86, 102, 118, 134, 150, 166, 182, 196, 202, 218, 234, or 250, wherein said antibody binds RANTES.

12. The antibody of claim 11, wherein said antibody is an IgG isotype.

13. An isolated fully human monoclonal antibody comprising a heavy chain variable sequence comprising the amino acid sequence of SEQ ID NO:2 and a light chain variable sequence comprising the amino acid sequence of SEQ ED NO:4.

14. The antibody of claim 13, wherein said antibody is an IgGl isotype.

15. The antibody of claim 13, wherein said antibody comprises a heavy chain sequence comprising the amino acid sequence of SEQ ID NO:263 and a light chain sequence comprising the amino acid sequence of SEQ ID NO:264.

16. An isolated fully human monoclonal antibody comprising a heavy chain variable sequence comprising the amino acid sequence of SEQ ID NO: 18 and a light chain variable sequence comprising the amino acid sequence of SEQ ID NO:4.

17. The antibody of claim 16, wherein said antibody is an IgGl isotype.

18. The antibody of claim 16, wherein said antibody comprises a heavy chain sequence comprising the amino acid sequence of SEQ ID NO:238 and a light chain sequence comprising the amino acid sequence of SEQ ID NO:254.

19. An isolated fully human monoclonal antibody comprising a heavy chain variable sequence comprising the amino acid sequence of SEQ ID NO:22 and a light chain variable sequence comprising the amino acid sequence of SEQ ID NO:24.

20. The antibody of claim 19, wherein said antibody is an IgGl isotype.

21. The antibody of claim 19, wherein said antibody comprises a heavy chain sequence comprising the amino acid sequence of SEQ ID NO: 186 and a light chain sequence comprising the amino acid sequence of SEQ ID NO: 187.

22. The antibody of claim 7, wherein said antibody comprises a V_H CDRl region comprising the amino acid sequence of SEQ ID NO: 8; a V_H CDR2 region comprising the amino acid sequence of SEQ ID NO:9, a V_H CDR3 region comprising the amino acid sequence of SEQ ID NO: 10; a V_L CDRl region comprising the amino acid sequence of SEQ ID NO: 14; a V_L CDR2 region comprising the amino acid sequence of SEQ ED NO: 15; and a V_L CDR3 region comprising an amino acid sequence of SEQ ID NO: 16.

23. The antibody of claim 7, wherein said antibody comprises a V_H CDRl region comprising the amino acid sequence of SEQ ID NO: 8; a V_H CDR2 region comprising the amino acid sequence of SEQ ID NO:9, a V_H CDR3 region comprising the amino acid sequence of SEQ ID NO:20; a V_L CDRl region comprising the amino acid sequence of SEQ ID NO: 14; a V_L CDR2 region comprising the amino acid sequence of SEQ ID NO: 15; and a V_L CDR3 region comprising an amino acid sequence of SEQ ID NO: 16.

24. The antibody of claim 7, wherein said antibody comprises a V_H CDRl region comprising the amino acid sequence of SEQ ID NO: 28; a V_H CDR2 region comprising the amino acid sequence of SEQ ID NO:29, a V_H CDR3 region comprising the amino acid sequence of SEQ ID NO:30; a V_L CDRl region comprising the amino acid sequence of SEQ ID NO: 34; a V_L CDR2 region comprising the amino acid sequence of SEQ ID NO:35; and a V_L CDR3 region comprising an amino acid sequence of SEQ ID NO:36.

25. A pharmaceutical composition comprising the antibody of claim 1 and a carrier.

26. An isolated antibody that binds human RANTES when human RANTES is bound to a glycosaminoglycan (GAG), wherein said antibody comprises:

(a) a V_H CDRl region comprising the amino acid sequence of SEQ ID NO: 8, 28. 44. 90, 106, 122 or 154;

(b) a V_H CDR2 region comprising the amino acid sequence of SEQ ID NO: 9, 29. 45. 91, 107, 123, 155, or 207;

(c) a V_H CDR3 region comprising the amino acid sequence of SEQ ID NO: 10, 20, 30, 64, 92, 124, 156, 188, or 208;

(d) a V_L CDRl region comprising the amino acid sequence of SEQ ID NO: 14, 34. 96, 128, 160, 176, 192, or 212;

(e) a V_L CDR2 region comprising the amino acid sequence of SEQ ID NO: 15, 35. 97, 129, 161, 177, 193, or 213; and

(f) a V_L CDR3 region comprising the amino acid sequence of SEQ ID NO: 16, 36. 98, 130, 162, 178, 194, or 214;

wherein said antibody binds RANTES in the context of GAG.

27. The antibody of claim 26, wherein said antibody is a monoclonal antibody or an antigen-binding fragment thereof.

28. The antibody of claim 26, wherein said antibody is a fully human monoclonal antibody or an antigen-binding fragment thereof.

29. The antibody of claim 26, wherein said antibody is an IgG isotype.

30. The antibody of claim 26, wherein said antibody is an IgGl isotype.

31. A method of alleviating a symptom of a clinical indication associated with ischemia or reperfusion injury in a subject, the method comprising administering an antagonist of RANTES to a subject in need thereof in an amount sufficient to alleviate the symptom of the clinical indication associated with ischemia or reperfusion injury in the subject.

32. The method of claim 31, wherein said subject is a human.

33. The method of claim 31, wherein said antagonist is a monoclonal antibody or an antigen-binding fragment thereof.

34. The method of claim 31, wherein said monoclonal antibody is an antibody according to any one of claims 1 to 24 or 26 to 30 or fragment thereof.

35. The method of claim 31, wherein said antagonist is a mutated RANTES polypeptide that modulates an activity of RANTES selected from the ability of RANTES to bind to a receptor selected from CCRl, CCR3, CCR4, and CCR5, the ability of RANTES to bind a glycosaminoglycan and the ability of RANTES to form oligomers.

36. A method of alleviating a symptom of an autoimmune disease or inflammatory disorder, the method comprising administering an antibody according to any one of claims 7 to 24 or 26 to 30 to a subject in need thereof in an amount sufficient to alleviate the symptom of the autoimmune disease or inflammatory disorder in the subject.

37. The method of claim 36, wherein said subject is a human.

Fig. 1 A

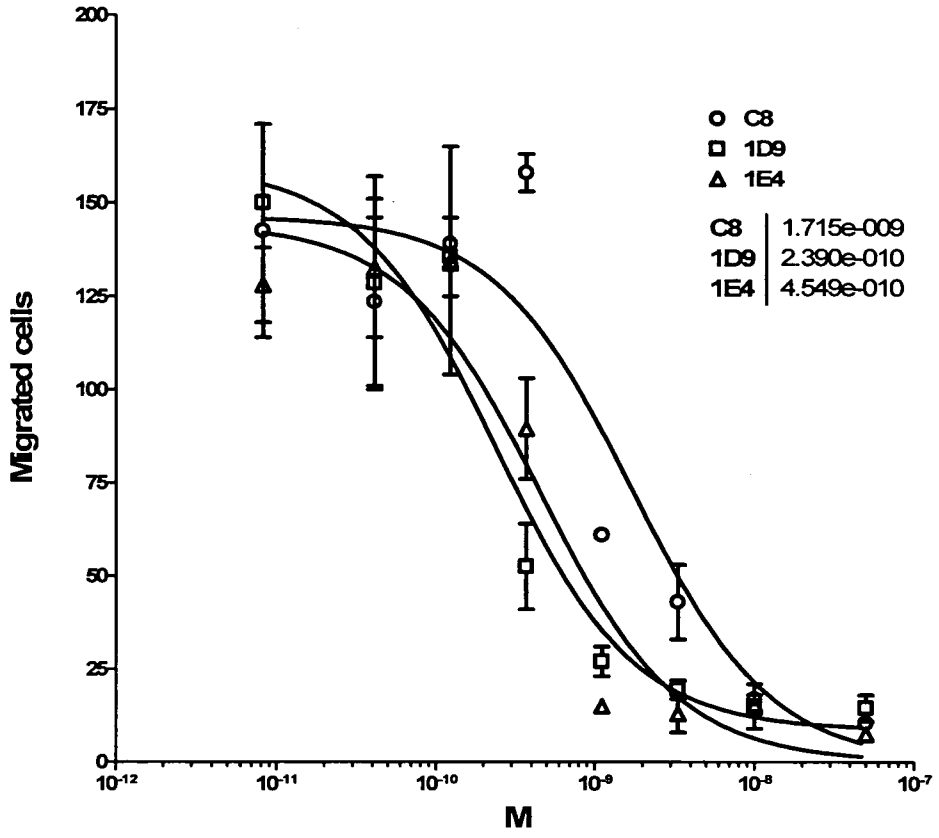

Migrated cells
200
175
150
125
100
75
50
25
0
10-12
10-11
10-10
10-9
10-8
10-7
M
C8
1D9
1E4
C8 1.715e-009
1D9 2.390e-010
1E4 4.549e-010

Fig. 1 B

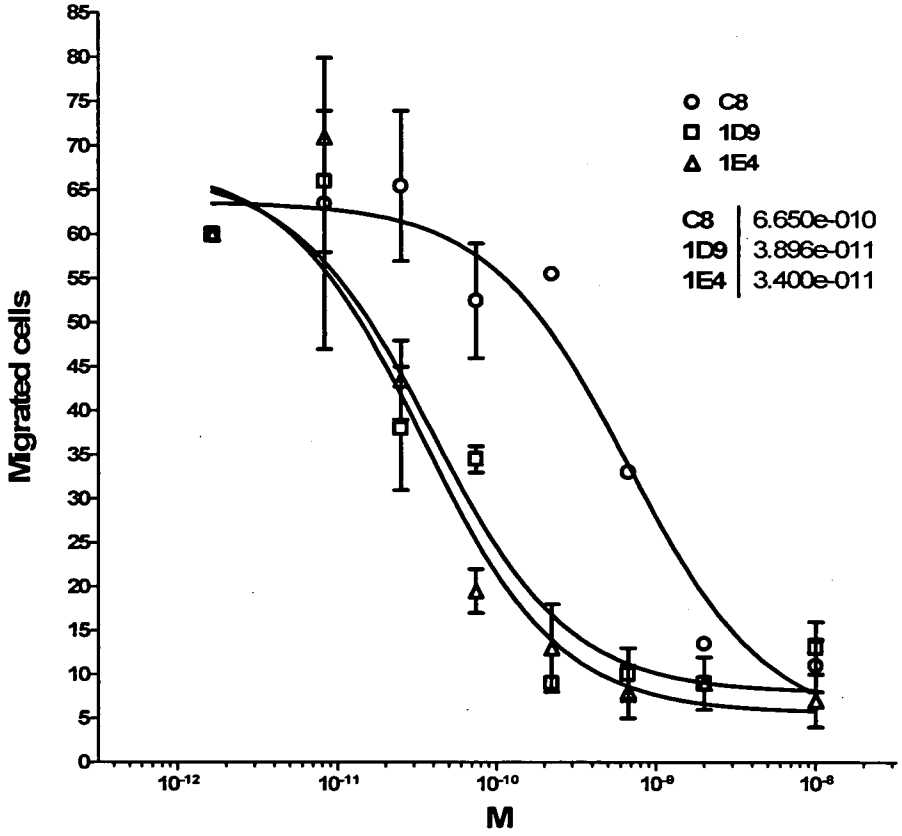

Migrated cells
M
C8
1D9
1E4
C8 6.650e-010
1D9 3.896e-011
1E4 3.400e-011

Fig. 1 C

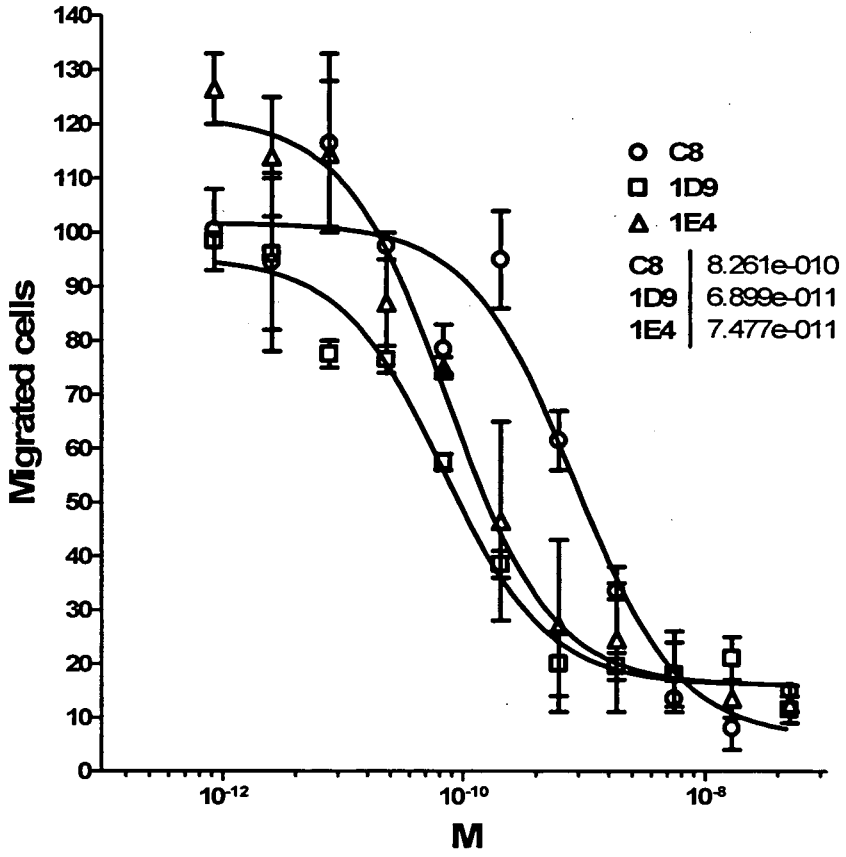

Migrated cells
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
10-12
10-10
10-8
M
C8
1D9
1E4
C8 8.261e-010
1D9 6.899e-011
1E4 7.477e-011

Fig. 2

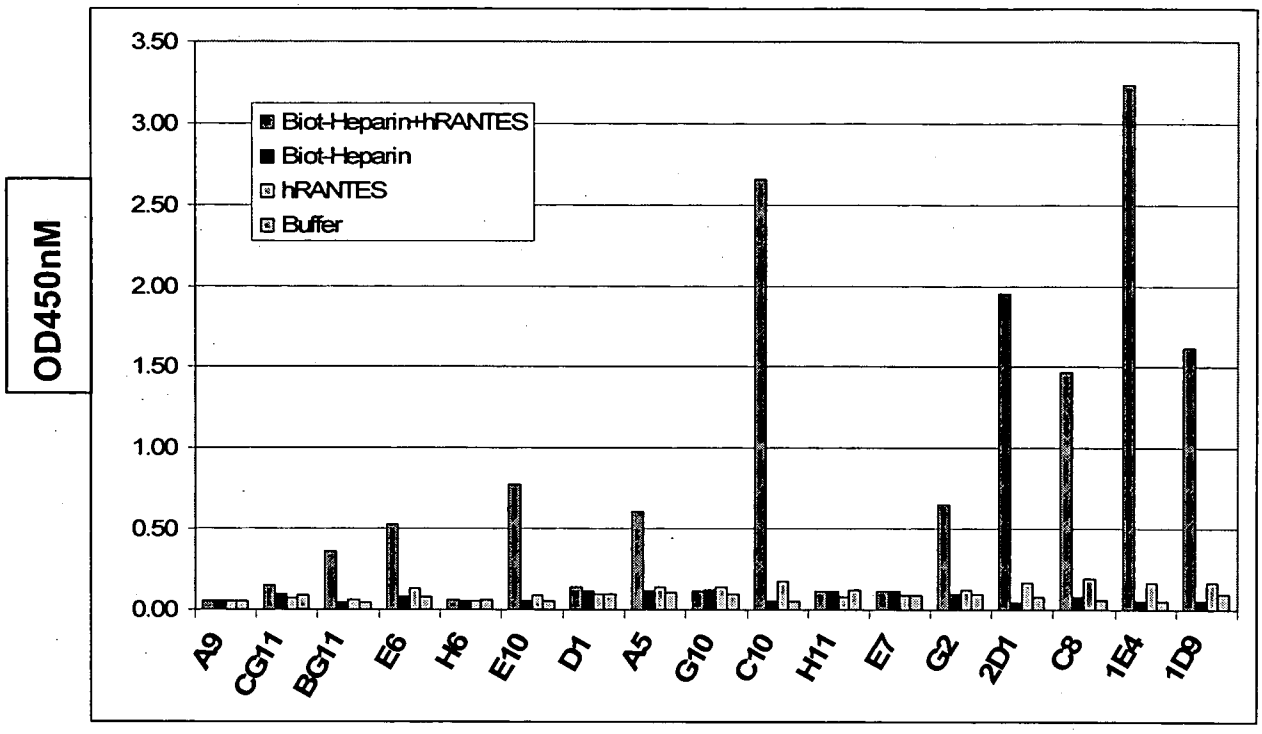

OD450nM
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Biot-Heparin+hRANTES
Biot-Heparin
hRANTES
Buffer
A9
CG11
BG11
E6
H6
E10
D1
A5
G10
C10
H11
E7
G2
2D1
C8
1E4
1D9

Fig.3A

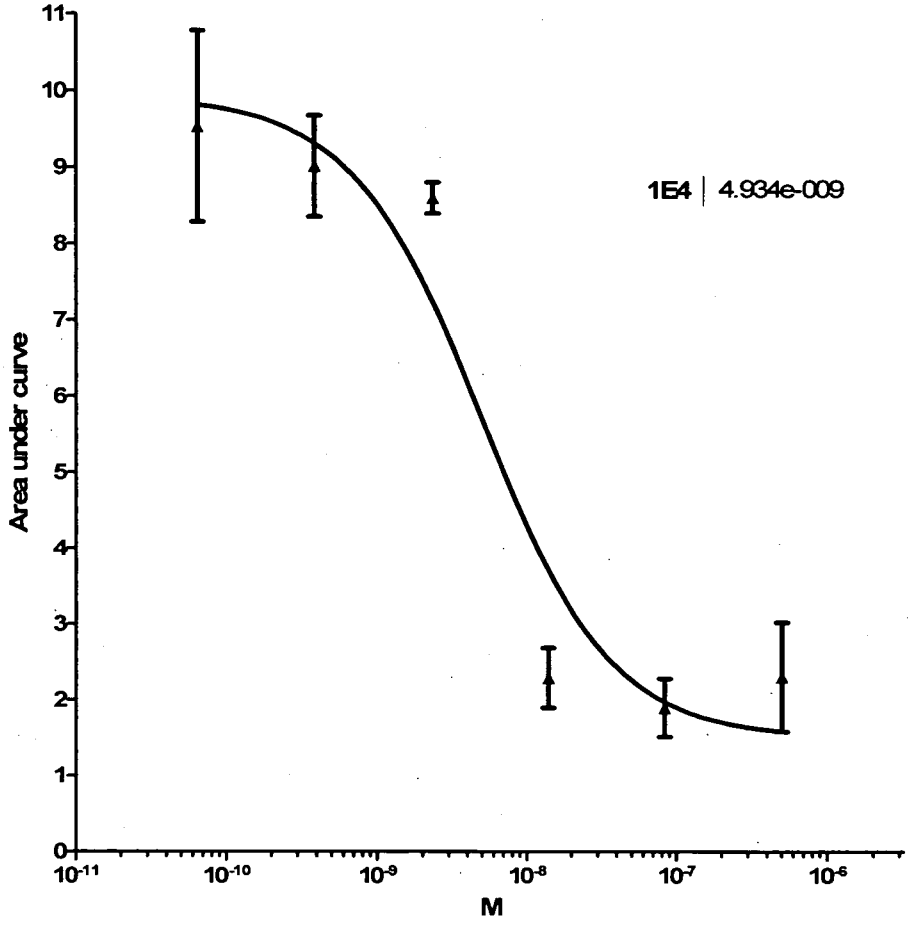

Area under curve
11
10
9
8
7
6
5
4
3
2
1
0
10-11
10-10
10-9
10-8
10-7
10-6
M
1E4 | 4.934e-009

Fig. 3B

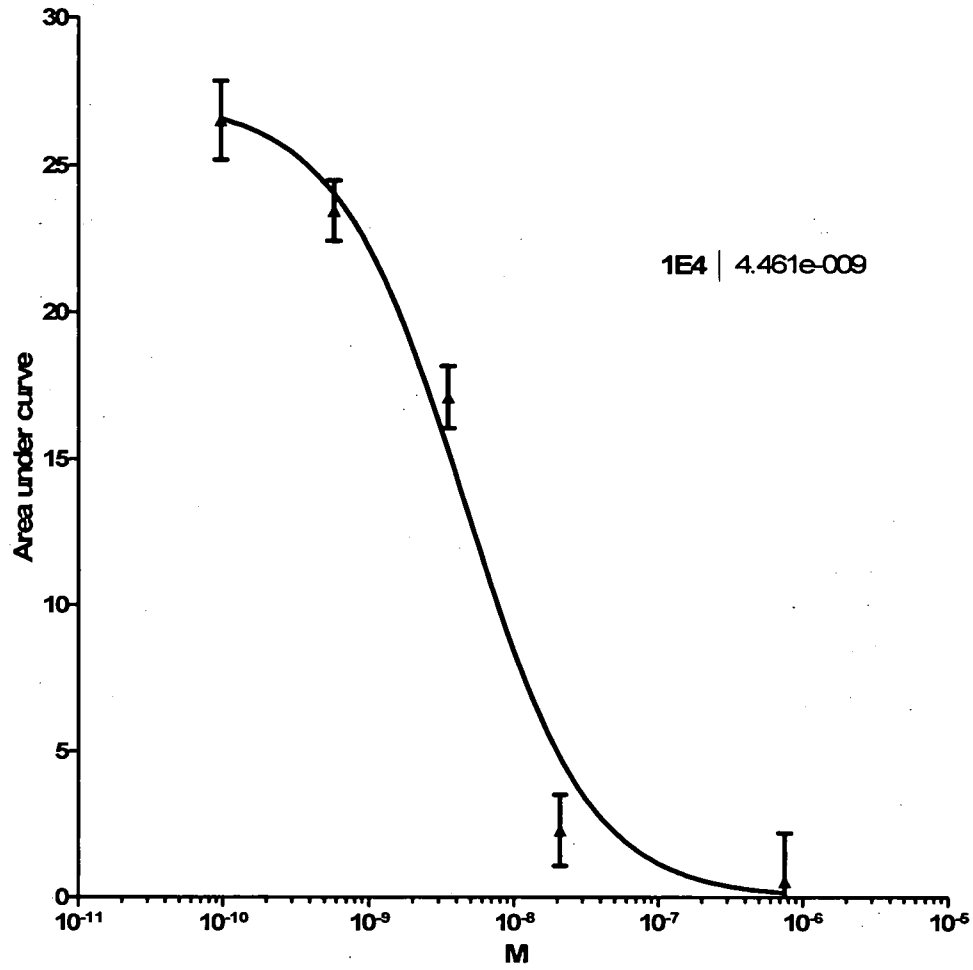

30
25
20
15
10
5
0
Area under curve
1E4 | 4.461e-009
10-11
10-10
10-9
10-8
10-7
10-6
10-5
M

Fig.3C

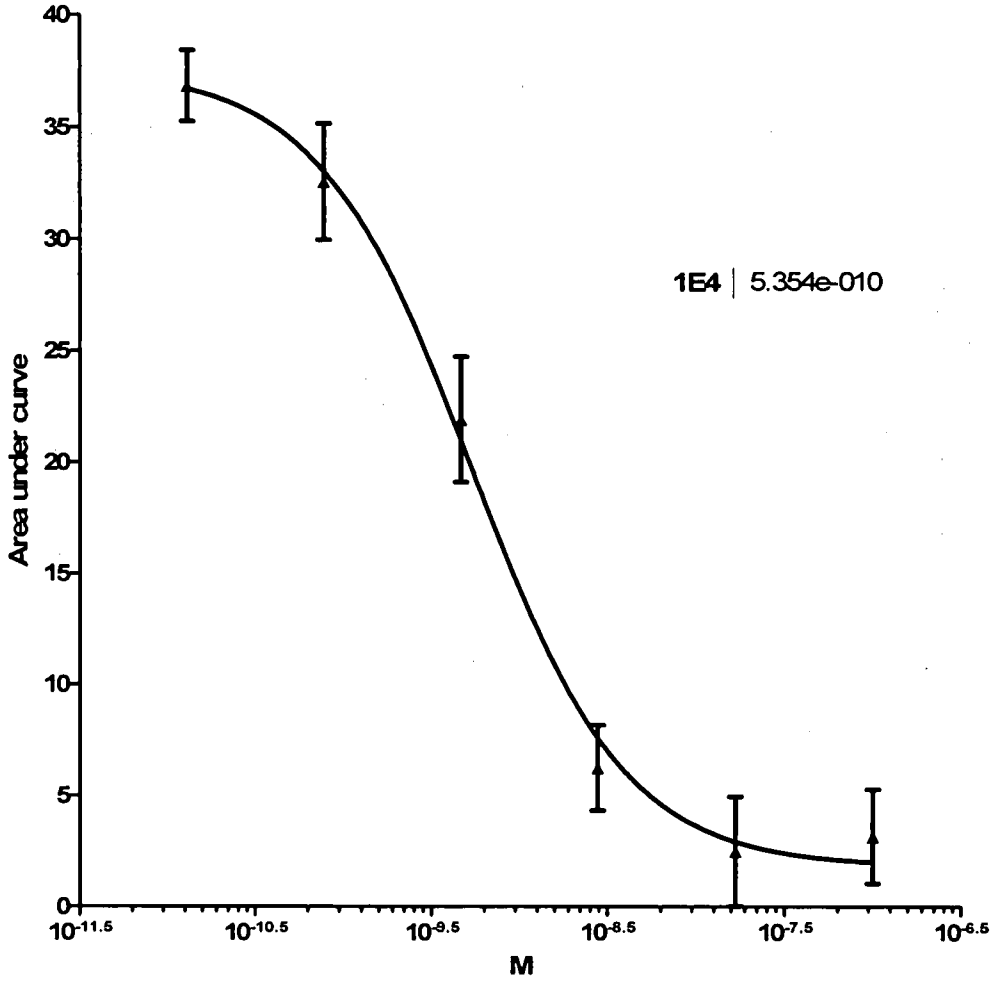

40
35
30
25
20
15
10
5
0
Area under curve
1E4 | 5.354e-010
10-11.5
10-10.5
10-9.5
10-8.5
10-7.5
10-6.5
M

Fig.4A

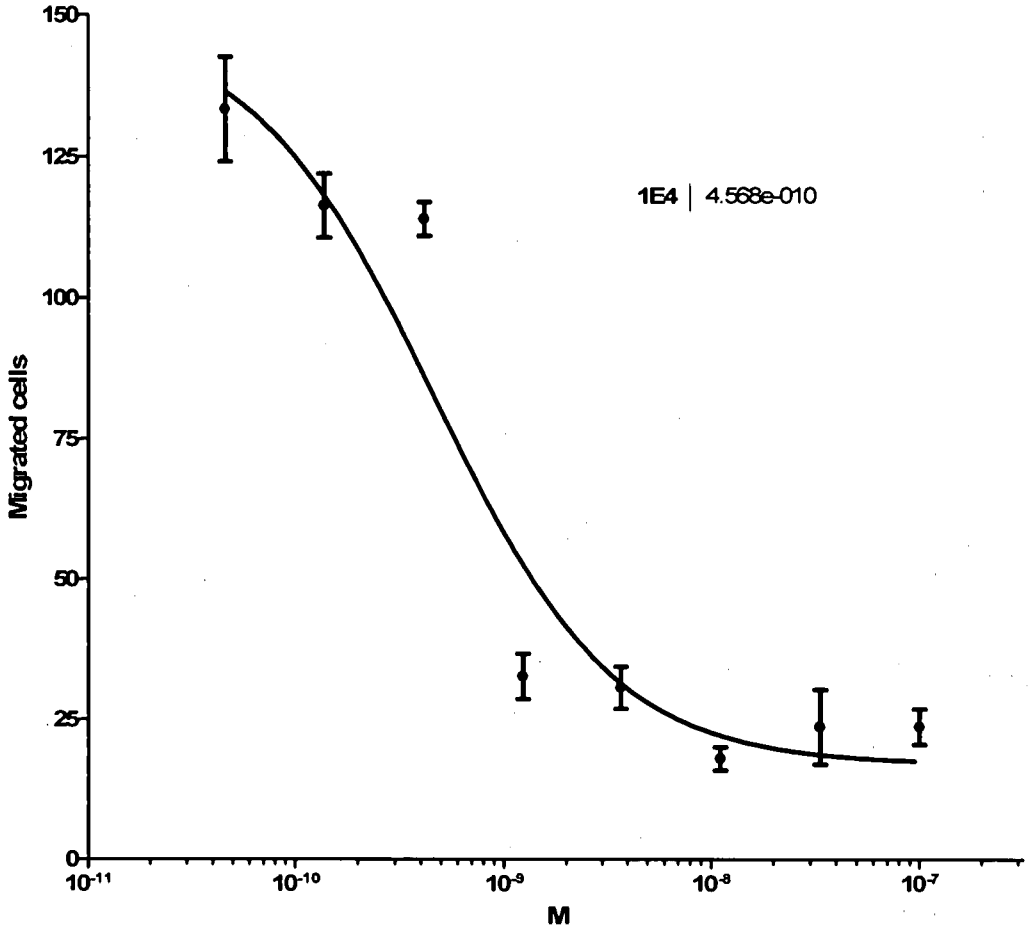

150
125
100
75
50
25
0
Migrated cells
1E4 | 4.568e-010
10-11
10-10
10-9
10-8
10-7
M

Fig. 4B

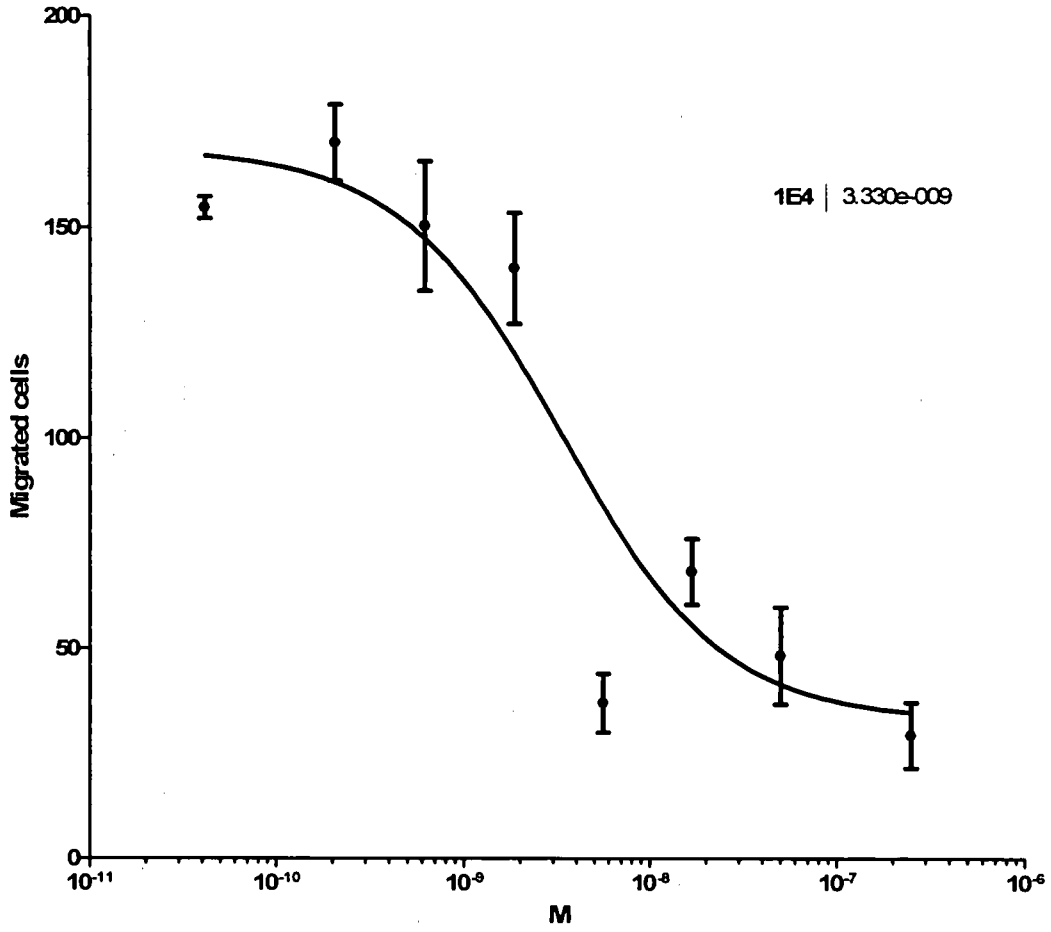

200
150
100
50
0
Migrated cells
1E4 | 3.330e-009
10-11
10-10
10-9
10-8
10-7
10-6
M

Fig. 4C

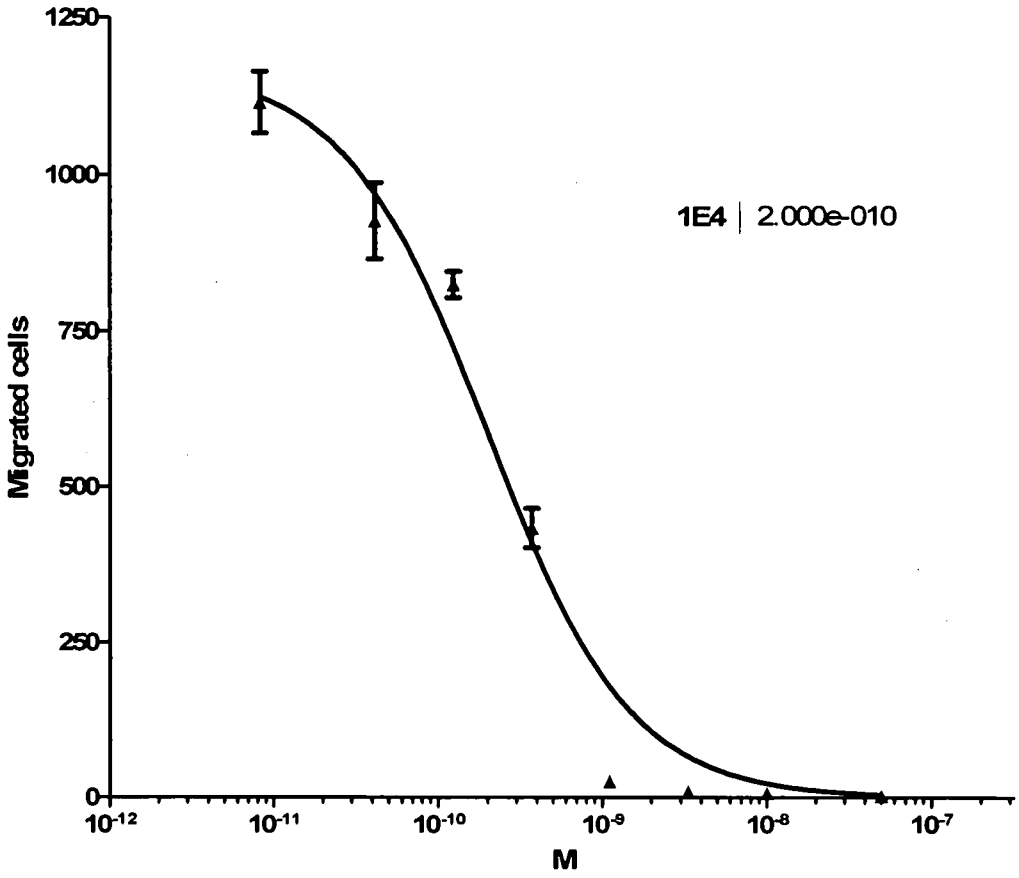

1250
1000
750
500
250
0
Migrated cells
1E4 | 2.000e-010
10-12
10-11
10-10
10-9
10-8
10-7
M

Fig.4D

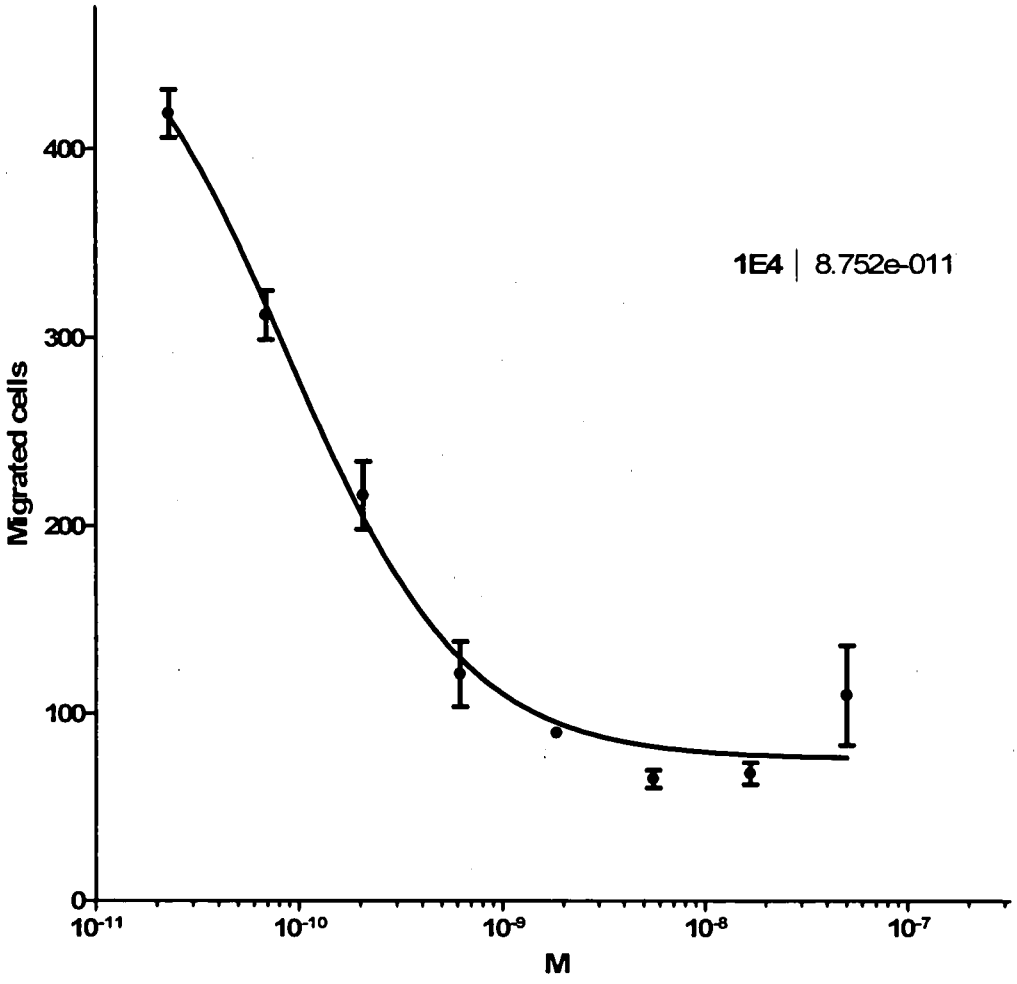

1E4 | 8.752e-011
Migrated cells
400
300
200
100
0
10-11
10-10
10-9
10-8
10-7
M

Fig. 5

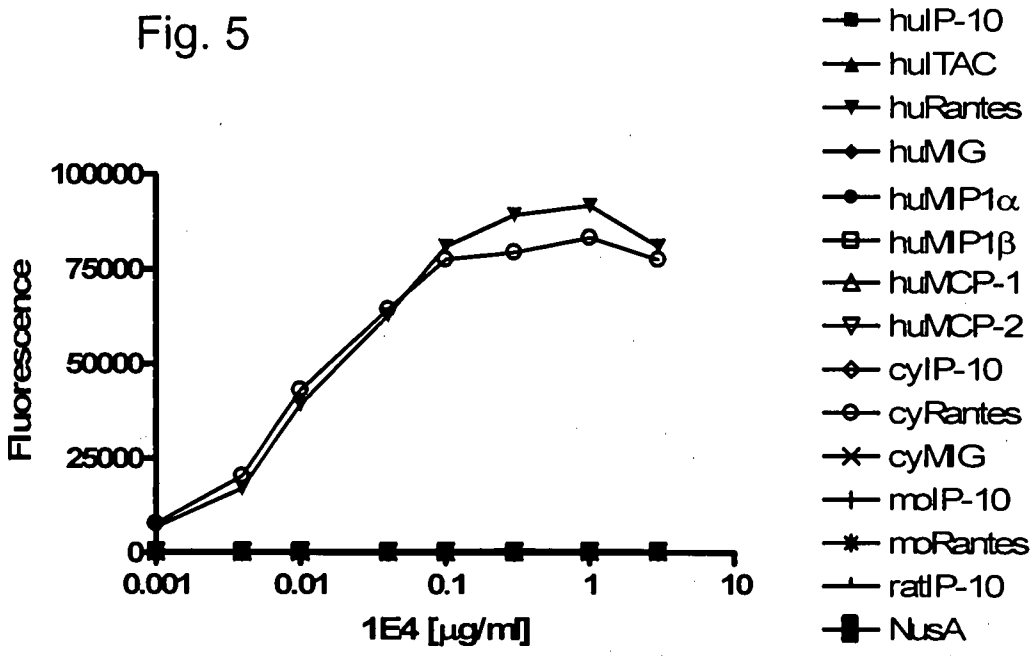

100000
75000
50000
25000
0
Fluorescence
0.001
0.01
0.1
1
10
1E4 [µg/ml]
huIP-10
huITAC
huRantes
huMIG
huMIP1α
huMIP1β
huMCP-1
huMCP-2
cyIP-10
cyRantes
cyMIG
moIP-10
moRantes
ratIP-10
NusA

Fig. 6

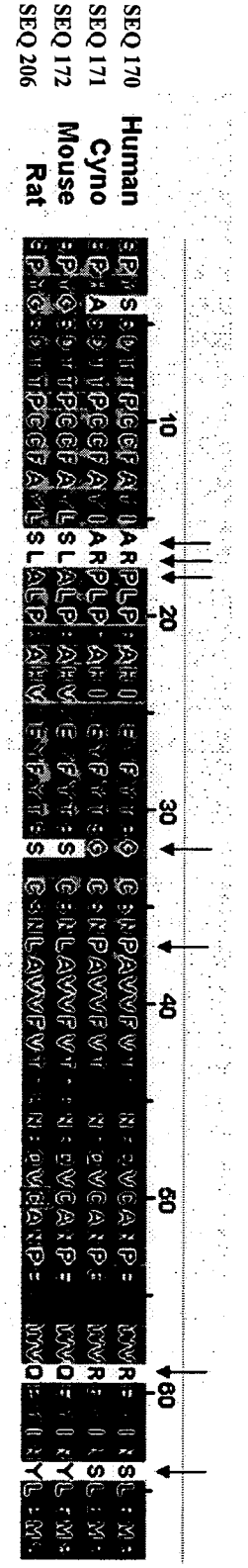

SEQ 170
SEQ 171
SEQ 172
SEQ 206
Human
Cyno
Mouse
Rat
10
20
30
40
50
60

Fig. 7

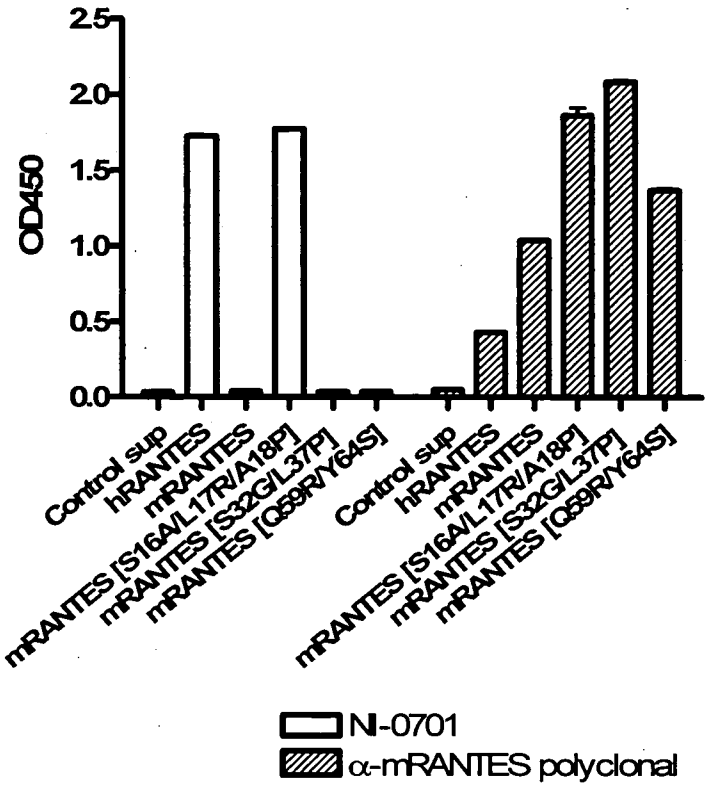

2.5
2.0
1.5
1.0
0.5
0.0
OD450
Control sup
hRANTES
mRANTES
mRANTES [S16A/L17R/A18P]
mRANTES [S32G/L37P]
mRANTES [Q59R/Y64S]
Control sup
hRANTES
mRANTES
mRANTES [S16A/L17R/A18P]
mRANTES [S32G/L37P]
mRANTES [Q59R/Y64S]
NI-0701
α-mRANTES polyclonal

Fig. 8

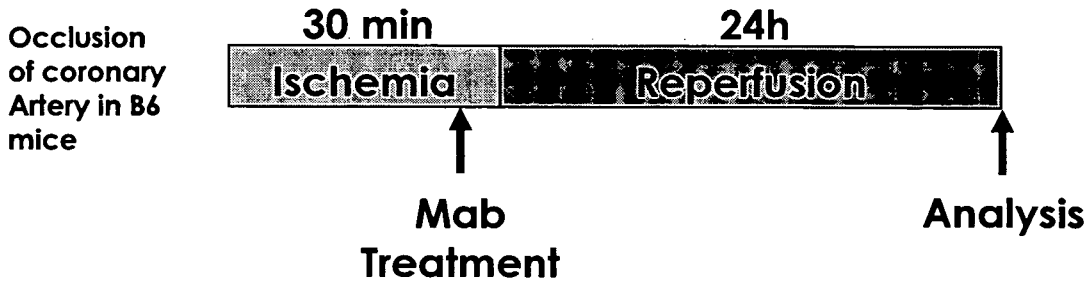

Occlusion of coronary Artery in B6 mice
30 min
24h
Ischemia
Reperfusion
Mab Treatment
Analysis

Fig. 9

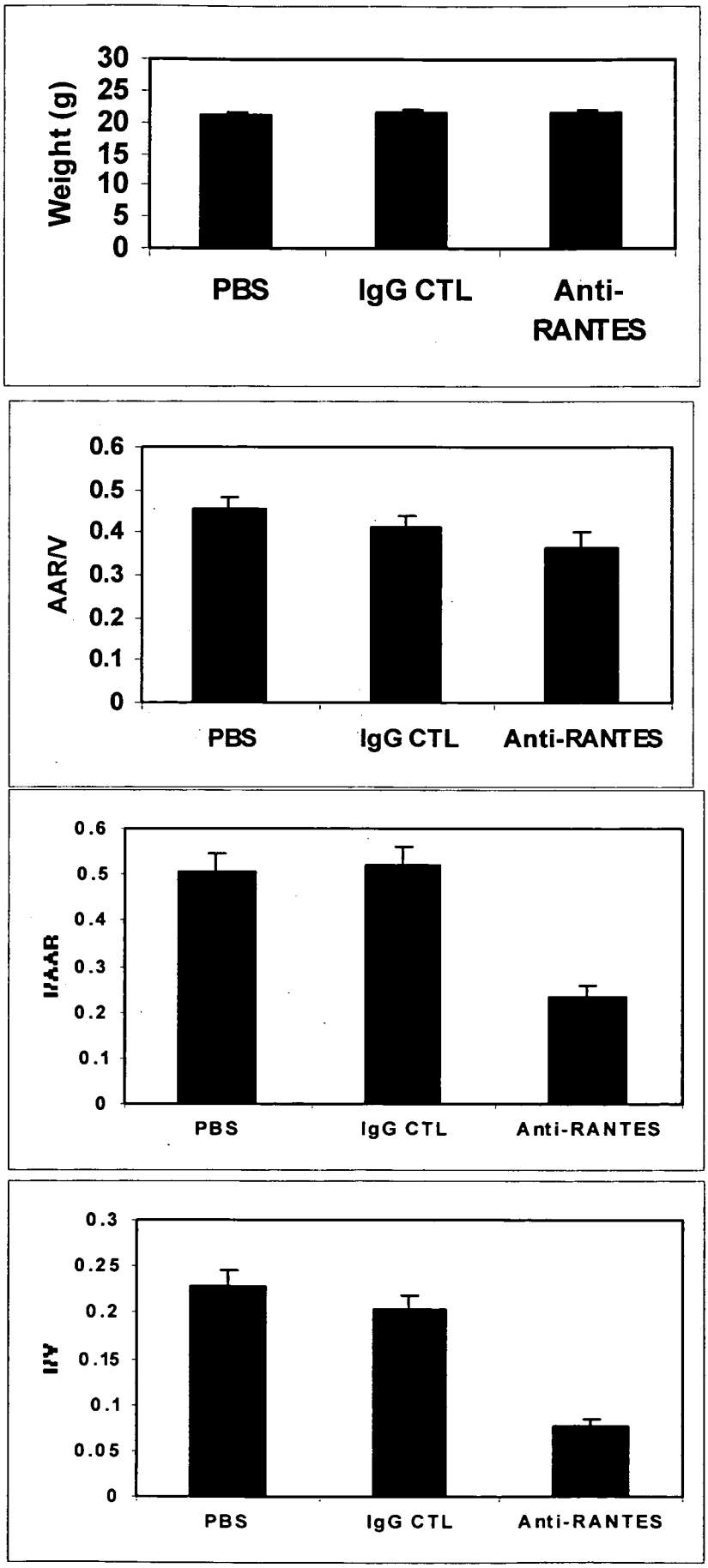
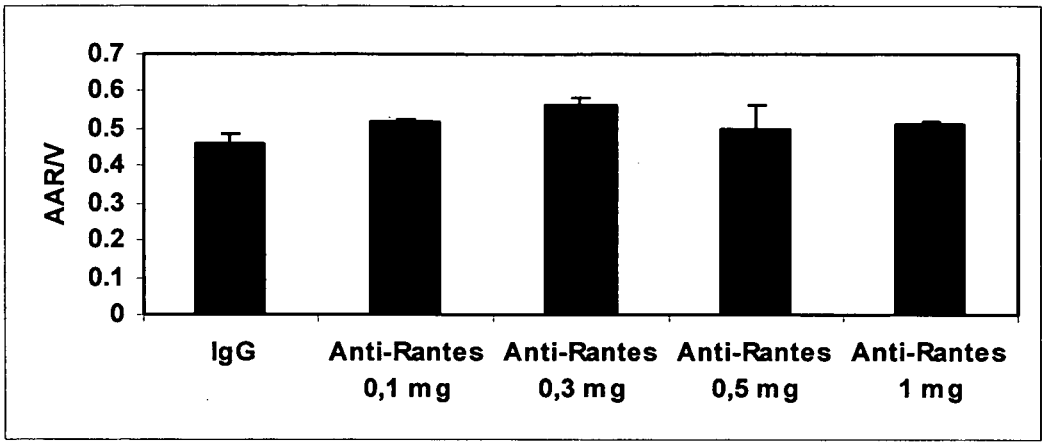
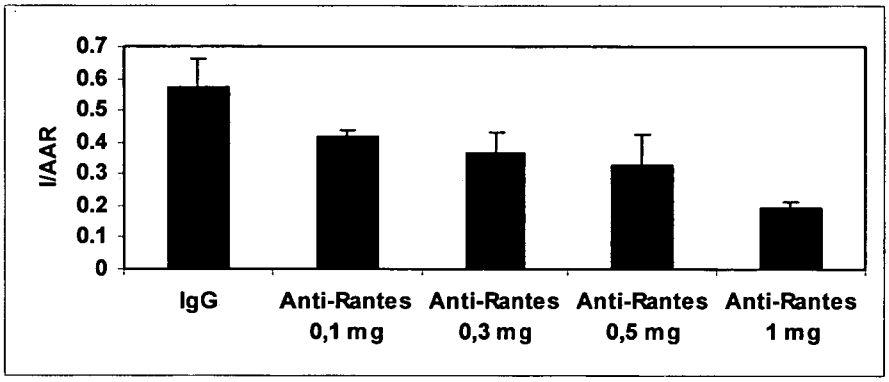

Weight (g)
30
25
20
15
10
5
0
PBS
IgG CTL
Anti-RANTES
AAR/V
0.6
0.5
0.4
0.3
0.2
0.1
0
PBS
IgG CTL
Anti-RANTES
IAAR
0.6
0.5
0.4
0.3
0.2
0.1
0
PBS
IgG CTL
Anti-RANTES
IIV
0.3
0.25
0.2
0.15
0.1
0.05
0
PBS
IgG CTL
Anti-RANTES

Fig. 10


AAR/V
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
IgG
Anti-Rantes 0,1 mg
Anti-Rantes 0,3 mg
Anti-Rantes 0,5 mg
Anti-Rantes 1 mg


I/AAR
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
IgG
Anti-Rantes 0,1 mg
Anti-Rantes 0,3 mg
Anti-Rantes 0,5 mg
Anti-Rantes 1 mg

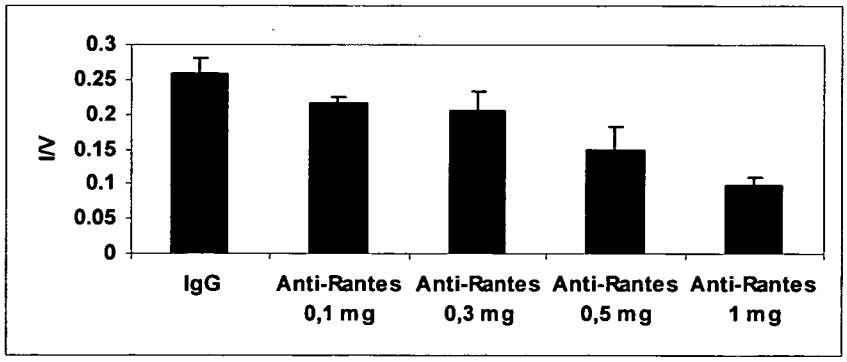

I/V
0.3
0.25
0.2
0.15
0.1
0.05
0
IgG
Anti-Rantes 0,1 mg
Anti-Rantes 0,3 mg
Anti-Rantes 0,5 mg
Anti-Rantes 1 mg

Fig. 11

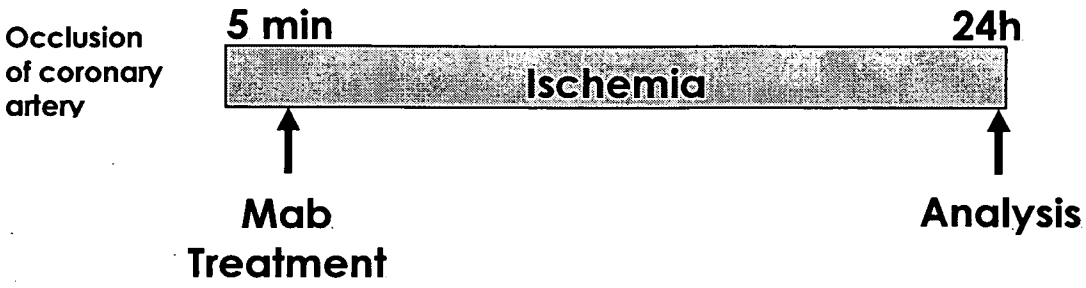
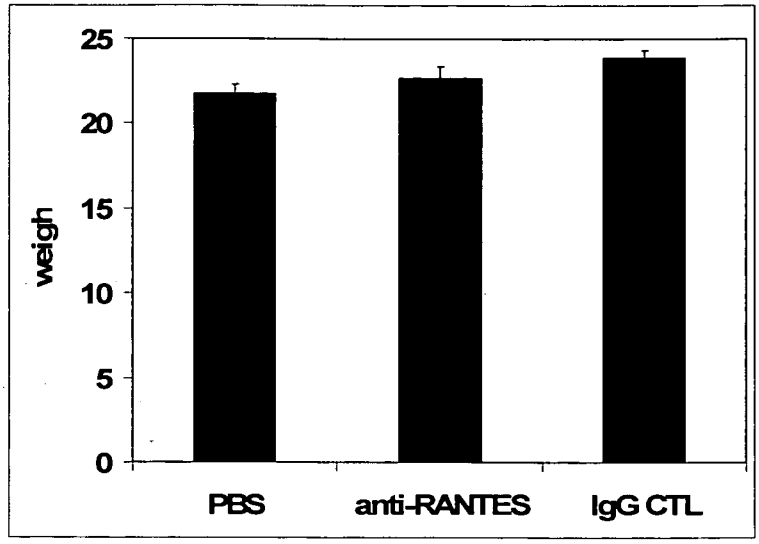
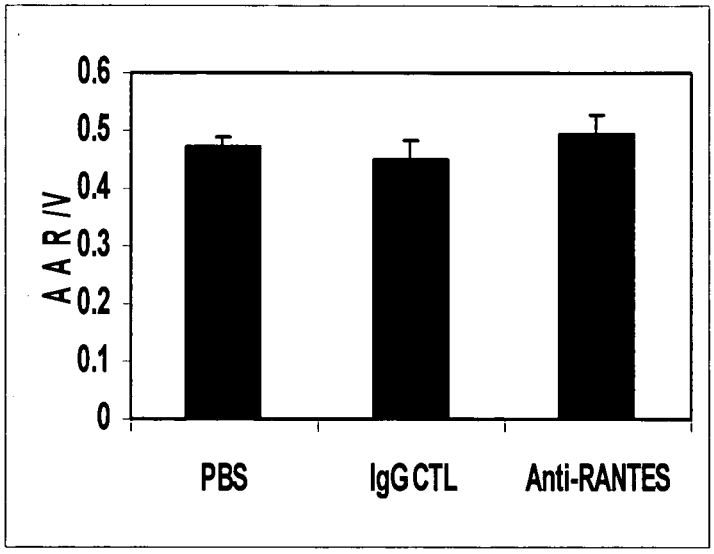

Occlusion of coronary artery
5 min
24h
Ischemia
Mab Treatment
Analysis

Fig. 12


25
20
15
10
5
0
weigh
PBS
anti-RANTES
IgG CTL


0.6
0.5
0.4
0.3
0.2
0.1
0
A A R /V
PBS
IgG CTL
Anti-RANTES

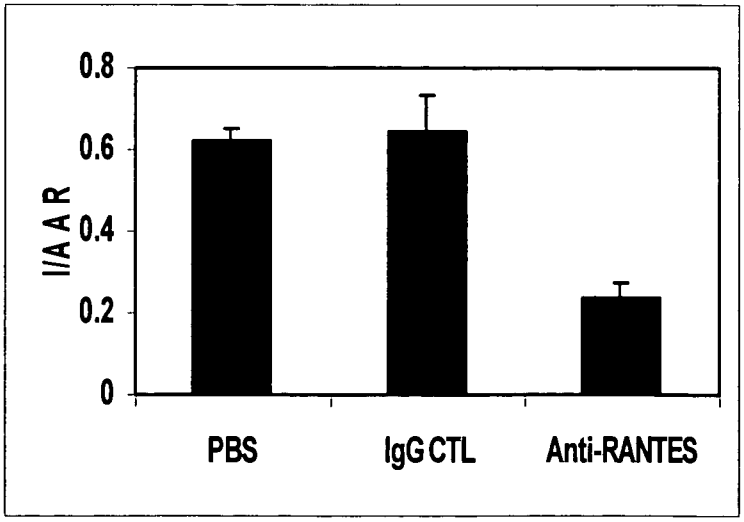

0.8
0.6
0.4
0.2
0
I/A A R
PBS
IgG CTL
Anti-RANTES

Fig. 12

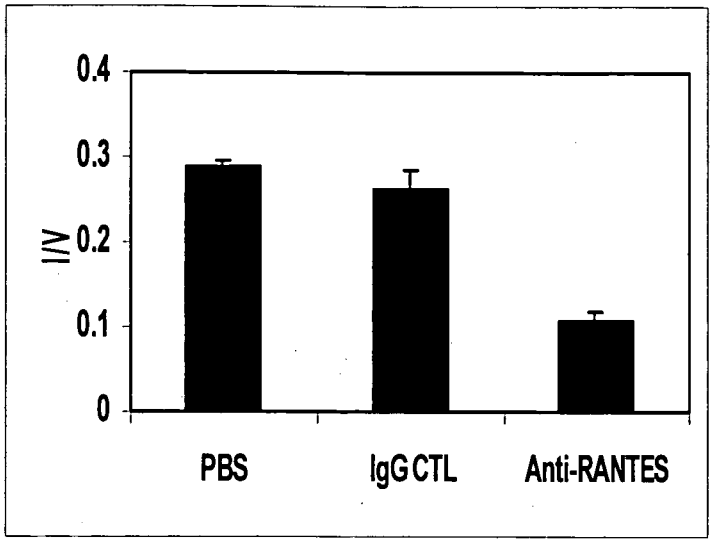
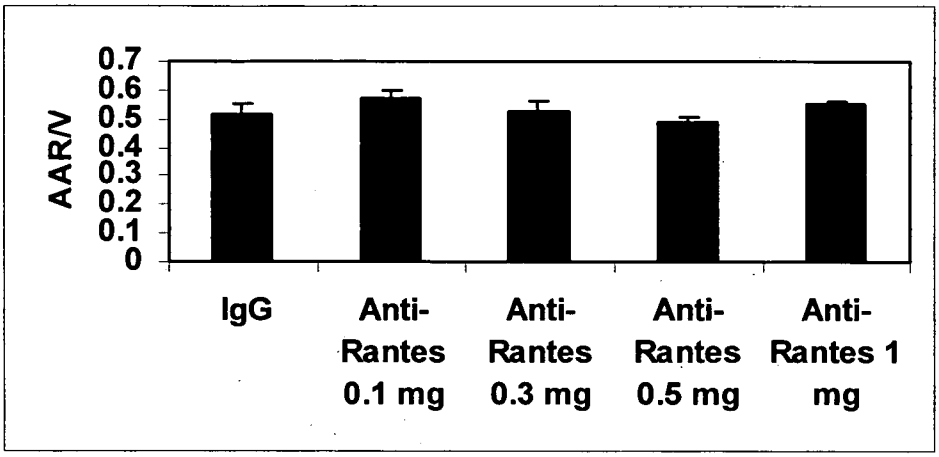
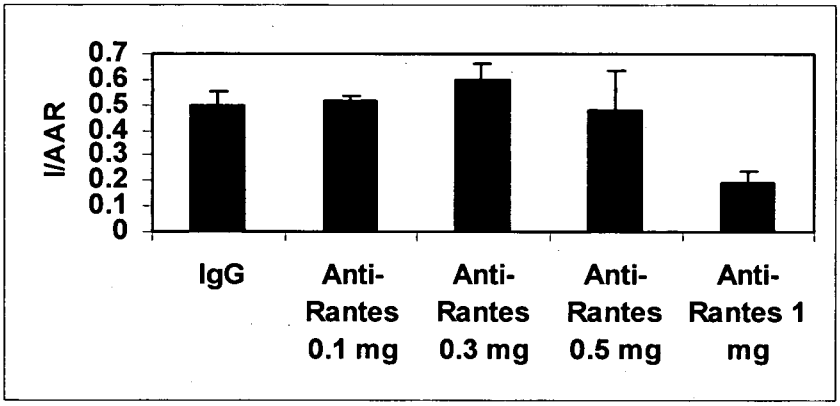
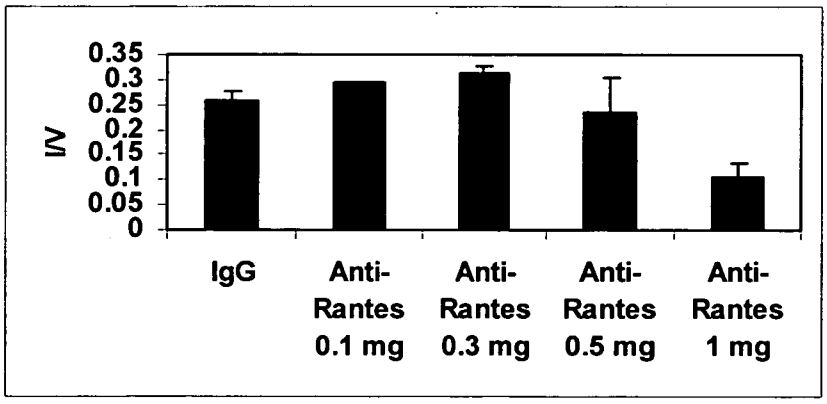


Fig. 13


AAR/V
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
IgG
Anti-Rantes 0.1 mg
Anti-Rantes 0.3 mg
Anti-Rantes 0.5 mg
Anti-Rantes 1 mg


I/AAR
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
IgG
Anti-Rantes 0.1 mg
Anti-Rantes 0.3 mg
Anti-Rantes 0.5 mg
Anti-Rantes 1 mg


I/V
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
IgG
Anti-Rantes 0.1 mg
Anti-Rantes 0.3 mg
Anti-Rantes 0.5 mg
Anti-Rantes 1 mg