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(54) **ANTIBODIES OPERABLY LINKED TO
SELECTED CHEMOATTRACTANTS**

(75) Inventors: **Kenton Abel**, Hacienda Heights, CA
(US); **Arthur J. Chirino**, Camarillo,
CA (US); **Omid Vafa**, Monrovia, CA
(US)

Correspondence Address:
Robin M. Silva
Dorsey & Whitney LLP
Intellectual Property Department
555 California Street, Suite 1000
San Francisco, CA 94104-1513 (US)

(73) Assignee: **Xencor, Inc.**

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(57) **ABSTRACT**

An antibody or fragment thereof operably linked to a one or more chemoattractants selected from the group consisting of: C5a or fragments thereof; C3a or fragments thereof; C4a or fragments thereof; and, formyl-Met-Leu-Phe (fMLP).

FIGURE 1A

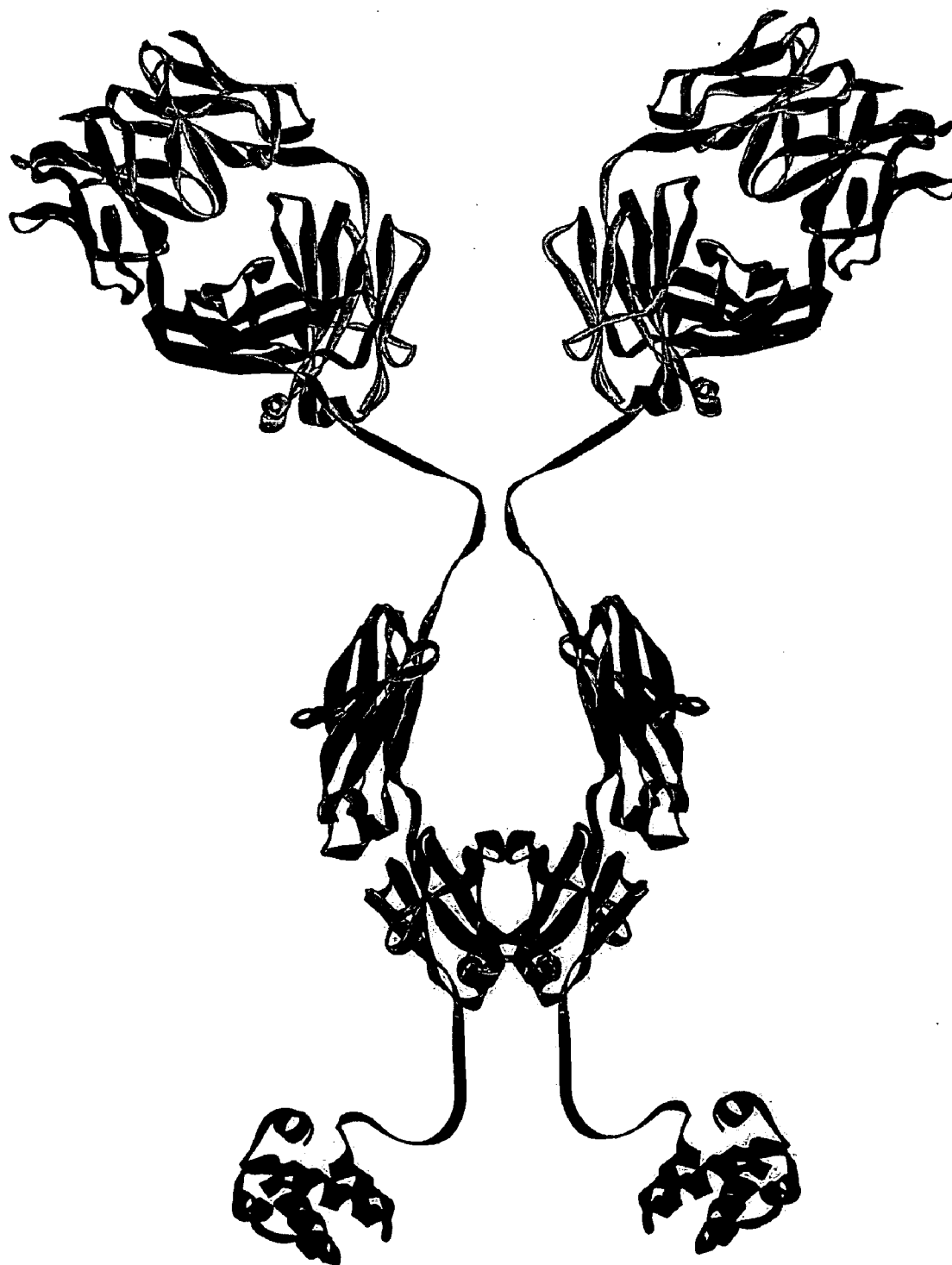


FIGURE 1B

SEQ. ID NO:1

Light Chain (intact)

QIVLSQSPAILSPGKVTMTTCRASSSVSYIHWQQKPGSSPKPWYATSNLASGVPVRFSGSGSGTSYSLTIS
RVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD
NALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

FIGURE 1C

SEQ. ID NO:2

Heavy Chain (IgG1 hinge) + G(SG)₅ + C5a (1-74)

QVQLQPGAEELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRLGWIGAIYPGNGDTSYNQKFKGKATLTADKS
SSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLV
KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKAEPKSCD
KTHHTCPPCPAPELLGGPDVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPLPEEKTIISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKG
FYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSLSPG
KSGSGSGSGSGLQKKIEEIAAKYKHSVVKCCYDGACVNNDTCEQRAARISLGPRCIKAFTECCVVASQLRA
NISHKDMQLGR

FIGURE 2A



FIGURE 2B

SEQ. ID NO:3

Light Chain + G(SG)₅ + C5a (1-74)

ELVMTQSPSSLSASVGRVNIACRASQGISSALAWYQQKPGKAPRLIYDASNLESGVPSRFSGSGSGTDFTLTI
SSLQPEDFAIYYCQQFNSYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
DNALQSGNSQESVTEQDSKSTYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGSGSGSGSGSG
MLQKKIEEIAKYHSVVKCCYDGACVNNDETCEQRAARISLGPRCIKAFTECCVVASQLRANISHKDMQLGR

FIGURE 2C

SEQ. ID NO:4

Heavy Chain (truncated at 227)

QVKLLEQSGAEVKKPGASVKVSCKASGYSFTSYGLHWVRQAPGORLEWMGWISAGTGNTKYSQKFRGRVTFTTRDT
SATTAYMGLSSLRPEDTAVYYCARDPYGGGKSEFDYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL
VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
DK

FIGURE 3A



FIGURE 3B

SEQ. ID NO:5

Light Chain + G(SG)₅ + C5a (1-74)

ELVMTQSPSSLSASVGDRVNIACRASQGISSALAWYQQKPGKAPRLLIYDASNLESGVPSRFSGSGSGTDFTLTIS
SSLQPEDFAIYYCQQFNSYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
DNALQSGNSQESVTEQDSKSTYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGSGSGSGSGSGSG
MLQKKIEEIIAKYHSVVKCCYDGACVNNDETCEQRAARISLGPRCIKAFTECCVVASQLRANISHKDMQLGR

FIGURE 3C

SEQ. ID NO:6

Heavy Chain (truncated at 240)

QVKLLEQSGAEVKKPGASVKVSKASGYSFTSYGLHWVRQAPGQRLEWMGWISAGTGNTKYSQKFRGRVTFTTRDT
SATTAYMGLSSLRPEDTAVYYCARDPYGGGKSEFDYWGQGLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL
VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
DKTHTCPPCPAPELL

FIGURE 4A

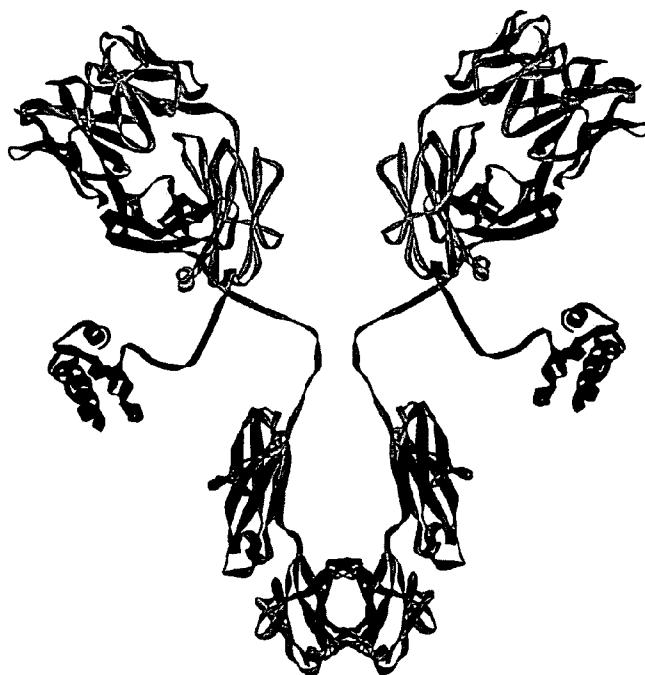


FIGURE 4B

SEQ. ID NO:7

Light Chain + G(SG)₅ + C5a (1-74)

QIVLSQSPAILSASPGEKVTMTCRASSSVSYIHWFQQKPGSSPKPWIYATSNLASGVPVRFSGSGSGTSYSLTIS
RVEAEDAATYYCQQWTSNPPTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD
NALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGSGSGSGSGSGSM
LQKKIEEIAKYHSVVKCCYDGACVNNDTCEQRAARISLGPRCIKAFTECCVVASQLRANISHKDMQLGR

FIGURE 4C

SEQ. ID NO:8

Heavy Chain (Intact)

QVQLQQPGAELVKPGASVKMSCKASGYTFTSYNMHWVKQTPGRGLEWIGAIYPGNGDTSYNQKFKGKATLTADKS
SSTAYMQLSSLTSEDSAVYYCARSTYYGGDWYFNVWGAGTTVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLV
KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKAEPKSCD
KTHTCPPCPAPELLGGPDVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPKEKTIKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKG
FYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMHEALHNHYTQKSLSLSPG
K

FIGURE 5A

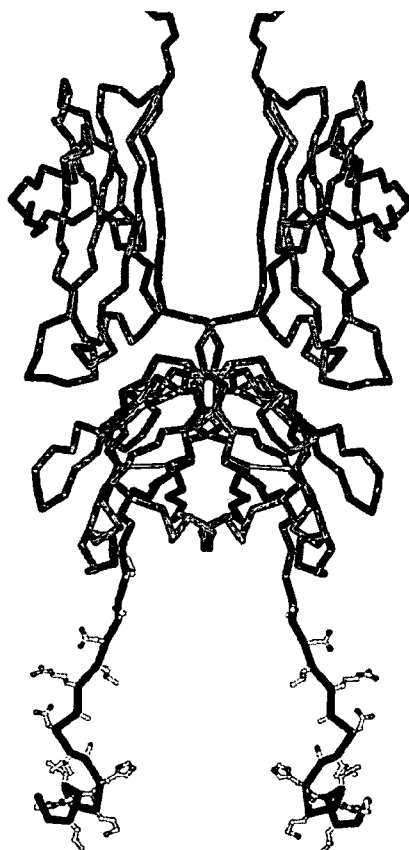


FIGURE 5B

SEQ. ID NO:9

Light Chain (intact)

ELVMTQSPSSLSASVGDRVNIACRASQGISSALAWYQQKPGKAPRLIIYDASNLESGVPSRFSGSGSGTDFTLTIS
SSLQPEDFAIYYCQQFNISYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
DNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC

FIGURE 5C

SEQ. ID NO:10

Heavy Chain + C5a (58-74)

QVKLEQSGAEVKKPGASVKVSCKASGYSFTSYGLHWVRQAPGQRLEWMGWISAGTGNTKYSQKFRGRVTFTRDT
SATTAYMGLSSLRPEDTAVYYCARDPYGGGKSEFDYWGGTGLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL
VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHENPEVKFNWYVDGVEVHNAKTKPREEQ
YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTTPFVLDSDGSFFLYSKLTVDKSRWQQGNVDFSCVMHEALHNHYTQKSLSLAS
QLRANISHKDMQLGR

FIGURE 6A

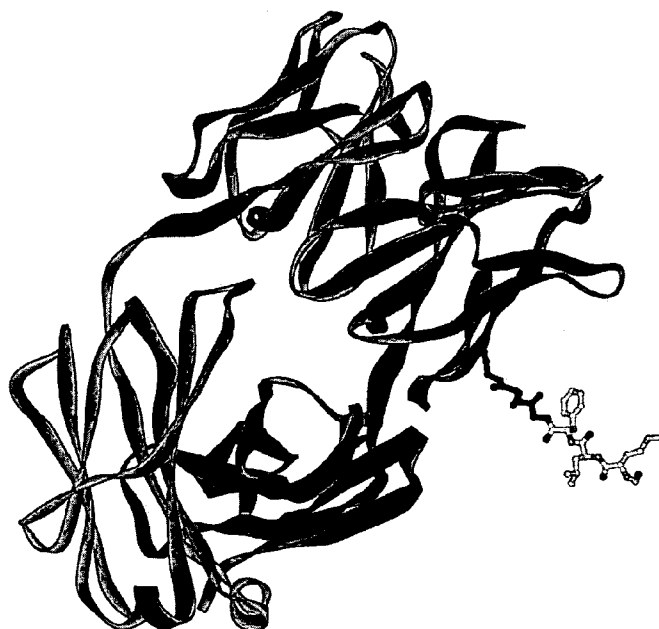


FIGURE 6B

SEQ. ID NO:11

Light Chain (intact)

ELVMTQSPSSLSASVGDVRVNIACRASQGISSALAWYQQKPGKAPRLIIYDASNLESGVPSRFSGSGSGTDFTLT
SSLPEDFAIYYCQQFNSYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
DNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

FIGURE 6C

SEQ. ID NO:12

(f)MLP + Heavy Chain (truncated at 227)

(f)MLFQVKLLEQSGAEVKKPGASVKVSCASGYSFTSYGLHWVRQAPGQRLEWMGWISAGTGNTKYSQKFRGRV
TFTRDTSATTAYMGLSSLRPEDTAVYYCARDPYGGGKSEFDYWGGTLVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKK
VEPKSCDK

FIGURE 7A



FIGURE 7B

SEQ. ID NO:13

Light Chain + C5a (58-74)

ELVMTQSPSSLSASVGDRVNIACRASQGISSALAWYQQKPGKAPRLIYDASNLESGVPSRFSGSGSGTDFTLTIS
SSLQPEDFAIYYCQQFNISYPLTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
DNALQSGNSQESVTEQDSKSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECASQLRANISHK
DMQLGR

FIGURE 7C

SEQ. ID NO:14

(f)MLP + Heavy Chain (truncated at 227)

(f)MLFQVKLLEQSGAEVKKPGASVKVSCKASGYSFTSYGLHWVRQAPGQRLEWMGWISAGTGNTKYSQKFRGRV
TFTRDTSATTAYMGLSSLRPEDTAVYYCARDPYGGGKSEFDYWGQGLVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK
VEPKSCDK

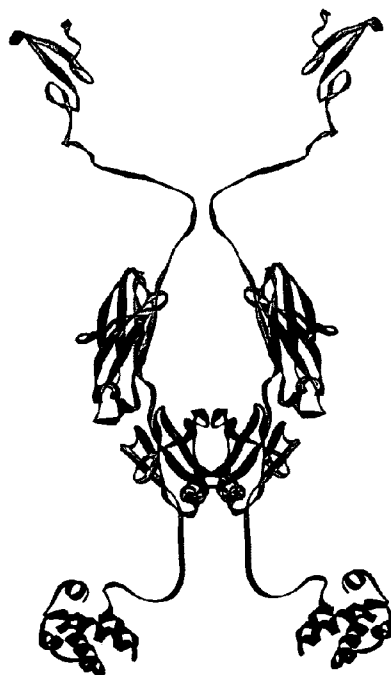


FIGURE 8A

FIGURE 8B

SEQ. ID NO:15

huEGF (1-53) + IgG1 hinge + C_H2 + C_H3 + G(SG)₅ + C5a (1-74)

NSDSECPLSHDGYCLHDGVCMIYIEALDKYACNCVVG YIGERCQYRDLKWWELRDKTHTCPPCPAPPELLGGPSVFL
FPPKPKDTLMISRTPEVTCVVDVSHENPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK
EYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCVMHEALHNHYTQKSLSLGS GSGSGSGMLQKKIEEIAAKY
KHSVVKCCYDGACVNDETCEQRAARISLGPRCIKAFTECCVVASQLRANISHKDMQLGR

FIGURE 9A

SEQ. ID NO:16

huC3a (1-77)

SVQLTEKRMDKVGKYPKELRKCCEDGMRENPMRFSCQRRTRFISLGEACKKVFLDCCNYITELRRQHARASHLGL
AR

FIGURE 9B

SEQ. ID NO:17

huC4a (1-77)

NVNFQKAINKLGQYASPTAKRCCQDGVTRLPMMSCEQRAARVQQPDCREPFLSCCQFAESLRKKS RDKGQAGL
QR

FIGURE 9C

SEQ. ID NO:18

huC5a (1-74)

MLQKKIEEIAAKYKHSVVKCCYDGACVNNDTCEQRAARISLGPRCIKAFTECCVVASQLRANISHKDMQLGR

ANTIBODIES OPERABLY LINKED TO SELECTED CHEMOATTRACTANTS

[0001] This application claims the benefit of under 35 U.S.C. § 119(e) to U.S. Ser. No. 60/627,445, filed Nov. 12, 2004, incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0002] 1. Field of the Invention

[0003] The present invention relates to conjugates of chemoattractants and antibodies or fragments thereof. The chemoattractants can be selected from among C5a or fragments thereof, C3a or fragments thereof, and C4a or fragments thereof.

[0004] 2. Description of Related Art

[0005] Antibodies are immunological proteins that bind a specific antigen. In most mammals, including humans and mice, antibodies are constructed from paired heavy and light polypeptide chains. Each chain is made up of individual immunoglobulin (Ig) domains, and thus the generic term immunoglobulin is used for such proteins. Each chain is made up of two distinct regions, referred to as the variable and constant regions. The light and heavy chain variable regions show significant sequence diversity between antibodies, and are responsible for binding the target antigen. The constant regions show less sequence diversity, and are responsible for binding a number of natural proteins to elicit important biochemical events. In humans there are five different classes of antibodies including IgA (which includes subclasses IgA1 and IgA2), IgD, IgE, IgG (which includes subclasses IgG1, IgG2, IgG3, and IgG4), and IgM. The distinguishing features between these antibody classes are their constant regions, although subtler differences may exist in the V region. **FIG. 1** shows an IgG1 antibody, used here as an example to describe the general structural features of immunoglobulins. IgG antibodies are tetrameric proteins composed of two heavy chains and two light chains. The IgG heavy chain is composed of four immunoglobulin domains linked from N- to C-terminus in the order V_H — C_{H1} — C_{H2} — C_{H3} , referring to the heavy chain variable domain, constant gamma 1 domain, constant gamma 2 domain, and constant gamma 3 domain respectively. The IgG light chain is composed of two immunoglobulin domains linked from N- to C-terminus in the order V_L — C_{L1} , referring to the light chain variable domain and the light chain constant domain respectively.

[0006] The variable region of an antibody contains the antigen binding determinants of the molecule, and thus determines the specificity of an antibody for its target antigen. The variable region is so named because it is the most distinct in sequence from other antibodies within the same class. The majority of sequence variability occurs in the complementarity determining regions (CDRs). There are 6 CDRs total, three each per heavy and light chain, designated V_H CDR1, V_H CDR2, V_H CDR3, V_L CDR1, V_L CDR2, and V_L CDR3. The variable region outside of the CDRs is referred to as the framework (FR) region. Although not as diverse as the CDRs, sequence variability does occur in the FR region between different antibodies. Overall, this characteristic architecture of antibodies provides a stable scaffold (the FR region) upon which substantial antigen binding diversity (the CDRs) can be explored by the

immune system to obtain specificity for a broad array of antigens. A number of high-resolution structures are available for a variety of variable region fragments from different organisms, some unbound and some in complex with antigen. The sequence and structural features of antibody variable regions are well characterized (Morea et al., 1997, *Biophys Chem* 68:9-16; Morea et al., 2000, *Methods* 20:267-279), incorporated by reference in its entirety, and the conserved features of antibodies have enabled the development of a wealth of antibody engineering techniques (Maynard et al., 2000, *Annu Rev Biomed Eng* 2:339-376), incorporated by reference in its entirety. For example, it is possible to graft the CDRs from one antibody, for example a murine antibody, onto the framework region of another antibody, for example a human antibody. This process, referred to in the art as “humanization”, enables generation of less immunogenic antibody therapeutics from nonhuman antibodies. Fragments consisting of the variable region can exist in the absence of other regions of the antibody, including for example the antigen binding fragment (Fab) consisting of V_H — C_{H1} and V_H — C_{L1} , the variable fragment (Fv) consisting of V_H and V_L , the single chain variable fragment (scFv) consisting of V_H and V_L linked together in the same chain, as well as a variety of other variable region fragments (Little et al., 2000, *Immunol Today* 21:364-370), incorporated by reference in its entirety.

[0007] There is a need to combine the advantages of antibodies with those of other proteins. In particular, there is a need to combine proteins having chemoattractant properties with antibodies. The present invention meets these and other needs.

SUMMARY OF THE INVENTION

[0008] In one aspect, a chemoattractant-antibody conjugate comprising an antibody operably linked to a chemoattractant or fragment thereof, the chemoattractant selected from the group consisting of C5a, C4a, and C3a. Exemplary fragments include residues 58-74 of the C5a chemoattractant. In further embodiments, the chemoattractant is linked to the antibody by a linker, such as a glycine-serine linker. The antibody can be any antibody or fragment, such as Fab fragments, Fc fragments, or antibody heavy chains. In additional embodiments, the cytotoxic activity of effector cells is enhanced by favorably modulating relevant receptors that mediate target cell killing (e.g. by ADCC, ADAP or CDC).

BRIEF DESCRIPTION OF THE FIGURES

[0009] **FIG. 1:** **FIG. 1A** is a ribbons representation of a three-dimensional model of an antibody-C5a fusion, wherein the antibody is an intact IgG1 and C5a is attached to the C-terminus of the heavy chain with a Gly-Ser linker. **FIG. 1B** and **FIG. 1C** are the amino acid sequences of the light chain and heavy chain, respectively.

[0010] **FIG. 2:** **FIG. 2A** is a ribbons representation of a three-dimensional model of an antibody-C5a fusion, wherein the antibody is a Fab fragment and C5a is attached to the C-terminus of the light chain with a Gly-Ser linker. **FIG. 2B** and **FIG. 2C** are the amino acid sequences of the light chain and heavy chain, respectively.

[0011] **FIG. 3:** **FIG. 3A** is a ribbons representation of a three-dimensional model of an antibody-C5a fusion,

wherein the antibody is a F(ab')₂ fragment and C5a is attached to the C-terminus of the light chain with a Gly-Ser linker. **FIG. 3B** and **FIG. 3C** are the amino acid sequences of the light chain and heavy chain, respectively.

[0012] **FIG. 4:** **FIG. 4A** is a ribbons representation of a three-dimensional model of an antibody-C5a fusion, wherein the antibody is an intact IgG1 and C5a is attached to the C-terminus of the light chain with a Gly-Ser linker. **FIG. 4B** and **FIG. 4C** are the amino acid sequences of the light chain and heavy chain, respectively.

[0013] **FIG. 5:** **FIG. 5A** is a carbon- α trace of a three-dimensional model of an antibody-C5a fusion, wherein the antibody is an intact IgG1 (only Fc region shown) and C5a is a fragment consisting of residues 58-74. The C5a is directly attached to the C-terminus of the heavy chain. **FIG. 5B** and **FIG. 5C** are the amino acid sequences of the light chain and heavy chain, respectively.

[0014] **FIG. 6:** **FIG. 6A** is a ribbons representation of a three-dimensional model of an antibody-fMLP fusion, wherein the antibody is a Fab fragment and fMLP is directly attached to the N-terminus of the heavy chain. **FIG. 6B** and **FIG. 6C** are the amino acid sequences of the light chain and heavy chain, respectively.

[0015] **FIG. 7:** **FIG. 7A** is a ribbons representation of a three-dimensional model of an antibody-C5a-fMLP fusion, wherein the antibody is a Fab region and the C5a consists of residues 58-74. The C5a is directly attached to the C-terminus of the light chain and fMLP is directly attached to the N-terminus of the heavy chain. **FIG. 7B** and **FIG. 7C** are the amino acid sequences of the light chain and heavy chain, respectively.

[0016] **FIG. 8:** **FIG. 8A** is a ribbons representation of a three-dimensional model of a humanEGF-antibody-C5a fusion. The human EGF is directly attached to the N-terminus of the IgG1 hinge region and the C5a is attached to the C-terminus of the light chain with a Gly-Ser linker. **FIG. 8B** is the amino acid of huEGF (1-53)+IgG1 hinge+C_H2+C_H3+G(SG)₅+C5a (1-74).

[0017] **FIG. 9:** **FIGS. 9A, 9B** and **9C** are the amino acid sequences of human C3a, C4a and C5a, respectively.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

[0018] The present invention is directed to chemoattractant-antibody conjugate including an antibody operably linked to a chemoattractant or fragment thereof selected from among C5a, C4a, and C3a.

[0019] General Definitions

[0020] In order that the invention may be more completely understood, several definitions are set forth below. Such definitions are meant to encompass grammatical equivalents.

[0021] By "ADCC" or "antibody dependent cell-mediated cytotoxicity" as used herein is meant the cell-mediated reaction wherein nonspecific cytotoxic cells that express Fc γ Rs recognize bound antibody on a target cell and subsequently cause lysis of the target cell.

[0022] By "ADCP" or antibody dependent cell-mediated phagocytosis as used herein is meant the cell-mediated

reaction wherein nonspecific cytotoxic cells that express Fc γ Rs recognize bound antibody on a target cell and subsequently cause phagocytosis of the target cell.

[0023] By "amino acid modification" herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence. The preferred amino acid modification herein is a substitution. By "amino acid substitution" or "substitution" herein is meant the replacement of an amino acid at a particular position in a parent polypeptide sequence with another amino acid. For example, the substitution 1332E refers to a variant polypeptide, in this case an Fc variant, in which the isoleucine at position 332 is replaced with a glutamic acid.

[0024] By "effector function" as used herein is meant a biochemical event that results from the interaction of an antibody Fc region with an Fc receptor or ligand. Effector functions include but are not limited to ADCC, ADCP, and CDC. By "effector cell" as used herein is meant a cell of the immune system that expresses one or more Fc receptors and mediates one or more effector functions. Effector cells include but are not limited to monocytes, macrophages, neutrophils, dendritic cells, eosinophils, mast cells, platelets, B cells, large granular lymphocytes, Langerhans' cells, natural killer (NK) cells, and $\gamma\gamma$ T cells, and may be from any organism including but not limited to humans, mice, rats, rabbits, and monkeys. By "library" herein is meant a set of Fc variants in any form, including but not limited to a list of nucleic acid or amino acid sequences, a list of nucleic acid or amino acid substitutions at variable positions, a physical library consisting of nucleic acids that encode the library sequences, or a physical library consisting of the Fc variant proteins, either in purified or unpurified form.

[0025] By "parent polypeptide" or "precursor polypeptide" (including Fc parent or precursors) as used herein is meant a polypeptide that is subsequently modified to generate a variant. Said parent polypeptide may be a naturally occurring polypeptide, or a variant or engineered version of a naturally occurring polypeptide. Parent polypeptide may refer to the polypeptide itself, compositions that comprise the parent polypeptide, or the amino acid sequence that encodes it. Accordingly, by "parent Fc polypeptide" as used herein is meant a Fc polypeptide that is modified to generate a variant, and by "parent antibody" as used herein is meant an antibody that is modified to generate a variant antibody.

[0026] As outlined above, certain positions of the Fc molecule can be altered. By "position" as used herein is meant a location in the sequence of a protein. Positions may be numbered sequentially, or according to an established format, for example the EU index as in Kabat. For example, position 297 is a position in the human antibody IgG1. Corresponding positions are determined as outlined above, generally through alignment with other parent sequences.

[0027] By "residue" as used herein is meant a position in a protein and its associated amino acid identity. For example, Asparagine 297 (also referred to as Asn297, also referred to as N297) is a residue in the human antibody IgG1.

[0028] By "target antigen" as used herein is meant the molecule that is bound specifically by the variable region of a given antibody. A target antigen may be a protein, carbohydrate, lipid, or other chemical compound.

[0029] By "target cell" as used herein is meant a cell that bind to a target molecule.

[0030] By “variable region” as used herein is meant the region of an immunoglobulin that comprises one or more Ig domains substantially encoded by any of the V_k , V_l , and/or V_H genes that make up the kappa, lambda, and heavy chain immunoglobulin genetic loci respectively.

[0031] By “variant polypeptide” as used herein is meant a polypeptide sequence that differs from that of a parent polypeptide sequence by virtue of at least one amino acid modification. Variant polypeptide may refer to the polypeptide itself, a composition consisting of the polypeptide, or the amino sequence that encodes it. Preferably, the variant polypeptide has at least one amino acid modification compared to the parent polypeptide, e.g. from about one to about ten amino acid modifications, and preferably from about one to about five amino acid modifications compared to the parent. The variant polypeptide sequence herein will preferably possess at least about 80% homology with a parent polypeptide sequence, and most preferably at least about 90% homology, more preferably at least about 95% homology.

[0032] Thus “amino acid”, or “peptide residue”, as used herein means both naturally occurring and synthetic amino acids. For example, homophenylalanine, citrulline and noreucine are considered amino acids for the purposes of the invention. “Amino acid” also includes imino acid residues such as proline and hydroxyproline. The side chain may be in either the (R) or the (S) configuration. In the preferred embodiment, the amino acids are in the (S) or L-configuration. If non-naturally occurring side chains are used, non-amino acid substituents may be used, for example to prevent or retard in vivo degradation.

[0033] By “protein” herein is meant at least two covalently attached amino acids, which includes proteins, polypeptides, oligopeptides and peptides. The protein may be made up of naturally occurring amino acids and peptide bonds, or synthetic peptidomimetic structures, i.e. “analogs”, such as peptoids (see Simon et al., 1992, *Proc Natl Acad Sci USA* 89(20):9367, incorporated by reference in its entirety) particularly when LC peptides are to be administered to a patient.

[0034] I. Chemoattractant-Antibody Conjugates

[0035] The present invention is directed to chemoattractant-antibody conjugates.

[0036] One way in which cells in the innate immune system are able to detect the presence of infection is by binding bacterial peptides containing N-formylmethionine, or fMet, a modified amino acid that initiates all proteins synthesized in prokaryotes. The receptor that recognizes these peptides is known as the fMet-Leu-Phe (fMLP) receptor, after a tripeptide for which it has a high affinity, though it is not restricted to binding just this tripeptide. The fMLP receptor belongs to an ancient and widely distributed family of receptors that have seven membrane-spanning segments; the best-characterized members of this family are the photoreceptors rhodopsin and bacteriorhodopsin. In the immune system, members of this family of receptors have a number of essential roles; the receptors for the anaphylotoxins and for chemokines belong to this family.

[0037] Chemokines can be produced by a wide variety of cell types in response to bacterial products, viruses, and agents that cause physical damage, such as silica or the urate

crystals that occur in gout. Thus, infection or physical damage to tissues sets in motion the production of chemokine gradients that can direct phagocytes to sites where they are needed. In addition, peptides that act as chemoattractants for neutrophils are made by bacteria themselves. All bacteria produce proteins with an amino-terminal N-formylated methionine, and, as discovered many years ago, the fMLP peptide is a potent chemotactic factor for inflammatory cells, especially neutrophils. The fMLP receptor is also a G protein-coupled receptor like the receptors for chemokines and for the complement fragments C5a, C3a, and C4a. The present conjugates are thus able to augment the function of chemoattractants with the function of antibodies, thereby providing novel therapeutic treatment for disease.

[0038] Accordingly, the present application is directed to chemoattractant-antibody conjugates. By “chemoattractant-antibody conjugate” or antibody-chemoattractant conjugate” as used herein is meant any covalently attached conjugate of an antibody with a chemoattractant selected from among: C3a or fragments thereof; C4a or fragments thereof; and C5a or fragments thereof. Examples of antibody-chemoattractant conjugates include but are not limited to: a Fab conjugated to fMLP on the N-terminus of the heavy chain; a Fc fused to a C5a fragment on the C-terminus of the heavy chain a (Gly-Ser)_n linker, a F(ab')₂ attached to fMLP on the ϵ -nitrogen of a Lys side chain; etc.

[0039] A. Chemoattractants

[0040] The chemoattractant-antibody conjugates of the present invention include C3a, C4a, and C5a chemoattractants or fragments thereof, covalently bonded to an antibody. **FIGS. 9a, b, and c** depict the sequences of C3a, C4a, and C5a, respectively.

[0041] There is a common mechanism for attracting neutrophils, whether by complement, chemokines, or bacterial peptides. Neutrophils are the first to arrive in large numbers at a site of infection, with monocytes and immature dendritic cells being recruited later. The complement peptide C5a, and the chemokines IL-8 and MCP-1 also activate their respective target cells, so that not only are neutrophils and macrophages brought to potential sites of infection but, in the process, they are armed to deal with any pathogens they may encounter. In particular, neutrophils exposed to IL-8 and the cytokine TNF- α are activated to produce the respiratory burst that generates oxygen radicals and nitric oxide, and to release their stored lysosomal contents, thus contributing both to host defense and to the tissue destruction and pus formation seen in local sites of infection with pyogenic bacteria. (See *Immunobiology*, vol. 5, Charles A. Janeway et al., Ed., Garland Publishing, New York, 2001, incorporated by reference in its entirety.)

[0042] C5a anaphylatoxin is the active fragment derived from C5 (a component of complement) cleavage by a convertase (C2b or Bb). C5a/C5aR interactions have been implicated in inflammation responses involving deposited IgG immune complexes (ICs), and C5aR is a key chemotactic receptor for leukocytes in host defense. C5a is a major vasodilator that increases blood flow and functions as a chemoattractant for killer neutrophil and monocyte infiltration to malignant sites (by increasing adhesion to vessel walls). Moreover, C5a functions as an autocrine factor in macrophage mediated tumoricidal activity/enhancing phagocytosis, and it also activates mast cells to release histamine and TNF- α .

[0043] C5a has been shown to augment antibody dependent cellular cytotoxicity (Otonello et al, Blood, Volume 87, Issue 12, pp. 5171-5178, 1996), incorporated by reference in its entirety. FcRs are known to play a critical role in inflammatory, autoimmune disease, and cancer. Recent findings show that C5a/C5aR interactions are directly involved in the regulation of FcγRs through the induction of FcγRIII and suppression of FcγRII on neutrophils/macrophages (Shushakova et al., J. Clin. Invest. 110: 1823-1830,) incorporated by reference in its entirety.

[0044] The present invention is also directed to conjugates including fragments of C5a. Fragments of C5a include any fragment having any function or activity of C5a as known herein. Particularly preferred are C5a fragments including amino acid residues 58-74 of C5a.

[0045] Like C5a, C3a is an anaphylatoxin that effects many of the same cells as C5a. C3a has its own transmembrane receptor. Once C3a and C5a are produced they undergo a rapid loss of activity in serum. This is primarily the result of serum carboxypeptidase cleavage of C-terminal Arg, which creates the desArg forms of these anaphylatoxins, which have much reduced activity.

[0046] C4a is also generated. C4a is less potent than C5a and C3a. The order of bioactivity of these fragment is C5a>C3a>>C4a.

[0047] Local inflammatory responses can be induced by small complement fragments, especially C5a. The small complement fragments are differentially active: C5a is more active than C3a, which is more active than C4a. C5a also has higher biological specificity than C3a and C4a. They cause local inflammatory responses by acting directly on local blood vessels, stimulating an increase in blood flow, increased vascular permeability, and increased binding of phagocytes to endothelial cells. C5a also activates mast cells to release mediators such as histamine and TNF-α that contribute to the inflammatory response. The increase in vessel diameter and permeability leads to the accumulation of fluid and protein. Fluid accumulation increases lymphatic drainage, bringing pathogens and their antigenic components to nearby lymph nodes. The antibodies, complement, and cells thus recruited participate in pathogen clearance by enhancing phagocytosis. The small complement fragments can also directly increase the activity of the phagocytes. (Immunobiology, 5th ed. Janeway, Charles A.; Travers, Paul; Walport, Mark; Shlomchik, Mark, New York and London: Garland Publishing, 2001, incorporated by reference in its entirety).

[0048] C5a can also enhance phagocytosis of opsonized microorganisms. Activation of complement, either by the alternative or the MB-lectin pathway, leads to the deposition of C3b on the surface of the microorganism. C3b can be bound by the complement receptor CR1 on the surface of phagocytes, but this on its own is insufficient to induce phagocytosis. Phagocytes also express receptors for the anaphylotoxin C5a, and binding of C5a will now activate the cell to phagocytose microorganisms bound through CR1. (Immunobiology, 5th ed. Janeway, Charles A.; Travers, Paul; Walport, Mark; Shlomchik, Mark, New York and London: Garland Publishing, 2001,) incorporated by reference in its entirety.

[0049] B. Antibodies

[0050] The chemoattractants-antibody conjugates include an antibody conjugated to the chemoattractant or fragment thereof.

[0051] By "antibody" herein is meant to include full length antibodies and antibody fragments, and may refer to a natural antibody from any organism, an engineered antibody, or an antibody generated recombinantly for experimental, therapeutic, or other purposes as further discussed below.

[0052] Traditional antibody structural units typically comprise a tetramer. Each tetramer is typically composed of two identical pairs of polypeptide chains, each pair having one "light" (typically having a molecular weight of about 25 kDa) and one "heavy" chain (typically having a molecular weight of about 50-70 kDa). 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. IgG has several subclasses, including, but not limited to IgG1, IgG2, IgG3, and IgG4. IgM has subclasses, including, but not limited to, IgM1 and IgM2. Thus, "isotype" as used herein is meant any of the subclasses of immunoglobulins defined by the chemical and antigenic characteristics of their constant regions. The known human immunoglobulin isotypes are IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgM1, IgM2, IgD, and IgE.

[0053] 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. In the variable region, three loops are gathered for each of the V domains of the heavy chain and light chain to form an antigen-binding site. Each of the loops is referred to as a complementarity-determining region (hereinafter referred to as a "CDR"), in which the variation in the amino acid sequence is most significant.

[0054] The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Kabat et al. collected numerous primary sequences of the variable regions of heavy chains and light chains. Based on the degree of conservation of the sequences, they classified individual primary sequences into the CDR and the framework and made a list thereof (see SEQUENCES OF IMMUNOLOGICAL INTEREST, 5th edition, NIH publication, No. 91-3242, E. A. Kabat et al.).

[0055] In the IgG subclass of immunoglobulins, there are several immunoglobulin domains in the heavy chain. By "immunoglobulin (Ig) domain" herein is meant a region of an immunoglobulin having a distinct tertiary structure. Of interest in the present invention are the heavy chain domains, including, the constant heavy (CH) domains and the hinge domains. In the context of IgG antibodies, the IgG isotypes each have three CH regions. Accordingly, "CH" domains in the context of IgG are as follows: "CH1" refers to positions 118-220 according to the EU index as in Kabat. "CH2" refers to positions 237-340 according to the EU index as in Kabat, and "CH3" refers to positions 341-447 according to the EU index as in Kabat.

[0056] Another type of Ig domain of the heavy chain is the hinge region. By "hinge" or "hinge region" or "antibody hinge region" or "immunoglobulin hinge region" herein is

meant the flexible polypeptide comprising the amino acids between the first and second constant domains of an antibody. Structurally, the IgG CH1 domain ends at EU position 220, and the IgG CH2 domain begins at residue EU position 237. Thus for IgG the antibody hinge is herein defined to include positions 221 (D221 in IgG1) to 236 (G236 in IgG1), wherein the numbering is according to the EU index as in Kabat. In some embodiments, for example in the context of an Fc region, the lower hinge is included, with the "lower hinge" generally referring to positions 226 or 230.

[0057] The different IgG subclasses have different affinities for the FcγRs, with IgG1 and IgG3 typically binding substantially better to the receptors than IgG2 and IgG4 (Jefferis et al., 2002, *Immunol Lett* 82:57-65), incorporated by reference in its entirety. All FcγRs bind the same region on IgG Fc, yet with different affinities: the high affinity binder FcγRI has a Kd for IgG1 of 10^{-8} M⁻¹, whereas the low affinity receptors FcγRII and FcγRIII generally bind at 10^{-6} and 10^{-5} respectively. The extracellular domains of FcγRIIIa and FcγRIIIb are 96% identical, however FcγRIIIb does not have an intracellular signaling domain. Furthermore, whereas FcγRI, FcγRIIa/c, and FcγRIIIa are positive regulators of immune complex-triggered activation, characterized by having an intracellular domain that has an immunoreceptor tyrosine-based activation motif (ITAM), FcγRIIb has an immunoreceptor tyrosine-based inhibition motif (ITIM) and is therefore inhibitory. Thus the former are referred to as activation receptors, and FcγRIIb is referred to as an inhibitory receptor. The receptors also differ in expression pattern and levels on different immune cells. Yet another level of complexity is the existence of a number of FcγR polymorphisms in the human proteome. A particularly relevant polymorphism with clinical significance is V158/F158 FcγRIIIa. Human IgG1 binds with greater affinity to the V158 allotype than to the F158 allotype. This difference in affinity, and presumably its effect on ADCC and/or ADCP, has been shown to be a significant determinant of the efficacy of the anti-CD20 antibody rituximab (Rituxan®, a registered trademark of IDEC Pharmaceuticals Corporation). Patients with the V158 allotype respond favorably to rituximab treatment; however, patients with the lower affinity F158 allotype respond poorly (Cartron et al., 2002, *Blood* 99:754-758), incorporated by reference in its entirety. Approximately 10-20% of humans are V158/V158 homozygous, 45% are V158/F158 heterozygous, and 35-45% of humans are F158/F158 homozygous (Lehrnbecher et al., 1999, *Blood* 94:4220-4232; Cartron et al., 2002, *Blood* 99:754-758), both incorporated by reference in its entirety. Thus 80-90% of humans are poor responders, that is they have at least one allele of the F158 FcγRIIIa.

[0058] By "full length antibody" herein is meant the structure that constitutes the natural biological form of an antibody, including variable and constant regions. For example, in most mammals, including humans and mice, the full length antibody of the IgG class is a tetramer and consists of two identical pairs of two immunoglobulin chains, each pair having one light and one heavy chain, each light chain consisting of immunoglobulin domains V_L and C_L, and each heavy chain consisting of immunoglobulin domains V_H, Cγ1, Cγ2, and Cγ3. In some mammals, for example in camels and llamas, IgG antibodies may consist of only two heavy chains, each heavy chain consisting of a variable domain attached to the Fc region. By "IgG" as used herein is meant a polypeptide belonging to the class of

antibodies that are substantially encoded by a recognized immunoglobulin gamma gene. In humans this class comprises IgG1, IgG2, IgG3, and IgG4. In mice this class comprises IgG1, IgG2a, IgG2b, IgG3.

[0059] The antibody may be a chimeric antibody and/or a humanized antibody. In general, both "chimeric antibodies" and "humanized antibodies" refer to antibodies that combine regions from more than one species. For example, "chimeric antibodies" traditionally comprise variable region(s) from a mouse (or rat, in some cases) and the constant region(s) from a human. "Humanized antibodies" generally refer to non-human antibodies that have had the variable-domain framework regions swapped for sequences found in human antibodies. Generally, in a humanized antibody, the entire antibody, except the CDRs, is encoded by a polynucleotide of human origin or is identical to such an antibody except within its CDRs. The CDRs, some or all of which are encoded by nucleic acids originating in a non-human organism, are grafted into the beta-sheet framework of a human antibody variable region to create an antibody, the specificity of which is determined by the engrafted CDRs. The creation of such antibodies is described in, e.g., WO 92/11018, Jones, 1986, *Nature* 321:522-525, Verhoeven et al., 1988, *Science* 239:1534-1536. "Backmutation" of selected acceptor framework residues to the corresponding donor residues is often required to regain affinity that is lost in the initial grafted construct (U.S. Pat. No. 5,530,101; U.S. Pat. No. 5,585,089; U.S. Pat. No. 5,693,761; U.S. Pat. No. 5,693,762; U.S. Pat. No. 6,180,370; U.S. Pat. No. 5,859,205; U.S. Pat. No. 5,821,337; U.S. Pat. No. 6,054,297; U.S. Pat. No. 6,407,213).

[0060] The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region, typically that of a human immunoglobulin. Humanized antibodies can also be generated using mice with a genetically engineered immune system. Roque et al., 2004, *Biotechnol. Prog.* 20:639-654. A variety of techniques and methods for humanizing and reshaping non-human antibodies are well known in the art (See Tsurushita & Vasquez, 2004, *Humanization of Monoclonal Antibodies*, Molecular Biology of B Cells, 533-545, Elsevier Science (USA), and references cited therein). Humanization methods include but are not limited to methods described in Jones et al., 1986, *Nature* 321:522-525; Riechmann et al., 1988, *Nature* 332:323-329; Verhoeven et al., 1988, *Science*, 239:1534-1536; Queen et al., 1989, *Proc Natl Acad Sci, USA* 86:10029-33; He et al., 1998, *J. Immunol.* 160: 1029-1035; Carter et al., 1992, *Proc Natl. Acad Sci USA* 89:4285-9; Presta et al., 1997, *Cancer Res.* 57(20):4593-9; Gorman et al., 1991, *Proc. Natl. Acad. Sci. USA* 88:4181-4185; O'Connor et al., 1998, *Protein Eng* 11:321-8, each of which is incorporated herein by reference in its entirety. Humanization or other methods of reducing the immunogenicity of nonhuman antibody variable regions may include resurfacing methods, as described for example in Roguska et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:969-973. In one embodiment, the parent antibody has been affinity matured, as is known in the art. Structure-based methods may be employed for humanization and affinity maturation, for example as described in U.S. Ser. No. 11/004,590. Selection based methods may be employed to humanize and/or affinity mature antibody variable regions, including but not limited to methods described in Wu et al., 1999, *J. Mol. Biol.* 294:151-162; Baca et al., 1997, *J. Biol. Chem.*

272(16):10678-10684; Rosok et al., 1996, *J. Biol. Chem.* 271(37): 22611-22618; Raderet et al., 1998, *Proc. Natl. Acad. Sci. USA* 95: 8910-8915; Krauss et al., 2003, *Protein Engineering* 16(10):753-759. Other humanization methods may involve the grafting of only parts of the CDRs, including but not limited to methods described in U.S. Ser. No. 09/810,502; Tan et al., 2002, *J. Immunol.* 169:1119-1125; De Pascalis et al., 2002, *J. Immunol.* 169:3076-3084. For a description of the concepts of chimeric and humanized antibodies see Clark et al., 2000 and references cited therein (Clark, 2000, *Immunol Today* 21:397-402), incorporated herein by reference in its entirety.

[0061] 2. Antibody Fragments

[0062] The term "antibody" includes antibody fragments, as are known in the art, such as Fab, Fd, dAb, Fab', F(ab')₂, Fc, Fv, scFv, or other subsequences of antibodies, either produced by the modification of whole antibodies or those synthesized de novo using recombinant DNA technologies. Specific antibody fragments include, but are not limited to, (i) the Fab fragment consisting of VL, VH, CL and CH1 domains, (ii) the Fd fragment consisting of the VH and CH1 domains, (iii) the Fv fragment consisting of the VL and VH domains of a single antibody; (iv) the dAb fragment (Ward et al., 1989, *Nature* 341:544-546) which consists of a single variable, (v) isolated CDR regions, (vi) F(ab')₂ fragments, a bivalent fragment comprising two linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site (Bird et al., 1988, *Science* 242:423-426, Huston et al., 1988, *Proc. Natl. Acad. Sci. U.S.A.* 85:5879-5883), (viii) bispecific single chain Fv dimers (PCT/US92/09965) and (ix) "diabodies" or "triabodies", multivalent or multispecific fragments constructed by gene fusion (Tomlinson et al., 2000, *Methods Enzymol.* 326:461-479; WO94/13804; Holliger et al., 1993, *Proc. Natl. Acad. Sci. U.S.A.* 90:6444-6448).

[0063] Alternatively, the antibodies can be a variety of structures, including, but not limited to, antibody fragments, monoclonal antibodies, bispecific antibodies, minibodies, domain antibodies, synthetic antibodies (sometimes referred to herein as "antibody mimetics"), chimeric antibodies, humanized antibodies, antibody fusions (sometimes referred to as "antibody conjugates"), and fragments of each, respectively.

[0064] 3. Fc Fragments

[0065] Antibodies also include Fc fusions. By "Fc fusion" as used herein is meant a protein wherein one or more polypeptides or other molecule is operably linked to an Fc region. For example, chemoattractants or fragments thereof can be operably linked to the Fc region. Fc fusion is herein meant to be synonymous with the terms "immunoadhesin", "Ig fusion", "Ig chimera", and "receptor globulin" (sometimes with dashes) as used in the prior art (Chamow et al., 1996, *Trends Biotechnol* 14:52-60; Ashkenazi et al., 1997, *Curr Opin Immunol* 9:195-200). An Fc fusion combines the Fc region of an immunoglobulin with a fusion partner, which in general can be any protein or small molecule. Virtually any protein or small molecule may be linked to Fc to generate an Fc fusion. Protein fusion partners may include, but are not limited to, the variable region of any antibody, the target-binding region of a receptor, an adhesion mol-

ecule, a ligand, an enzyme, a cytokine, a chemokine, or some other protein or protein domain. Small molecule fusion partners may include any therapeutic agent that directs the Fc fusion to a therapeutic target. Such targets may be any molecule, preferably an extracellular receptor, that is implicated in disease. Fc fusions can include more than one polypeptide operably linked to the Fc region.

[0066] In addition to Fc fusions, antibody fusions include the fusion of the constant region of the heavy chain with one or more fusion partners (again including the variable region of any antibody), while other antibody fusions are substantially or completely full length antibodies with fusion partners. In one embodiment, a role of the fusion partner is to mediate target binding, and thus it is functionally analogous to the variable regions of an antibody (and in fact can be). Virtually any protein or small molecule may be linked to Fc to generate an Fc fusion (or antibody fusion). Protein fusion partners may include, but are not limited to, the target-binding region of a receptor, an adhesion molecule, a ligand, an enzyme, a cytokine, a chemokine, or some other protein or protein domain. Small molecule fusion partners may include any therapeutic agent that directs the Fc fusion to a therapeutic target. Such targets may be any molecule, preferably an extracellular receptor, that is implicated in disease.

[0067] By "Fc" or "Fc region", as used herein is meant the polypeptide comprising the constant region of an antibody excluding the first constant region immunoglobulin domain and in some cases, part of the hinge. Thus Fc refers to the last two constant region immunoglobulin domains of IgA, IgD, and IgG, and the last three constant region immunoglobulin domains of IgE and IgM, and the flexible hinge N-terminal to these domains. For IgA and IgM, Fc may include the J chain. For IgG, as illustrated in **FIG. 1**, Fc comprises immunoglobulin domains Cgamma2 and Cgamma3 (Cg2 and Cg3) and the lower hinge region between Cgamma1 (Cg1) and Cgamma2 (Cg2). Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually defined to include residues C226 or P230 to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat. Fc may refer to this region in isolation, or this region in the context of an Fc polypeptide, as described below. By "Fc polypeptide" as used herein is meant a polypeptide that comprises all or part of an Fc region. Fc polypeptides include antibodies, Fc fusions, isolated Fcs, and Fc fragments.

[0068] Particularly preferred are full-length antibodies that comprise Fc variants as described in U.S. Ser. No. 60/627,774, Lazar et al., titled "Optimized Fc Variants" and filed Nov. 12, 2004, incorporated herein by reference in its entirety.

[0069] The Fc region interacts with a number of Fc receptors and ligands, imparting an array of important functional capabilities referred to as effector functions. For IgG the Fc region, as shown in **FIG. 1**, comprises Ig domains Cγ2 and Cγ3 and the N-terminal hinge leading into Cγ2. An important family of Fc receptors for the IgG class are the Fc gamma receptors (FcγRs). These receptors mediate communication between antibodies and the cellular arm of the immune system (Raghavan et al., 1996, *Annu Rev Cell Dev Biol* 12:181-220; Ravetch et al., 2001, *Annu Rev Immunol* 19:275-290), both incorporated by reference in its entirety. In humans this protein family includes FcγRI

(CD64), including isoforms FcγRIa, FcγRIb, and FcγRIc; FcγRII (CD32), including isoforms FcγRIIa (including allotypes H131 and R131), FcγRIIb (including FcγRIIb-1 and FcγRIIb-2), and FcγRIIc; and FcγRIII (CD16), including isoforms FcγRIIIa (including allotypes V158 and F158) and FcγRIIIb (including allotypes FcγRIIIb-NA1 and FcγRIIIb-NA2) (Jefferis et al., 2002, *Immunol Lett* 82:57-65), incorporated by reference in its entirety. These receptors typically have an extracellular domain that mediates binding to Fc, a membrane spanning region, and an intracellular domain that may mediate some signaling event within the cell. These receptors are expressed in a variety of immune cells including monocytes, macrophages, neutrophils, dendritic cells, eosinophils, mast cells, platelets, B cells, large granular lymphocytes, Langerhans' cells, natural killer (NK) cells, and γγ T cells. Formation of the Fc/FcγR complex recruits these effector cells to sites of bound antigen, typically resulting in signaling events within the cells and important subsequent immune responses such as release of inflammation mediators, B cell activation, endocytosis, phagocytosis, and cytotoxic attack. The ability to mediate cytotoxic and phagocytic effector functions is a potential mechanism by which antibodies destroy targeted cells. The cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause lysis of the target cell is referred to as antibody dependent cell-mediated cytotoxicity (ADCC) (Raghavan et al., 1996, *Annu Rev Cell Dev Biol* 12:181-220; Ghetie et al., 2000, *Annu Rev Immunol* 18:739-766; Ravetch et al., 2001, *Annu Rev Immunol* 19:275-290), each of which is incorporated by reference in its entirety. The cell-mediated reaction wherein nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause phagocytosis of the target cell is referred to as antibody dependent cell-mediated phagocytosis (ADCP). A number of structures have been solved of the extracellular domains of human FcγRs, including FcγRIIa (pdb accession code 1H9V)(Sondermann et al., 2001, *J Mol Biol* 309:737-749) (pdb accession code 1FCG)(Maxwell et al., 1999, *Nat Struct Biol* 6:437-442), FcγRIIb (pdb accession code 2FCB-)(Sondermann et al., 1999, *Embo J* 18:1095-1103); and FcγRIIIb (pdb accession code 1E4J)(Sondermann et al., 2000, *Nature* 406:267-273.), each of which is incorporated by reference in its entirety. All FcγRs bind the same region on Fc, at the N-terminal end of the Cy2 domain and the preceding hinge, shown in **FIG. 2**. This interaction is well characterized structurally (Sondermann et al., 2001, *J Mol Biol* 309:737-749), and several structures of the human Fc bound to the extracellular domain of human FcγRIIIb have been solved (pdb accession code 1E4K)(Sondermann et al., 2000, *Nature* 406:267-273.) (pdb accession codes 1IIS and 1IIX)(Radaev et al., 2001, *J Biol Chem* 276:16469-16477), as well as has the structure of the human IgE Fc/FcεRIα complex (pdb accession code 1F6A)(Garman et al., 2000, *Nature* 406:259-266), each of which is incorporated by reference in its entirety.

[0070] In certain embodiments, the Fc fusion is an Fc variant. By "Fc variant" as used herein is meant an Fc sequence that differs from that of a parent Fc sequence by virtue of at least one amino acid modification. An Fc variant may only encompass an Fc region, or may exist in the context of an antibody, Fc fusion, or other polypeptide that is substantially encoded by Fc. Fc variant may refer to the Fc polypeptide itself, compositions consisting of the Fc

variant polypeptide, or the amino acid sequence that encodes it. In a preferred embodiment, the variant proteins of the invention comprise an Fc variant, as described herein, and as such, may comprise an antibody (and the corresponding derivatives) with the Fc variant, or an Fc fusion protein that comprises the Fc variant. In addition, in some cases, the Fc is a variant as compared to a wild-type Fc, or to a "parent" variant.

[0071] Fc variants can be modified in various ways. Mutagenesis studies have been carried out on Fc towards various goals, with substitutions typically made to alanine (referred to as alanine scanning) or guided by sequence homology substitutions (Duncan et al., 1988, *Nature* 332:563-564; Lund et al., 1991, *J Immunol* 147:2657-2662; Lund et al., 1992, *Mol Immunol* 29:53-59; Jefferis et al., 1995, *Immunol Lett* 44:111-117; Lund et al., 1995, *Faseb J* 9:115-119; Jefferis et al., 1996, *Immunol Lett* 54:101-104; Lund et al., 1996, *J Immunol* 157:4963-4969; Armour et al., 1999, *Eur J Immunol* 29:2613-2624; Shields et al., 2001, *J Biol Chem* 276:6591-6604; Jefferis et al., 2002, *Immunol Lett* 82:57-65) (U.S. Pat. No. 5,624,821; U.S. Pat. No. 5,885,573; PCT WO 00/42072; PCT WO 99/58572), each of which is incorporated by reference in its entirety. The majority of substitutions reduce or ablate binding with FcγRs. However some success has been achieved at obtaining Fc variants with higher FcγR affinity. (See for example U.S. Pat. No. 5,624,821, and PCT WO 00/42072). For example, Winter and colleagues substituted the human amino acid at position 235 of mouse IgG2b antibody (a glutamic acid to leucine mutation) that increased binding of the mouse antibody to human FcγRI by 100-fold (Duncan et al., 1988, *Nature* 332:563-564; U.S. Pat. No. 5,624,821, each incorporated by reference in its entirety). Shields et al. used alanine scanning mutagenesis to map Fc residues important to FcγR binding, followed by substitution of select residues with non-alanine mutations (Shields et al., 2001, *J Biol Chem* 276:6591-6604; Presta et al., 2002, *Biochem Soc Trans* 30:487-490; PCT WO 00/42072, each of which is incorporated by reference in its entirety). Several mutations disclosed in this study, including S298A, E333A, and K334A, show enhanced binding to the activating receptor FcγRIIIa and reduced binding to the inhibitory receptor FcγRIIb. These mutations were combined to obtain double and triple mutation variants that show additive improvements in binding. The best variant disclosed in this study is a S298A/E333A/K334A triple mutant with approximately a 1.7-fold increase in binding to F158 FcγRIIIa, a 5-fold decrease in binding to FcγRIIb, and a 2.1-fold enhancement in ADCC.

[0072] An Fc polypeptide may be a multimeric Fc polypeptide, comprising two or more Fc regions, one or some or all of which may comprise Fc variants. The advantage of such a molecule is that it provides multiple binding sites for Fc receptors with a single protein molecule. In one embodiment, Fc regions may be linked using a chemical engineering approach. For example, Fab's and Fc's may be linked by thioether bonds originating at cysteine residues in the hinges, generating molecules such as FabFc₂ (Kan et al., 2001, *J. Immunol.*, 2001, 166: 1320-1326; Stevenson et al., 2002, *Recent Results Cancer Res.* 159: 104-12; U.S. Pat. No. 5,681,566, each incorporated by reference in its entirety). Fc regions may be linked using disulfide engineering and/or chemical cross-linking, for example as described in Caron et al., 1992, *J. Exp. Med.* 176:1191-1195, and Shopes, 1992, J.

Immunol. 148(9):2918-22, each incorporated by reference in its entirety. In a preferred embodiment, Fc regions may be linked genetically. For example multiple C $\square$ 2 domains have been fused between the Fab and Fc regions of an antibody (White et al., 2001, *Protein Expression and Purification* 21: 446-455).

[0073] In a preferred embodiment, antibodies are linked genetically to generated tandemly linked Fc polypeptides as described in U.S. Ser. No. 60/531,752, filed Dec. 22, 2003, entitled "Fc Polypeptides with novel Fc receptor binding sites", both of which are incorporated by reference in their entirety. Tandemly linked Fc polypeptides may comprise two or more Fc regions, preferably one to three, most preferably two Fc regions. It may be advantageous to explore a number of engineering constructs in order to obtain homo- or hetero-tandemly linked Fc polypeptides with the most favorable structural and functional properties. Tandemly linked Fc polypeptides may be homo-tandemly linked Fc polypeptides, that is an Fc polypeptide of one isotype is fused genetically to another Fc polypeptide of the same isotype. In an alternate embodiment, Fc polypeptides from different isotypes may be tandemly linked, referred to as hetero-tandemly linked Fc polypeptides. For example, because of the capacity to target Fc γ R and Fc α RI receptors, an Fc polypeptide that binds both Fc γ Rs and Fc α RI may provide a significant clinical improvement. Any number of Fc polypeptides from any of the recognized immunoglobulin constant region genes, including mu (μ), delta (δ), gamma (γ), sigma (σ), and alpha (α), which encode the IgM, IgD, IgG, IgE, and IgA isotypes respectively, may be linked tandemly, in any order, to generate a homo- or hetero-tandemly linked Fc polypeptide. As will be appreciated by one skilled in the art, the properties of any given tandemly linked Fc polypeptide will depend on the construct. For example, it is anticipated that because there are multiple FcRn binding sites on tandemly linked Fc polypeptides that comprise two or more IgG Fc polypeptides, pharmacokinetics may be enhanced. Likewise, because there are multiple binding sites for Fc γ Rs and C1q on tandemly linked Fc polypeptides that comprise two or more IgG Fc polypeptides, Fc γ R and C1q-mediated reactions such as ADCC, ADCP, and CDC may be enhanced. Likewise, it is anticipated that because there are binding sites for Fc γ Rs and Fc γ RI on tandemly linked Fc polypeptides that comprise one or more IgG Fc polypeptides and one or more IgA Fc polypeptides, Fc receptor-mediated reactions such as ADCC may be enhanced. An array of valuable properties may be realized by combining Fc polypeptides in various combinations using the concepts of engineering homo- and hetero-tandemly linked Fc polypeptides.

[0074] The amino acids of the Fc region can be modified. In one embodiment, amino acid modifications to the antibody enhance effector function. Thus the Fc polypeptide may be combined with other amino acid modifications in the Fc polypeptide that provide altered or optimized interaction with one or more Fc ligands, including but not limited to Fc γ Rs, C1q, FcRn, FcR homologues (FcRHs) (reference), and/or as yet undiscovered Fc ligands. Additional modifications may provide altered or optimized affinity and/or specificity to the Fc ligands. Additional modifications may provide altered or optimized effector functions, including but not limited to ADCC, ADCP, CDC, and/or serum half-life. Such combination may provide additive, synergistic, or novel properties in antibodies or Fc fusions. In one embodi-

ment, the conjugates of the present invention may be combined with Fc variants (Duncan et al., 1988, *Nature* 332:563-564; Lund et al., 1991, *J Immunol* 147:2657-2662; Lund et al., 1992, *Mol Immunol* 29:53-59; Alegre et al., 1994, *Transplantation* 57:1537-1543; Hutchins et al., 1995, *Proc Natl Acad Sci U S A* 92:11980-11984; Jefferis et al., 1995, *Immunol Lett* 44:111-117; Lund et al., 1995, *Faseb J* 9:115-119; Jefferis et al., 1996, *Immunol Lett* 54:101-104; Lund et al., 1996, *J Immunol* 157:4963-4969; Armour et al., 1999, *Eur J Immunol* 29:2613-2624; Idusogie et al., 2000, *J Immunol* 164:4178-4184; Reddy et al., 2000, *J Immunol* 164:1925-1933; Xu et al., 2000, *Cell Immunol* 200:16-26; Idusogie et al., 2001, *J Immunol* 166:2571-2575; Shields et al., 2001, *J Biol Chem* 276:6591-6604; Jefferis et al., 2002, *Immunol Lett* 82:57-65; Presta et al., 2002, *Biochem Soc Trans* 30:487-490; Hinton et al., 2004, *J Biol Chem* 279:6213-6216) (U.S. Pat. No. 5,624,821; U.S. 5,885,573; U.S. Pat. No. 6,194,551; PCT WO 00/42072; PCT WO 99/58572; US 2004/0002587 A1), U.S. Pat. No. 6,737,056, PCT US2004/000643, U.S. Ser. No. 10/370,749, and PCT/US2004/005112), each of which is incorporated by reference in its entirety. For example, as disclosed in U.S. Pat. No. 6,737,056, PCT US2004/000643, U.S. Ser. No. 10/370,749, and PCT/US2004/005112, the substitutions S298A, S298D, K326E, K326D, E333A, K334A, and P396L provide optimized Fc γ R binding and/or enhanced ADCC, and thus may be considered additional modifications to be combined with Fc variants. Furthermore, as disclosed in Idusogie et al., 2001, "Engineered Antibodies with Increased Activity to Recruit Complement" *J. Immunology* 166:2571-2572, substitutions K326W, K326Y, and E333S provide enhanced binding to the complement protein C1q and enhanced CDC.

[0075] 4. Diabodies

[0076] In certain embodiments, the antibodies of the invention multispecific antibody, and notably a bispecific antibody, also sometimes referred to as "diabodies". These are antibodies that bind to two (or more) different antigens. Diabodies can be manufactured in a variety of ways known in the art (Holliger and Winter, 1993, *Current Opinion Biotechnol.* 4:446-449), e.g., prepared chemically or from hybrid hybridomas.

[0077] 5. Minibodies

[0078] In certain embodiments, the antibody is a minibody. Minibodies are minimized antibody-like proteins comprising a scFv joined to a CH3 domain. Hu et al., 1996, *Cancer Res.* 56:3055-3061. In some cases, the scFv can be joined to the Fc region, and may include some or all of the hinge region.

[0079] 6. Covalently Modified Antibodies and Fragments

[0080] The full length antibodies or antibody fragments may be modified. For example, the molecules may be stabilized by the incorporation of disulphide bridges linking the VH and VL domains (Reiter et al., 1996, *Nature Biotech.* 14:1239-1245).

[0081] Covalent modifications of antibodies are included within the scope of this invention, and are generally, but not always, done post-translationally. For example, several types of covalent modifications of the antibody are introduced into the molecule by reacting specific amino acid

residues of the antibody with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues.

[0082] Cysteiny residues most commonly are reacted with α -haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteiny residues also are derivatized by reaction with bromotrifluoroacetone, α -bromo- β -(5-imidazolyl)propionic acid, chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercur-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole.

[0083] Histidyl residues are derivatized by reaction with diethylpyrocarbonate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1M sodium cacodylate at pH 6.0.

[0084] Lysiny residues and amino terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysiny residues. Other suitable reagents for derivatizing α -amino-containing residues include imidoesters such as methyl picolinimidate; pyridoxal phosphate; pyridoxal; chloroborohydride; trinitrobenzenesulfonic acid; O-methylisourea; 2,4-pentanedione; and transaminase-catalyzed reaction with glyoxylate.

[0085] Arginy residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginine residues requires that the reaction be performed in alkaline conditions because of the high pKa of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as the arginine epsilon-amino group.

[0086] The specific modification of tyrosyl residues may be made, with particular interest in introducing spectral labels into tyrosyl residues by reaction with aromatic diazonium compounds or tetranitromethane. Most commonly, N-acetylimidazole and tetranitromethane are used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively. Tyrosyl residues are iodinated using 125I or 131I to prepare labeled proteins for use in radioimmunoassay, the chloramine T method described above being suitable.

[0087] Carboxyl side groups (aspartyl or glutamyl) are selectively modified by reaction with carbodiimides ($R'-N=C=N-R'$), where R and R' are optionally different alkyl groups, such as 1-cyclohexyl-3-(2-morpholinyl-4-ethyl) carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residues are converted to asparaginy and glutaminy residues by reaction with ammonium ions.

[0088] Derivatization with bifunctional agents is useful for crosslinking antibodies to a water-insoluble support matrix or surface for use in a variety of methods, in addition to methods described below. Commonly used crosslinking agents include, e.g., 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithio-bis (succinimidylpropionate), and bifunctional maleimides

such as bis-N-maleimido-1,8-octane. Derivatizing agents such as methyl-3-[(p-azidophenyl)dithio]propionimidate yield photoactivatable intermediates that are capable of forming crosslinks in the presence of light. Alternatively, reactive water-insoluble matrices such as cyanogen bromide-activated carbohydrates and the reactive substrates described in U.S. Pat. Nos. 3,969,287; 3,691,016; 4,195,128; 4,247,642; 4,229,537; and 4,330,440 are employed for protein immobilization.

[0089] Glutaminy and asparaginy residues are frequently deamidated to the corresponding glutamyl and aspartyl residues, respectively. Alternatively, these residues are deamidated under mildly acidic conditions. Either form of these residues falls within the scope of this invention.

[0090] Other modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threony residues, methylation of the α -amino groups of lysine, arginine, and histidine side chains (T. E. Creighton, *Proteins: Structure and Molecular Properties*, W. H. Freeman & Co., San Francisco, pp. 79-86 [1983]), acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

[0091] Antibodies can be aglycosylated. By "aglycosylated antibody" as used herein is meant an antibody that lacks carbohydrate attached at position 297 of the Fc region, wherein numbering is according to the EU system as in Kabat. The aglycosylated antibody may be a deglycosylated antibody, that is an antibody for which the Fc carbohydrate has been removed, for example chemically or enzymatically. Alternatively, the aglycosylated antibody may be a nonglycosylated or unglycosylated antibody, that is an antibody that was expressed without Fc carbohydrate, for example by mutation of one or residues that encode the glycosylation pattern or by expression in an organism that does not attach carbohydrates to proteins, for example bacteria.

[0092] Removal of carbohydrate moieties present on the starting antibody may be accomplished chemically or enzymatically. Chemical deglycosylation requires exposure of the protein to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the polypeptide intact. Chemical deglycosylation is described by Hakimuddin et al., 1987, *Arch. Biochem. Biophys.* 259:52 and by Edge et al., 1981, *Anal. Biochem.* 118:131. Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura et al., 1987, *Meth. Enzymol.* 138:350. Glycosylation at potential glycosylation sites may be prevented by the use of the compound tunicamycin as described by Duskin et al., 1982, *J. Biol. Chem.* 257:3105. Tunicamycin blocks the formation of protein-N-glycoside linkages.

[0093] Alternatively, the antibody portion of the chemoattractant-antibody conjugates can be modified to include one or more engineered glycoforms. By "engineered glycoform" as used herein is meant a carbohydrate composition that is covalently attached to an IgG, wherein said carbohydrate composition differs chemically from that of a parent IgG. Engineered glycoforms may be useful for a variety of purposes, including but not limited to enhancing or reducing effector function. Engineered glycoforms may be generated

by a variety of methods known in the art (Umaña et al., 1999, *Nat Biotechnol* 17:176-180; Davies et al., 2001, *Biotechnol Bioeng* 74:288-294; Shields et al., 2002, *J Biol Chem* 277:26733-26740; Shinkawa et al., 2003, *J Biol Chem* 278:3466-3473); (U.S. Pat. No. 6,602,684; U.S. Ser. No. 10/277,370; U.S. Ser. No. 10/113,929; PCT WO 00/61739A1; PCT WO 01/29246A1; PCT WO 02/31140A1; PCT WO 02/30954A1); (Potelligent™ technology [Biowa, Inc., Princeton, NJ]; GlycoMab™ glycosylation engineering technology [GLYCART biotechnology AG, Zürich, Switzerland]). Many of these techniques are based on controlling the level of fucosylated and/or bisecting oligosaccharides that are covalently attached to the Fc region, for example by expressing an IgG in various organisms or cell lines, engineered or otherwise (for example Lec-13 CHO cells or rat hybridoma YB2/O cells), by regulating enzymes involved in the glycosylation pathway (for example FUT8 [α 1,6-fucosyltransferase] and/or β 1-4-N-acetylglucosaminyltransferase III [GnTIII]), or by modifying carbohydrate(s) after the IgG has been expressed. Engineered glycoform typically refers to the different carbohydrate or oligosaccharide; thus an IgG variant, for example an antibody or Fc fusion, can include an engineered glycoform. Alternatively, engineered glycoform may refer to the IgG variant that comprises the different carbohydrate or oligosaccharide. As is known in the art, glycosylation patterns can depend on both the sequence of the protein (e.g., the presence or absence of particular glycosylation amino acid residues, discussed below), or the host cell or organism in which the protein is produced. Particular expression systems are discussed below.

[0094] Glycosylation of polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tri-peptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tri-peptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose, to a hydroxylamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

[0095] Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tri-peptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the starting sequence (for O-linked glycosylation sites). For ease, the antibody amino acid sequence is preferably altered through changes at the DNA level, particularly by mutating the DNA encoding the target polypeptide at pre-selected bases such that codons are generated that will translate into the desired amino acids.

[0096] Another means of increasing the number of carbohydrate moieties on the antibody is by chemical or enzymatic coupling of glycosides to the protein. These procedures are advantageous in that they do not require production of the protein in a host cell that has glycosylation capabilities for N- and O-linked glycosylation. Depending

on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan, or (f) the amide group of glutamine. These methods are described in WO 87/05330 published Sep. 11, 1987, and in Aplin and Wriston, 1981, *CRC Crit. Rev. Biochem.*, pp. 259-306.

[0097] 7. Conjugated Antibodies

[0098] The antibody portion of the chemoattractant antibody conjugates can be modified with a conjugate partner. Conjugate partners can be proteinaceous or non-proteinaceous; the latter generally being generated using functional groups on the antibody and on the conjugate partner. For example linkers are known in the art; for example, homo- or hetero-bifunctional linkers as are well known (see, 1994 Pierce Chemical Company catalog, technical section on cross-linkers, pages 155-200, incorporated herein by reference).

[0099] Suitable conjugates include, but are not limited to, labels as described below, drugs and cytotoxic agents including, but not limited to, cytotoxic drugs (e.g., chemotherapeutic agents) or toxins or active fragments of such toxins. Suitable toxins and their corresponding fragments include diphtheria A chain, exotoxin A chain, ricin A chain, abrin A chain, curcin, croton, phenomycin, enomycin and the like. Cytotoxic agents also include radiochemicals made by conjugating radioisotopes to antibodies, or binding of a radionuclide to a chelating agent that has been covalently attached to the antibody. Additional embodiments utilize calicheamicin, auristatins, geldanamycin, maytansine, and duocarmycins and analogs; for the latter, see U.S. 2003/0050331, hereby incorporated by reference in its entirety.

[0100] Additional nonproteinaceous polymers include, but are not limited to, various polyols such as polyethylene glycol, polypropylene glycol or polyoxyalkylenes, in the manner set forth in U.S. Pat. Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337. In addition, as is known in the art, amino acid substitutions may be made in various positions within the antibody to facilitate the addition of polymers such as PEG. See for example, U.S. Publication No. 2005/0114037, incorporated herein by reference in its entirety. Other modifications of the conjugates of the present invention are contemplated herein. For example, the antibody or Fc fusion may be linked to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol (PEG), polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol. Other modifications of the conjugates of the present invention are contemplated herein. In a preferred embodiment, additional modifications are made to remove covalent degradation sites such as deamidation (i.e. deamidation of glutaminyl and asparaginyl residues to the corresponding glutamyl and aspartyl residues), oxidation, and proteolytic degradation sites. Deamidation sites that are particular useful to remove are those that have enhance propensity for deamidation, including, but not limited to asparaginyl and glutamyl residues followed by glycines (NG and QG motifs, respectively). In such cases, substitution of either residue can significantly reduce the tendency for deamidation. Common oxidation sites include methionine and cysteine residues.

[0101] 8. Labeled Antibodies

[0102] In some embodiments, the covalent modification of the antibodies of the invention comprises the addition of one or more labels. In some cases, these are considered antibody fusions.

[0103] The term “labelling group” means any detectable label. In some embodiments, the labelling group is coupled to the antibody via spacer arms of various lengths to reduce potential steric hindrance. Various methods for labelling proteins are known in the art and may be used in performing the present invention.

[0104] In general, labels fall into a variety of classes, depending on the assay in which they are to be detected: a) isotopic labels, which may be radioactive or heavy isotopes; b) magnetic labels (e.g., magnetic particles); c) redox active moieties; d) optical dyes; enzymatic groups (e.g. horseradish peroxidase, β -galactosidase, luciferase, alkaline phosphatase); e) biotinylated groups; and f) predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags, etc.). In some embodiments, the labelling group is coupled to the antibody via spacer arms of various lengths to reduce potential steric hindrance. Various methods for labelling proteins are known in the art and may be used in performing the present invention.

[0105] Specific labels include optical dyes, including, but not limited to, chromophores, phosphors and fluorophores, with the latter being specific in many instances. Fluorophores can be either “small molecule” fluors, or proteinaceous fluors.

[0106] By “fluorescent label” is meant any molecule that may be detected via its inherent fluorescent properties. Suitable fluorescent labels include, but are not limited to, fluorescein, rhodamine, tetramethylrhodamine, eosin, erythrosin, coumarin, methyl-coumarins, pyrene, Malachite green, stilbene, Lucifer Yellow, Cascade BlueJ, Texas Red, IAEDANS, EDANS, BODIPY FL, LC Red 640, Cy 5, Cy 5.5, LC Red 705, Oregon green, the Alexa-Fluor dyes (Alexa Fluor 350, Alexa Fluor 430, Alexa Fluor 488, Alexa Fluor 546, Alexa Fluor 568, Alexa Fluor 594, Alexa Fluor 633, Alexa Fluor 660, Alexa Fluor 680), Cascade Blue, Cascade Yellow and R-phycoerythrin (PE) (Molecular Probes, Eugene, Oreg.), FITC, Rhodamine, and Texas Red (Pierce, Rockford, Ill.), Cy5, Cy5.5, Cy7 (Amersham Life Science, Pittsburgh, Pa.). Suitable optical dyes, including fluorophores, are described in Molecular Probes Handbook by Richard P. Haugland, hereby incorporated by reference in its entirety.

[0107] Suitable proteinaceous fluorescent labels also include, but are not limited to, green fluorescent protein, including a Renilla, Ptilosarcus, or Aequorea species of GFP (Chalfie et al., 1994, Science 263:802-805), EGFP (Clontech Laboratories, Inc., Genbank Accession Number U55762), blue fluorescent protein (BFP, Quantum Biotechnologies, Inc. 1801 de Maisonneuve Blvd. West, 8th Floor, Montreal, Quebec, Canada H3H 1J9; Stauber, 1998, Biotechniques 24:462-471; Heim et al., 1996, Curr. Biol. 6:178-182), enhanced yellow fluorescent protein (EYFP, Clontech Laboratories, Inc.), luciferase (Ichiki et al., 1993, J. Immunol. 150:5408-5417), β galactosidase (Nolan et al., 1988, Proc.

Natl. Acad. Sci. U.S.A. 85:2603-2607) and Renilla (WO92/15673, WO95/07463, WO98/14605, WO98/26277, WO99/49019, U.S. Pat. Nos. 5,292,658, 5,418,155, 5,683,888, 5,741,668, 5,777,079, 5,804,387, 5,874,304, 5,876,995, 5,925,558). All of the above-cited references are expressly incorporated herein by reference in their entirety.

[0108] Small molecule fusion and conjugate partners may include any therapeutic agent that directs the conjugates to a therapeutic target. Such targets may be any molecule, preferably an extracellular receptor, that is implicated in disease. Two families of surface receptors that are targets of a number of approved small molecule drugs are G-Protein Coupled Receptors (GPCRs), and ion channels, including K⁺, Na⁺, Ca⁺ channels. Nearly 70% of all drugs currently marketed worldwide target GPCRs. Thus the conjugates of the present invention may be fused to a small molecule that targets, for example, one or more GABA receptors, purinergic receptors, adrenergic receptors, histaminergic receptors, opioid receptors, chemokine receptors, glutamate receptors, nicotinic receptors, the 5HT (serotonin) receptor, and estrogen receptors. A fusion or conjugate partner may be a small-molecule mimetic of a protein that targets a therapeutically useful target. Specific examples of particular drugs that may serve as antibody fusion and conjugate partners can be found in L. S. Goodman et al., Eds., *Goodman and Gilman's The Pharmacological Basis of Therapeutics* (McGraw-Hill, New York, ed. 9, 1996, incorporated by reference in its entirety). Fusion and conjugate partners include not only small molecules and proteins that bind known targets for existing drugs, but orphan receptors that do not yet exist as drug targets. The completion of the genome and proteome projects are proving to be a driving force in drug discovery, and these projects have yielded a trove of orphan receptors. There is enormous potential to validate these new molecules as drug targets, and develop protein and small molecule therapeutics that target them. Such protein and small molecule therapeutics are contemplated as antibody fusion and conjugate partners that employ the conjugates of the present invention.

[0109] In yet another embodiment, the conjugates of the present invention may be conjugated to a “receptor” (such streptavidin) for utilization in tumor pretargeting. The antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a “ligand” (e.g. avidin) which is conjugated to a cytotoxic agent (e.g. a radionucleotide). In an alternate embodiment, the Fc polypeptide is conjugated or operably linked to an enzyme in order to employ Antibody Dependent Enzyme Mediated Prodrug Therapy (ADEPT). ADEPT may be used by conjugating or operably linking the Fc polypeptide to a prodrug-activating enzyme that converts a prodrug (e.g. a peptidyl chemotherapeutic agent, see PCT WO 81/01145, incorporated by reference in its entirety) to an active anti-cancer drug. See, for example, PCT WO 88/07378 and U.S. Pat. No. 4,975,278, each incorporated by reference in its entirety. The enzyme component of the immunoconjugate useful for ADEPT includes any enzyme capable of acting on a prodrug in such a way so as to convert it into its more active, cytotoxic form. Enzymes that are useful in the method of this invention include but are not limited to alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful

for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratin protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), that are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as β -galactosidase and neuraminidase useful for converting glycosylated prodrugs into free drugs; β -lactamase useful for converting drugs derivatized with α -lactams into free drugs; and penicillin amidases, such as penicillin V amidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art as "abzymes", can be used to convert the prodrugs of the invention into free active drugs (see, for example, Massey, 1987, *Nature* 328: 457-458). Fc polypeptide-abzyme conjugates can be prepared for delivery of the abzyme to a tumor cell population. A variety of additional conjugates are contemplated for the Fc polypeptides of the present invention. A variety of chemotherapeutic agents, anti-angiogenic agents, tyrosine kinase inhibitors, and other therapeutic agents are described herein.

[0110] C. Linkers

[0111] The chemoattractant and antibody portions can be attached covalently, or can be attached via a linker. The chemoattractant may be linked to any region of an antibody, including at the N- or C-termini, or at some residue in-between the termini of the antibody. In a preferred embodiment, a fusion or conjugate partner is linked at the N- or C-terminus of the antibody, most preferably the N-terminus. A variety of linkers may find use in the present invention to covalently link the chemoattractant to the antibody. In addition, fusion and conjugate partners may also be attached by a linker.

[0112] By "linker", "linker sequence", "spacer", "tethering sequence" or grammatical equivalents thereof, herein is meant a molecule or group of molecules (such as a monomer or polymer) that connects two molecules and often serves to place the two molecules in a preferred configuration. A number of strategies may be used to covalently link molecules together. These include, but are not limited to polypeptide linkages between N- and C-termini of proteins or protein domains, linkage via disulfide bonds, and linkage via chemical cross-linking reagents. In one aspect of this embodiment, the linker is a peptide bond, generated by recombinant techniques or peptide synthesis. Choosing a suitable linker for a specific case where two polypeptide chains are to be connected depends on various parameters, including but not limited to the nature of the two polypeptide chains (e.g., whether they naturally oligomerize), the distance between the N- and the C-termini to be connected if known, and/or the stability of the linker towards proteolysis and oxidation. Furthermore, the linker may contain amino acid residues that provide flexibility. Thus, the linker peptide may predominantly include the following amino acid residues: Gly, Ser, Ala, or Thr. The linker peptide should have a length that is adequate to link two molecules in such a way that they assume the correct conformation relative to one another so that they retain the desired activity. Suitable lengths for this purpose include at least one and not more

than 50 amino acid residues. Preferably, the linker is from about 1 to 30 amino acids in length, with linkers of 1 to 20 amino acids in length being most preferred. In addition, the amino acid residues selected for inclusion in the linker peptide should exhibit properties that do not interfere significantly with the activity of the polypeptide. Thus, the linker peptide on the whole should not exhibit a charge that would be inconsistent with the activity of the polypeptide, or interfere with internal folding, or form bonds or other interactions with amino acid residues in one or more of the monomers that would seriously impede the binding of receptor monomer domains. Useful linkers include glycine-serine, or GS linkers. By "Gly-Ser" or "GS" linkers is meant a polymer of glycines and serines in series (including, for example, $(\text{Gly-Ser})_n$, $(\text{GSGGS})_n$, $(\text{GGGGS})_n$ and $(\text{GGGS})_n$ (SEQ ID NOs: 19-21), where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers such as the tether for the shaker potassium channel, and a large variety of other flexible linkers, as will be appreciated by those in the art. Glycine-serine polymers are preferred since both of these amino acids are relatively unstructured, and therefore may be able to serve as a neutral tether between components. Secondly, serine is hydrophilic and therefore able to solubilize what could be a globular glycine chain. Third, similar chains have been shown to be effective in joining subunits of recombinant proteins such as single chain antibodies.

[0113] Suitable linkers may also be identified by screening databases of known three-dimensional structures for naturally occurring motifs that can bridge the gap between two polypeptide chains. In a preferred embodiment, the linker is not immunogenic when administered in a human patient. Thus linkers may be chosen such that they have low immunogenicity or are thought to have low immunogenicity. For example, a linker may be chosen that exists naturally in a human. In a preferred embodiment, the linker has the sequence of the hinge region of an antibody, that is the sequence that links the antibody Fab and Fc regions; alternatively the linker has a sequence that comprises part of the hinge region, or a sequence that is substantially similar to the hinge region of an antibody. Another way of obtaining a suitable linker is by optimizing a simple linker, e.g., $(\text{Gly4Ser})_n$ (SEQ ID NO: 20), through random mutagenesis. Alternatively, once a suitable polypeptide linker is defined, additional linker polypeptides can be created to select amino acids that more optimally interact with the domains being linked. Other types of linkers that may be used in the present invention include artificial polypeptide linkers and inteins. In another embodiment, disulfide bonds are designed to link the two molecules. In another embodiment, linkers are chemical cross-linking agents. For example, a variety of bifunctional protein coupling agents may be used, including but not limited to N-succinimidyl-3-(2-pyridyldithiol)propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate, iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), 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 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., 1971, *Science*

238:1098. Chemical linkers may enable chelation of an isotope. For example, Carbon-14-labeled 1-isothiocyanato-benzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody (see PCT WO 94/11026, incorporated by reference in its entirety). The linker may be cleavable, facilitating release of the cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, dimethyl linker or disulfide-containing linker (Chari et al., 1992, *Cancer Research* 52: 127-131, incorporated by reference in its entirety) may be used. Alternatively, a variety of nonproteinaceous polymers, including but not limited to polyethylene glycol (PEG), polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol, may find use as linkers, that is may find use to link the conjugates of the present invention to a fusion or conjugate partner to generate an Fc fusion, or to link the antibodies and Fc fusions to a conjugate. It is noted that the aforementioned description of linkers for Fc fusions may also find use to generate conjugates, as described more fully below.

[0114] Additional modifications to the chemoattractant-antibody conjugates include the use of unnatural amino acids incorporated using, for example, the technologies developed by Schultz and colleagues, including but not limited to methods described by Cropp & Shultz, 2004, *Trends Genet.* 20(12):625-30, Anderson et al., 2004, *Proc. Natl. Acad. Sci. U.S.A.* 101(2):7566-71, Zhang et al., 2003, 303(5656):371-3, and Chin et al., 2003, *Science* 301(5635):964-7, each of which is incorporated by reference in its entirety. In some embodiments, these modifications enable manipulation of various functional, biophysical, immunological, or manufacturing properties discussed above. In additional embodiments, these modifications enable additional chemical modification for other purposes. Other modifications of the antibodies are contemplated herein. For example, the antibody may be linked to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol (PEG), polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol. Additional amino acid modifications may be made to enable specific or non-specific chemical or posttranslational modification of the Fc polypeptides. Such modifications, include, but are not limited to PEGylation and glycosylation. Specific substitutions that can be utilized to enable PEGylation include, but are not limited to, introduction of novel cysteine residues or unnatural amino acids such that efficient and specific coupling chemistries can be used to attach a PEG or otherwise polymeric moiety. Introduction of specific glycosylation sites can be achieved by introducing novel N-X-T/S sequences into the Fc polypeptides of the present invention.

[0115] In one embodiment, the chemoattractant-antibody conjugates are administered with one or more additional molecules comprising an additional antibody. The additional antibodies can have efficacy in treating the same disease or an additional comorbidity; for example two antibodies may be administered that recognize two antigens that are over-expressed in a given type of cancer. Examples of antibodies that may be co-administered include, but are not limited to, anti 17-1A cell surface antigen antibodies such as Panorex™, anti- $\alpha 4\beta 7$ integrin antibodies such as LDP-02, anti- $\alpha V\beta 3$ integrin antibodies such as Vitaxin™, anti-complement factor 5 (C5) antibodies such as 5G1.1, anti-CA125 antibodies

such as OvaRex, anti-CD3 antibodies such as Nuvion, anti-CD4 antibodies such as IDEC-151, MDX-CD4, OKT4A, anti-CD20 antibodies such as Bexocar, Rituxan®, Zevalin®, anti-CD22 antibodies such as Lymphocide™, anti-CD23 antibodies such as IDEC-152, anti-CD25 antibodies such as Zenapax® (daclizumab), anti-CD33 antibodies such as Smart M195, anti-CD40L antibodies such as Antova™, IDEC-131, anti-CD44 antibodies such as Blvatumumab, anti-CD52 antibodies such as Campath® (alemtuzumab), anti-CD80 antibodies such as IDEC-114, anti-CEA antibodies, anti-CTLA-4 antibodies such as MDX-101, anti-EGFR antibodies such as ABX-EGF, Cetuximab, IMC-C225, Merck Mab 425, anti-EpCAM antibodies such as Crucell's anti-EpCAM, ING-1, IS-IL-2, anti-Her2 antibodies such as Herceptin®, MDX-210, anti-ICAM antibodies such as ICM3, anti-GD2 ganglioside antibodies such as TriGem, anti-gpIIIb/IIIa antibodies such as ReoPro, anti-HLA antibodies such as Oncolym®, Smart 1D10, anti-Muc1 antibodies such as BravaRex, TriAb, anti-PEM antigen antibodies such as Theragyn and Therex, anti-SK-1 antigen antibodies such as Monopharm C, anti-TNF- α antibodies such as CDP571, CDP870, D2E7, anti-TGF- β antibodies such as CAT-152, anti-VLA-4 antibodies such as Antegren™. Furthermore, anti-idiotypic antibodies including but not limited to the GD3 epitope antibody BEC2, the gp72 epitope antibody 105AD7, may be used.

[0116] In a preferred embodiment, additional modifications are made to improve biophysical properties (e.g. stability, solubility, oligomeric state) of the chemoattractant-antibody conjugates. Modifications can include, for example, substitution of exposed nonpolar amino acids with polar amino acids for higher solubility. The conjugates can also be combined with variants that reduce the oligomeric state or size of the antibody or Fc fusion, such that tumor penetration is enhanced, or in vivo clearance rates are increased as desired.

[0117] Additional modifications to the chemoattractant-antibody conjugates include modifications to reduce immunogenicity in humans. Such modifications include, but are not limited to, modifications that reduce binding of processed peptides derived from the parent sequence to MHC proteins, and modifications that reduce the propensity of the intact molecule to interact with B cell receptors and circulating antibodies.

[0118] Additional modifications include those that improve expression and/or purification yields from hosts or host cells commonly used for production of biologics. These include, but are not limited to various mammalian cell lines (e.g. CHO), yeast cell lines, bacterial cell lines, and plants. Additional modifications include modifications that remove or reduce the ability of heavy chains to form inter-chain disulfide linkages. Additional modifications include modifications that remove or reduce the ability of heavy chains to form intra-chain disulfide linkages.

[0119] Additional modifications include the use of unnatural amino acids incorporated using, for example, the technologies developed by Schultz and colleagues. In some embodiments, these modifications enable manipulation of various functional, biophysical, immunological, or manufacturing properties discussed above. In additional embodiments, these modifications enable additional chemical modification for other purposes.

[0120] Additional modifications include amino acid substitutions or other modifications that modulate the in vivo pharmacokinetic properties of a conjugates. These include, but are not limited to, modifications that enhance affinity for the neonatal Fc receptor FcRn (U.S. Ser. No. 10/020354; WO2001US0048432; EP2001000997063; U.S. Pat. No. 6,277,375; U.S. Ser. No. 09/933497; WO1997US0003321; U.S. Pat. No. 6,737,056; WO2000US0000973; Shields et al. *J. Biol. Chem.*, 276(9), 6591-6604 (2001); Zhou et al. *J. Mol. Biol.*, 332, 901-913 (2003), each of which is incorporated by reference in its entirety). These further include modifications that modify FcRn affinity in a pH-specific manner. In some embodiments, where enhanced in vivo half-life is desired, modifications that specifically enhance FcRn affinity at lower pH (5.5-6) relative to higher pH (7-8) are preferred (Hinton et al. *J. Biol. Chem.* 279(8), 6213-6216 (2004); Dall'Acqua et al. *J. Immunol.* 169, 5171-5180 (2002); Ghetie et al. *Nat. Biotechnol.*, 15(7), 637-640 (1997); WO2003US0033037; WO2004US0011213, each of which is incorporated by reference in its entirety). Additionally preferred modifications are those that maintain the wild-type Fc's improved binding at lower pH relative to the higher pH. In alternative embodiments, where rapid in vivo clearance is desired, modifications that reduce affinity for FcRn are preferred. (U.S. Pat. No. 6,165,745; WO1993US0003895; EP1993000910800; WO1997US0021437; Medesan et al., *J. Immunol.*, 158(5), 2211-2217 (1997); Ghetie and Ward, *Annu. Rev. Immunol.*, 18, 739-766 (2000); Martin et al. *Molecular Cell*, 7, 867-877 (2001); Kim et al. *Eur. J. Immunol.* 29, 2819-2825 (1999), each of which is incorporated by reference in its entirety).

[0121] Additional modifications that can be combined with conjugates of the present invention include modifications to enable specific or non-specific chemical or post-translational modification of the conjugates. Such modifications, include, but are not limited to, PEGylation and glycosylation. Specific substitutions that can be utilized to enable PEGylation include, but are not limited to, introduction of cysteine residues or unnatural amino acids such that efficient and specific coupling chemistries can be used to attach a PEG or otherwise polymeric moiety. Introduction of specific glycosylation sites can be achieved by introducing novel N-X-T/S sequences into the variants.

[0122] The chemoattractant-antibody conjugates can also be used to treat tumors. There are a number of possible mechanisms by which the antibodies destroy tumor cells, including anti-proliferation via blockage of needed growth pathways, intracellular signaling leading to apoptosis, enhanced down regulation and/or turnover of receptors, CDC, ADCC, ADCCP, and promotion of an adaptive immune response (Cragg et al., 1999, *Curr Opin Immunol* 11:541-547; Glennie et al., 2000, *Immunol Today* 21:403-410), each incorporated by reference in its entirety. Anti-tumor efficacy may be due to a combination of these mechanisms, and their relative importance in clinical therapy appears to be cancer dependent. Despite this arsenal of anti-tumor weapons, the potency of antibodies as anti-cancer agents is unsatisfactory, particularly given their high cost. Patient tumor response data show that monoclonal antibodies provide only a small improvement in therapeutic success over normal single-agent cytotoxic chemotherapeutics. For example, just half of all relapsed low-grade non-Hodgkin's lymphoma patients respond to the anti-CD20 antibody rituximab (McLaughlin et al., 1998, *J Clin Oncol* 16:2825-2833), incorporated by

reference in its entirety. Of 166 clinical patients, 6% showed a complete response and 42% showed a partial response, with median response duration of approximately 12 months. Trastuzumab (Herceptin®, a registered trademark of Genentech), an anti-HER2/neu antibody for treatment of metastatic breast cancer, has less efficacy. The overall response rate using trastuzumab for the 222 patients tested was only 15%, with 8 complete and 26 partial responses and a median response duration and survival of 9 to 13 months (Cobleigh et al., 1999, *J Clin Oncol* 17:2639-2648), incorporated by reference in its entirety. Currently for anticancer therapy, any small improvement in mortality rate defines success. Thus there is a significant need to enhance the capacity of antibodies to destroy targeted cancer cells.

[0123] A promising means for enhancing the anti-tumor potency of antibodies is via enhancement of their ability to mediate cytotoxic effector functions such as ADCC, ADCCP, and CDC. The importance of FcγR-mediated effector functions for the anti-cancer activity of antibodies has been demonstrated in mice (Clynes et al., 1998, *Proc Natl Acad Sci U S A* 95:652-656; Clynes et al., 2000, *Nat Med* 6:443-446), both incorporated by reference in its entirety, and the affinity of interaction between Fc and certain FcγRs correlates with targeted cytotoxicity in cell-based assays (Shields et al., 2001, *J Biol Chem* 276:6591-6604; Presta et al., 2002, *Biochem Soc Trans* 30:487-490; Shields et al., 2002, *J Biol Chem* 277:26733-26740), each of which is incorporated by reference in its entirety. Additionally, a correlation has been observed between clinical efficacy in humans and their allotype of high (V158) or low (F158) affinity polymorphic forms of FcγRIIIa (Carton et al., 2002, *Blood* 99:754-758). Together these data suggest that an antibody with an Fc region optimized for binding to certain FcγRs may better mediate effector functions and thereby destroy cancer cells more effectively in patients. The balance between activating and inhibiting receptors is an important consideration, and optimal effector function may result from an Fc with enhanced affinity for activation receptors, for example FcγRI, FcγRIIa/c, and FcγRIIIa, yet reduced affinity for the inhibitory receptor FcγRIIb. Furthermore, because FcγRs can mediate antigen uptake and processing by antigen presenting cells, enhanced Fc/FcγR affinity may also improve the capacity of antibody therapeutics to elicit an adaptive immune response.

[0124] Choosing the right target antigen for therapy is a complex process and encompasses many variables. For anti-cancer treatment it is desirable to have a target whose expression is restricted to the cancerous cells. If not completely restricted, then greatly up regulated in expression. Once a target with the desired expression pattern has been identified, an antibody must be chosen or generated that is specific for that antigen. Once these have been accomplished, the non-variable domain regions of the antibody can be tailored to suit the characteristics of the antigen. Many different modifications of antibodies have now been reported including truncation to produce Fab and Fab'2 fragments, scFv constructs, diabodies, bispecific antibodies, toxin or enzyme conjugated antibodies, altered glycoform antibodies, antibodies with amino acid changes in the Fc region. The exact nature of the antibody modification (if any) that will optimize efficacy for the selected target will depend on the characteristics of the target.

[0125] Some targets that have proven especially amenable to antibody therapy are those with signaling functions. For example antibody cross-linking of the Her-2/neu antigen generates an apoptotic signal that results in cancer cell death. In some cases such as the CD30 antigen this clustering with free antibody is insufficient to cause apoptosis in vitro. For in vitro assays sufficient clustering can be mediated by cross-linking the antibody or by immobilizing it at high density to a surface such as the well of a microtiter plate. However, in vivo this effect may be mediated by binding of the antibody to the Fc receptors expressed on a nearby cell. Antibody Fc regions that bind more tightly to Fc receptors may more effectively cluster the signaling target and lead to enhanced induction of apoptosis.

[0126] This can be experimentally tested in the following manner. To cells expressing the target that signals, add the conjugate with and without enhanced Fc receptor binding. Also, add an Fc receptor and a corresponding antibody that will cluster the Fc receptor. Alternative means can be used to cluster the Fc receptor such as immobilization on a bead, over-expression in a non-effector cell line. After allowing apoptosis to occur, measure the relative apoptosis of target expressing cells.

[0127] Antibodies that cause cell death through their interaction with targets may have an additional benefit. The signals released by such dying cells attract macrophages and other cells of the immune system. These cells can then take-up the dead or dying cells in an antibody mediated manner. This has been shown to result in cross-presentation of antigen and the potential for a host immune response against the target cells. Such auto-antibodies in response to antibody therapy have been reported for the antigen targets Her-2 and CD20. For this reason it may be advantageous to have Fc regions with altered receptor specificities to specifically stimulate cross-presentation and an immune response rather than the undesired effect of tolerance induction.

[0128] Other therapeutic antibodies exert their effects by blocking signaling of the receptor by inhibiting the binding between a receptor and its cognate ligand. Such antibodies are used to treat many disease states. In this case it may be advantageous to utilize antibodies that do not recruit any host immune functions. A secondary effect of such an antibody may be actually inducing signalling itself through receptor clustering. In this case the desired therapeutic effect of blocking signalling would be abrogated by antibody mediated signalling. As discussed above this clustering may be enhanced by antibody interaction with cells containing an Fc receptor. In this case an antibody that bound less tightly or not at all to the Fc receptor would be preferable. Such an antibody would not mediate signaling and its function thereby be restricted to blocking receptor ligand interactions. Signaling receptors for which this would be most appropriate would likely be monomeric receptors which can only be dimerized but not substantially clustered by a primary antibody. Multimeric receptors may be significantly clustered by the primary antibody and may not require additional clustering by Fc receptor binding.

[0129] Another mechanism of action of therapeutic antibodies is to cause receptor down-regulation. Such may be the case with the insulin-like growth factor receptor. Cell growth depends on continued signaling through the receptor

while in its absence cells cease to grow. One effect of antibodies directed against this receptor is to down-regulate its expression and thereby shut off signaling. Cell recovery from cytotoxic therapy requires stimulation of this receptor. Down-regulating the receptor prevents these cells from recovery and renders the cytotoxic therapy substantially more effective. For antibodies for which this is the primary mechanism of action, decreased Fc receptor binding may prevent the sequestration of antibody by nontarget binding to Fc receptors.

Targets That Do Not Signal

[0130] Although many therapeutically effective antibodies work in part by signaling through their target antigen, this is not always the case. For example, some target classes such as cell surface glycoforms do not generate any biological signal. However, altered glycoforms are often associated with disease states such as cancer. In other cases, interaction of antibodies with different epitopes of the same target antigen will confer different signaling effects. In such cases, Fc polypeptides of the present invention may find utility in providing novel routes of efficacy in otherwise non-efficacious molecules.

[0131] One approach that has been taken in generating therapeutic antibodies to such targets is to couple the antibody to a cytotoxic agent such as a radio-isotope or in some cases an enzyme that will process a substrate to produce a cytotoxic agent in the vicinity of the tumor. If such a cytotoxic agent is utilized it may be advantageous to have no or altered Fc receptor binding by the Fc portion of the antibody. This may help to minimize the generation of an immune response to the toxic agent or enzyme.

[0132] As mentioned above for signaling antibodies the death of cells will result in the recruitment of the host immune cells. Antibody mediated cross-presentation in such a case may be increased with immune response rather than immune tolerance if in addition to a cytotoxic moiety the therapeutic antibody has increased Fc receptor binding affinity or altered receptor specificity.

[0133] As an alternative to a cytotoxic moiety, altered Fc variants of the present invention that increase recruitment of immune functions may be inherently less toxic to the host while still effective at mediating cell killing. Such Fc variants may be more efficient at recruiting NK cells or at activating phagocytosis or initiating CDC.

Targets That Internalize

[0134] Another significant target type are those that internalize either as a normal function or in response to antibody binding. For such targets many efforts have been made to couple cytotoxic agents such as RNase, ricin and calicheamicin. These reagents can only exert their effect after internalization. For reagents such as this any Fc receptor binding may result in antibody being sequestered by binding nonproductively to Fc receptors. In this case it may be advantageous to utilize Fc regions with decreased affinity for Fc receptors.

[0135] Conversely, antibody pre-association with Fc receptors prior to their binding to target antigen presented on cells may serve to inhibit internalization of the target. In this case increased Fc receptor affinity may serve to improve pre-association and thereby recruitment of effector cells and the host immune response.

Soluble or Shed Targets

[0136] In the case of targets that are soluble rather than cell surface bound the recruitment of effector functions would not result in any cell death. However, there may be utility in stimulating the generation of host antibodies to the target. For some disease states successful treatment may require administration of the therapeutic antibody for extremely long periods of time. Such therapy may be prohibitively costly or cumbersome. In this case the stimulation of host immune response and the generation of antibodies may result in improved efficacy of the therapeutic. This might be applicable as an adjuvant to vaccine therapy. Antibody Fc regions mediating such an effect may have increased affinity for Fc receptors or altered Fc receptor specificity.

Antibodies Where ADCC is a Component of Therapeutic Mechanism

[0137] In one embodiment, the conjugates of the present invention function therapeutically, in whole or in part, through ADCC activity and include: anti-CD20 antibodies such as Bexocar, Rituxan®, Zevalin®, anti-CD33 antibodies such as Smart M195, anti-CD22 antibodies such as Lymphocide™, anti-CD30 antibodies such as AC-10 and SGN-30, anti-EGFR antibodies such as ABX-EGF, Cetuximab, IMC-C225, Merck Mab 425, anti-EpCAM antibodies such as Crucell's anti-EpCAM, anti-HER2 antibodies such as Herceptin and MDX-210, and anti-CEA antibodies such as cantumab and Pentacea.

Antibodies Where CDC is a Component of Therapeutic Mechanism

[0138] In one embodiment, the conjugates of the present invention function therapeutically, in whole or in part, through CDC activity and include: anti-CEA antibodies such as cantumab and Pentacea, anti-CD20 antibodies such as Bexocar, Rituxan®, Zevalin®, anti-EpCAM antibodies such as Crucell's anti-EpCAM and Edrecolomab, and anti-CD52 antibodies such as Campath® (alemtuzumab).

[0139] Conjugates including C5a, C3a, C4a and/or fMLP generates a novel therapeutic for the treatment of malignant tumors and other diseases. Thus, in a preferred embodiment, antibody-C5a mediated recruitment of complement function and effector cells may result in a concerted attack on malignant tumors by enabling one or more of the following characteristics: more effective tumor target permeation; cellular lysis; and clearance by enhancing vasopermeability, effector function, and phagocytosis, respectively.

[0140] While C5a has been an inhibitory target for the treatment of sepsis, the localization of C5a to malignant tumor sites using antibodies is favorable for various reasons. While not wishing to be bound by a particular theory or mechanism, it is believed that as a vasodilator, higher concentrations of C5a at the tumor site facilitates the recruitment of effector cells to the tumor site as well as enhance their permeation into solid tumors. The C5a molecule has been implicated as a modulator of Fc receptors (up regulation of FcγRIIIa, down regulation of FcγRIIb), and therefore may enhance the cytotoxic potential of effector cells at the tumor site by shifting the balance towards effector cell activation. Moreover, the up regulation of these receptors has potentially lasting consequences at the tumor site, in view of the 'multiple hit' hypothesis which implies that

effector cells are 'recycled' in targeting cancer cells in a series rather than a one time killing phenomenon (e.g. one neutrophil, one cancer cell). Therapeutically, the conjunction of antibodies with C5a adds significant cytotoxic potential, promotes vascular access for a plethora of immune effector cells that target cancers sites and promotes clearance.

[0141] Additionally, previous studies have implicated excessive systemic C5a with compromised immune functions and sepsis. Localizing C5a activity to the tumor site using antibodies will minimize this systemic response and favor the beneficial targeting activity discussed.

Diseases

[0142] The conjugates described herein can be used to treat cancers or cancerous tissues, autoimmune diseases.

[0143] By "cancer" and "cancerous" herein refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to carcinoma, lymphoma, blastoma, sarcoma (including liposarcoma), neuroendocrine tumors, mesothelioma, schwannoma, meningioma, adenocarcinoma, melanoma, and leukemia or lymphoid malignancies.

[0144] More particular examples of such cancers include hematologic malignancies, such as Hodgkin's lymphoma; non-Hodgkin's lymphomas (Burkitt's lymphoma, small lymphocytic lymphoma/chronic lymphocytic leukemia, mycosis fungoides, mantle cell lymphoma, follicular lymphoma, diffuse large B-cell lymphoma, marginal zone lymphoma, hairy cell leukemia and lymphoplasmacytic leukemia), tumors of lymphocyte precursor cells, including B-cell acute lymphoblastic leukemia/lymphoma, and T-cell acute lymphoblastic leukemia/lymphoma, thymoma, tumors of the mature T and NK cells, including peripheral T-cell leukemias, adult T-cell leukemia/T-cell lymphomas and large granular lymphocytic leukemia, Langerhans cell histiocytosis, myeloid neoplasias such as acute myelogenous leukemias, including AML with maturation, AML without differentiation, acute promyelocytic leukemia, acute myelomonocytic leukemia, and acute monocytic leukemias, myelodysplastic syndromes, and chronic myeloproliferative disorders, including chronic myelogenous leukemia; tumors of the central nervous system such as glioma, glioblastoma, neuroblastoma, astrocytoma, medulloblastoma, ependymoma, and retinoblastoma; solid tumors of the head and neck (e.g. nasopharyngeal cancer, salivary gland carcinoma, and esophageal cancer), lung (e.g. small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung and squamous carcinoma of the lung), digestive system (e.g. gastric or stomach cancer including gastrointestinal cancer, cancer of the bile duct or biliary tract, colon cancer, rectal cancer, colorectal cancer, and anal carcinoma), reproductive system (e.g. testicular, penile, or prostate cancer, uterine, vaginal, vulval, cervical, ovarian, and endometrial cancer), skin (e.g. melanoma, basal cell carcinoma, squamous cell cancer, actinic keratosis), liver (e.g. liver cancer, hepatic carcinoma, hepatocellular cancer, and hepatoma), bone (e.g. osteoclastoma, and osteolytic bone cancers) additional tissues and organs (e.g. pancreatic cancer, bladder cancer, kidney or renal cancer, thyroid cancer, breast cancer, cancer of the peritoneum, and Kaposi's sarcoma), and tumors of the vascular system (e.g. angiosarcoma and hemangiopericytoma).

[0145] By “autoimmune diseases” herein include allo-genic islet graft rejection, alopecia areata, ankylosing spondylitis, antiphospholipid syndrome, autoimmune Addison’s disease, antineutrophil cytoplasmic autoantibodies (ANCA), autoimmune diseases of the adrenal gland, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune myocarditis, autoimmune neutropenia, autoimmune oophoritis and orchitis, autoimmune thrombocytopenia, autoimmune urticaria, Behcet’s disease, bullous pemphigoid, cardiomyopathy, Castleman’s syndrome, celiac spruce-dermatitis, chronic fatigue immune dysfunction syndrome, chronic inflammatory demyelinating polyneuropathy, Churg-Strauss syndrome, cicatricial pemphigoid, CREST syndrome, cold agglutinin disease, Crohn’s disease, dermatomyositis, discoid lupus, essential mixed cryoglobulinemia, factor VIII deficiency, fibromyalgia-fibromyositis, glomerulonephritis, Grave’s disease, Guillain-Barre, Good-pasture’s syndrome, graft-versus-host disease (GVHD), Hashimoto’s thyroiditis, hemophilia A, idiopathic pulmonary fibrosis, idiopathic thrombocytopenia purpura (ITP), IgA neuropathy, IgM polyneuropathies, immune mediated thrombocytopenia, juvenile arthritis, Kawasaki’s disease, lichen plantus, lupus erthematosus, Meniere’s disease, mixed connective tissue disease, multiple sclerosis, type 1 diabetes mellitus, myasthenia gravis, pemphigus vulgaris, pernicious anemia, polyarteritis nodosa, polychondritis, polyglandular syndromes, polymyalgia rheumatica, polymyositis and dermatomyositis, primary agammaglobulinemia, primary biliary cirrhosis, psoriasis, psoriatic arthritis, Reynald’s phenomenon, Reiter’s syndrome, rheumatoid arthritis, sarcoidosis, scleroderma, Sjorgen’s syndrome, solid organ transplant rejection, stiff-man syndrome, systemic lupus erythematosus, takayasu arteritis, temporal arteritis/giant cell arteritis, thrombotic thrombocytopenia purpura, ulcerative colitis, uveitis, vasculitides such as dermatitis herpetiformis vasculitis, vitiligo, and Wegner’s granulomatosis.

[0146] By “infectious diseases” herein include diseases caused by pathogens such as viruses, bacteria, fungi, protozoa, and parasites. Infectious diseases may be caused by viruses including adenovirus, cytomegalovirus, dengue, Epstein-Barr, hanta, hepatitis A, hepatitis B, hepatitis C, herpes simplex type I, herpes simplex type II, human immunodeficiency virus, (HIV), human papilloma virus (HPV), influenza, measles, mumps, papova virus, polio, respiratory syncytial virus, rinderpest, rhinovirus, rotavirus, rubella, SARS virus, smallpox, viral meningitis, and the like. Infections diseases may also be caused by bacteria including *Bacillus anthracis*, *Borrelia burgdorferi*, *Campylobacter jejuni*, *Chlamydia trachomatis*, *Clostridium botulinum*, *Clostridium tetani*, *Diphtheria*, *E. coli*, *Legionella*, *Helicobacter pylori*, *Mycobacterium rickettsia*, *Mycoplasma nesisseria*, *Pertussis*, *Pseudomonas aeruginosa*, *S. pneumonia*, *Streptococcus*, *Staphylococcus*, *Vibria cholerae*, *Yersinia pestis*, and the like. Infectious diseases may also be caused by fungi such as *Aspergillus fumigatus*, *Blastomyces dermatitidis*, *Candida albicans*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Penicillium marneffe*, and the like. Infectious diseases may also be caused by protozoa and parasites such as chlamydia, kokzidioa, leishmania, malaria, rickettsia, trypanosoma, and the like.

Dosing

[0147] The dosing amounts and frequencies of administration are, in a preferred embodiment, selected to be therapeutically or prophylactically effective. As is known in the art, adjustments for conjugate degradation, systemic versus localized delivery, and rate of new protease synthesis, as well as the age, body weight, general health, sex, diet, time of administration, drug interaction and the severity of the condition may be necessary, and will be ascertainable with routine experimentation by those skilled in the art.

[0148] The concentration of the conjugates of the present invention in the formulation may vary from about 0.1 to 100 weight %. In a preferred embodiment, the concentration of the antibody or Fc fusion is in the range of 0.003 to 1.0 molar. In order to treat a patient, a therapeutically effective dose of the conjugate of the present invention may be administered. By “therapeutically effective dose” herein is meant a dose that produces the effects for which it is administered. The exact dose will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques. Dosages may range from 0.0001 to 100 mg/kg of body weight or greater, for example 0.1, 1, 10, or 50 mg/kg of body weight, with 1 to 10 mg/kg being preferred.

[0149] In some embodiments, only a single dose of the conjugate of the present invention is used.

[0150] In other embodiments, multiple doses of the conjugate of the present invention are administered. The elapsed time between administrations may be less than 1 hour, about 1 hour, about 1-2 hours, about 2-3 hours, about 3-4 hours, about 6 hours, about 12 hours, about 24 hours about 48 hours, about 2-4 days, about 4-6 days, about 1 week, about 2 weeks, or more than 2 weeks.

[0151] In other embodiments the conjugate of the present invention are administered in metronomic dosing regimes, either by continuous infusion or frequent administration without extended rest periods. Such metronomic administration may involve dosing at constant intervals without rest periods. Typically such regimens encompass chronic low-dose or continuous infusion for an extended period of time, for example 1-2 days, 1-2 weeks, 1-2 months, or up to 6 months or more. The use of lower doses may minimize side effects and the need for rest periods.

[0152] In certain embodiments the conjugate of the present invention and one or more other prophylactic or therapeutic agents are cyclically administered to the patient. Cycling therapy involves administration of a first agent at one time, a second agent at a second time, optionally additional agents at additional times, optionally a rest period, and then repeating this sequence of administration one or more times. The number of cycles is typically from 2-10. Cycling therapy may reduce the development of resistance to one or more agents, may minimize side effects, or may improve treatment efficacy.

Methods of Administration

[0153] Administration of the pharmaceutical composition comprising a conjugate of the present invention, preferably in the form of a sterile aqueous solution, may be done in a variety of ways, including, but not limited to orally, subcutaneously, intravenously, intranasally, intraotically, transder-

mally, topically (e.g., gels, salves, lotions, creams, etc.), intraperitoneally, intramuscularly, intrapulmonary, vaginally, parenterally, rectally, or intraocularly. In some instances, for example for the treatment of wounds, inflammation, etc., the antibody or Fc fusion may be directly applied as a solution or spray. As is known in the art, the pharmaceutical composition may be formulated accordingly depending upon the manner of introduction.

Subcutaneous

[0154] Subcutaneous administration may be preferable in some circumstances because the patient may self-administer the pharmaceutical composition. Many antibody therapeutics are not sufficiently potent to allow for formulation of a therapeutically effective dose in the maximum acceptable volume for subcutaneous administration. This problem may be addressed in part by the use of protein formulations comprising arginine-HCl, histidine, and polysorbate (see WO 04091658, which is incorporated by reference in its entirety). Conjugates of the present invention may be more amenable to subcutaneous administration due to, for example, increased potency, improved serum half-life, or enhanced solubility.

Intervenous

[0155] As is known in the art, antibody therapeutics are often delivered by IV infusion or bolus. The conjugates of the present invention may also be delivered using such methods. For example, administration may be by intravenous infusion with 0.9% sodium chloride as an infusion vehicle.

Inhaled

[0156] Pulmonary delivery may be accomplished using an inhaler or nebulizer and a formulation comprising an aerosolizing agent. For example, AERx® inhalable technology commercially available from Aradigm, or Inhance™ pulmonary delivery system commercially available from Nektar Therapeutics may be used. Conjugates of the present invention may be more amenable to intrapulmonary delivery. FcRn is present in the lung, and may promote transport from the lung to the bloodstream (e.g. Syntonix WO 04004798, Bitonti et.al. (2004) Proc. Nat. Acad. Sci. 101:9763-8, each incorporated by reference in its entirety). Accordingly, antibodies or Fc fusions that bind FcRn more effectively in the lung or that are released more efficiently in the bloodstream may have improved bioavailability following intrapulmonary administration. Conjugates of the present invention may also be more amenable to intrapulmonary administration due to, for example, improved solubility or altered isoelectric point.

Oral Delivery

[0157] Furthermore, conjugates of the present invention may be more amenable to oral delivery due to, for example, improved stability at gastric pH and increased resistance to proteolysis. Furthermore, FcRn appears to be expressed in the intestinal epithelia of adults (Dickinson et.al. (1999) J. Clin. Invest. 104:903-11), incorporated by reference in its entirety, so conjugates of the present invention with improved FcRn interaction profiles may show enhanced bioavailability following oral administration. FcRn mediated transport of antibodies and Fc fusions may also occur at other mucus membranes such as those in the gastrointes-

tinal, respiratory, and genital tracts (Yoshida et. al. (2004) Immunity 20:769-83), each incorporated by reference in its entirety.

Controlled Release

[0158] In addition, any of a number of delivery systems are known in the art and may be used to administer the conjugates of the present invention. Examples include, but are not limited to, encapsulation in liposomes, microparticles, microspheres (e.g. PLA/PGA microspheres), and the like. Alternatively, an implant of a porous, non-porous, or gelatinous material, including membranes or fibers, may be used. Sustained release systems may comprise a polymeric material or matrix such as polyesters, hydrogels, poly(vinylalcohol), polylactides, copolymers of L-glutamic acid and ethyl-L-gutamate, ethylene-vinyl acetate, lactic acid-glycolic acid copolymers such as the LUPRON DEPOT®, and poly-D(-)-3-hydroxybutyric acid. It is also possible to administer a nucleic acid encoding the antibody or Fc fusion of the current invention, for example by retroviral infection, direct injection, or coating with lipids, cell surface receptors, or other transfection agents. In all cases, controlled release systems may be used to release the antibody or Fc fusion at or close to the desired location of action.

Monotherapy

[0159] In one embodiment, a conjugate of the present invention is administered to a patient having a disease involving inappropriate expression of a protein or other molecule. Within the scope of the present invention this is meant to include diseases and disorders characterized by aberrant proteins, due for example to alterations in the amount of a protein present, protein localization, posttranslational modification, conformational state, the presence of a mutant or pathogen protein, etc. Similarly, the disease or disorder may be characterized by alterations molecules including but not limited to polysaccharides and gangliosides. An overabundance may be due to any cause, including but not limited to overexpression at the molecular level, prolonged or accumulated appearance at the site of action, or increased activity of a protein relative to normal. Included within this definition are diseases and disorders characterized by a reduction of a protein. This reduction may be due to any cause, including but not limited to reduced expression at the molecular level, shortened or reduced appearance at the site of action, mutant forms of a protein, or decreased activity of a protein relative to normal. Such an overabundance or reduction of a protein can be measured relative to normal expression, appearance, or activity of a protein, and said measurement may play an important role in the development and/or clinical testing of the conjugates of the present invention.

Combination Therapies

[0160] Conjugates of the present invention may be administered concomitantly with one or more other therapeutic regimens or agents. The additional therapeutic regimes or agents may be used to improve the efficacy or safety of the antibody or Fc fusion. Also, the additional therapeutic regimes or agents may be used to treat the same disease or a comorbidity rather than to alter the action of the antibody or Fc fusion. For example, a conjugate of the present invention may be administered to the patient along with chemotherapy, radiation therapy, or both chemotherapy and

radiation therapy. The conjugate of the present invention may be administered in combination with one or more other prophylactic or therapeutic agents, including but not limited to cytotoxic agents, chemotherapeutic agents, cytokines, growth inhibitory agents, anti-hormonal agents, kinase inhibitors, anti-angiogenic agents, cardioprotectants, immunostimulatory agents, immunosuppressive agents, agents that promote proliferation of hematological cells, angiogenesis inhibitors, protein tyrosine kinase (PTK) inhibitors, additional antibody or Fc fusion proteins, FcγRIIb or other Fc receptor inhibitors, or other therapeutic agents.

[0161] The terms “in combination with” and “co-administration” are not limited to the administration of said prophylactic or therapeutic agents at exactly the same time. Instead, it is meant that the conjugate of the present invention and the other agent or agents are administered in a sequence and within a time interval such that they may act together to provide a benefit that is increased versus treatment with only either the conjugate of the present invention or the other agent or agents. It is preferred that the antibody or Fc fusion and the other agent or agents act additively, and especially preferred that they act synergistically. Such molecules are suitably present in combination in amounts that are effective for the purpose intended. The skilled medical practitioner can determine empirically, or by considering the pharmacokinetics and modes of action of the agents, the appropriate dose or doses of each therapeutic agent, as well as the appropriate timings and methods of administration.

Other Antibodies and Proteins

[0162] In one embodiment, the conjugate of the present invention are administered with one or more additional molecules comprising antibodies or Fc. The conjugate of the present invention may be co-administered with one or more other antibodies that have efficacy in treating the same disease or an additional comorbidity; for example two antibodies may be administered that recognize two antigens that are overexpressed in a given type of cancer, or two antigens that mediate pathogenesis of an autoimmune or infectious disease.

Anti-Cancer Antibodies

[0163] Examples of anti-cancer antibodies that may be co-administered include, but are not limited to, anti 17-IA cell surface antigen antibodies such as Panorex™ (edrecolomab); anti-4-1BB antibodies; anti-4Dc antibodies; anti-A33 antibodies such as A33 and CDP-833; anti-α4β1 integrin antibodies such as natalizumab; anti-α4β7 integrin antibodies such as LDP-02; anti-αVβ1 integrin antibodies such as F-200, M-200, and SJ-749; anti-αVβ3 integrin antibodies such as abciximab, CNTO-95, Mab-17E6, and Vitaxin™; anti-complement factor 5 (C5) antibodies such as 5G1.1; anti-CA125 antibodies such as OvaRex® (oregiovomab); anti-CD3 antibodies such as Nuvion® (visilizumab) and Rexomab; anti-CD4 antibodies such as IDEC-151, MDX-CD4, OKT4A; anti-CD6 antibodies such as Oncolysin B and Oncolysin CD6; anti-CD7 antibodies such as HB2; anti-CD19 antibodies such as B43, MT-103, and Oncolysin B; anti-CD20 antibodies such as 2H7, 2H7.v16, 2H7.v114, 2H7.v115, Bexxar® (tositumomab), Rituxan® (rituximab), and Zevalin® (Ibritumomab tiuxetan); anti-CD22 antibodies such as Lymphocide™ (epratuzumab); anti-CD23 antibodies such as IDEC-152; anti-CD25 antibodies such as basiliximab and Zenapax® (daclizumab);

anti-CD30 antibodies such as AC10, MDX-060, and SGN-30; anti-CD33 antibodies such as Mylotarg® (gemtuzumab ozogamicin), Oncolysin M, and Smart M195; anti-CD38 antibodies; anti-CD40 antibodies such as SGN-40 and toralizumab; anti-CD40L antibodies such as 5c8, Antova™, and IDEC-131; anti-CD44 antibodies such as bivatuzumab; anti-CD46 antibodies; anti-CD52 antibodies such as Campath® (alemtuzumab); anti-CD55 antibodies such as SC-1; anti-CD56 antibodies such as huN901-DM1; anti-CD64 antibodies such as MDX-33; anti-CD66e antibodies such as XR-303; anti-CD74 antibodies such as IMMU-110; anti-CD80 antibodies such as galiximab and IDEC-114; anti-CD89 antibodies such as MDX-214; anti-CD123 antibodies; anti-CD138 antibodies such as B-B4-DM1; anti-CD146 antibodies such as AA-98; anti-CD148 antibodies; anti-CEA antibodies such as cT84.66, labetuzumab, and Pentacea™; anti-CTLA-4 antibodies such as MDX-101; anti-CXCR4 antibodies; anti-EGFR antibodies such as ABX-EGF, Erbitux® (cetuximab), IMC-C225, and Merck Mab 425; anti-EpCAM antibodies such as Crucell's anti-EpCAM, ING-1, and IS-IL-2; anti-ephrin B2/EphB4 antibodies; anti-Her2 antibodies such as Herceptin®, MDX-210; anti-FAP (fibroblast activation protein) antibodies such as sibrotuzumab; anti-ferritin antibodies such as NXT-211; anti-FGF-1 antibodies; anti-FGF-3 antibodies; anti-FGF-8 antibodies; anti-FGFR antibodies, anti-fibrin antibodies; anti-G250 antibodies such as WX-G250 and Rencarex®; anti-GD2 ganglioside antibodies such as EMD-273063 and TriGem; anti-GD3 ganglioside antibodies such as BEC2, KW-2871, and mitumomab; anti-gpIIb/IIIa antibodies such as ReoPro; anti-heparinase antibodies; anti-Her2/ErbB2 antibodies such as Herceptin® (trastuzumab), MDX-210, and pertuzumab; anti-HLA antibodies such as Oncolym®, Smart 1D10; anti-HM1.24 antibodies; anti-ICAM antibodies such as ICM3; anti-IgA receptor antibodies; anti-IGF-1 antibodies such as CP-751871 and EM-164; anti-IGF-1R antibodies such as IMC-A12; anti-IL-6 antibodies such as CNTO-328 and elsilimomab; anti-IL-15 antibodies such as HuMax™-IL15; anti-KDR antibodies; anti-laminin 5 antibodies; anti-Lewis Y antigen antibodies such as Hu3S193 and IGN-311; anti-MCAM antibodies; anti-Muc1 antibodies such as BravaRex and TriAb; anti-NCAM antibodies such as ERIC-1 and ICRT; anti-PEM antigen antibodies such as Theragyn and Therex; anti-PSA antibodies; anti-PSCA antibodies such as IG8; anti-Ptk antibodies; anti-PTN antibodies; anti-RANKL antibodies such as AMG-162; anti-RLIP76 antibodies; anti-SK-1 antigen antibodies such as Monopharm C; anti-STEAP antibodies; anti-TAG72 antibodies such as CC49-SCA and MDX-220; anti-TGF-β antibodies such as CAT-152; anti-TNF-α antibodies such as CDP571, CDP870, D2E7, Humira® (adalimumab), and Remicade® (infliximab); anti-TRAIL-R1 and TRAIL-R2 antibodies; anti-VE-cadherin-2 antibodies; and anti-VLA-4 antibodies such as Antegren™. Furthermore, anti-idiotypic antibodies including but not limited to the GD3 epitope antibody BEC2 and the gp72 epitope antibody 105AD7, may be used. In addition, bispecific antibodies including but not limited to the anti-CD3/CD20 antibody Bi20 may be used.

Antibodies for Autoimmune and Inflammatory Diseases and Transplant Rejection/GVHD

[0164] Examples of antibodies that may be co-administered to treat autoimmune or inflammatory disease, transplant rejection, GVHD, and the like include, but are not limited to, anti-α4β7 integrin antibodies such as LDP-02,

anti-beta2 integrin antibodies such as LDP-01, anti-complement (C5) antibodies such as 5G1.1, anti-CD2 antibodies such as BTI-322, MEDI-507, anti-CD3 antibodies such as OKT3, SMART anti-CD3, anti-CD4 antibodies such as IDEC-151, MDX-CD4, OKT4A, anti-CD11a antibodies, anti-CD14 antibodies such as IC14, anti-CD18 antibodies, anti-CD23 antibodies such as IDEC 152, anti-CD25 antibodies such as Zenapax, anti-CD40L antibodies such as 5c8, Antova, IDEC-131, anti-CD64 antibodies such as MDX-33, anti-CD80 antibodies such as IDEC-114, anti-CD147 antibodies such as ABX-CBL, anti-E-selectin antibodies such as CDP850, anti-gpIIb/IIIa antibodies such as ReoPro/Abcixima, anti-ICAM-3 antibodies such as ICM3, anti-ICE antibodies such as VX-740, anti-FcR1 antibodies such as MDX-33, anti-IgE antibodies such as rhuMab-E25, anti-IL-4 antibodies such as SB-240683, anti-IL-5 antibodies such as SB-240563, SCH55700, anti-IL-8 antibodies such as ABX-IL8, anti-interferon gamma antibodies, and anti-TNF α antibodies such as CDP571, CDP870, D2E7, Infliximab, MAK-195F, anti-VLA-4 antibodies such as Antegren. Examples of other Fc-containing molecules that may be co-administered to treat autoimmune or inflammatory disease, transplant rejection, GVHD, and the like include, but are not limited to, the p75 TNF receptor/Fc fusion Enbrel® (etanercept) and Regeneron's IL-1 trap.

Antibodies for Infectious Diseases

[0165] Examples of antibodies that may be co-administered to treat infectious diseases include, but are not limited to, anti-anthrax antibodies such as ABthrax, anti-CMV antibodies such as CytoGam and sevirumab, anti-cryptosporidium antibodies such as CryptoGAM, Sporidin-G, anti-helicobacter antibodies such as Pyloran, anti-hepatitis B antibodies such as HepeX-B, Nabi-HB, anti-HIV antibodies such as HRG-214, anti-RSV antibodies such as felvizumab, HNK-20, palivizumab, RespiGam, and anti-staphylococcus antibodies such as Aurexis, Aurograb, BSYX-A110, and SE-Mab.

Chemotherapeutic Agents

[0166] In one embodiment, the conjugate of the present invention are administered with a chemotherapeutic agent. By "chemotherapeutic agent" as used herein is meant a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include but are not limited to alkylating agents such as thiotepa and cyclophosphamide (CYTOXAN™); alkyl sulfonates such as busulfan, improsulfan and piposulfan; androgens such as calusterone, dromostanolone propionate, epitostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; antibiotics such as aclacinomysins, actinomycin, anthracycline, azaserine, bleomycins, cactinomycin, calicheamicin, carubicin, caminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY 117018, onapristone, and toremifene (Fareston); anti-

metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; folic acid replenisher such as frolic acid; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; proteins such as arginine deiminase and asparaginase; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; taxanes, e.g. paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, N.J.) and docetaxel (TAXOTERE®, Rhne-Poulenc Rorer, Antony, France); topoisomerase inhibitor RFS 2000; thymidylate synthase inhibitor (such as Tomudex); additional chemotherapeutics including aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; difluoromethylornithine (DMFO); elformithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguanzone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK®; razoxane; sizofuran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; retinoic acid; esperamicins; capecitabine. Pharmaceutically acceptable salts, acids or derivatives of any of the above may also be used.

[0167] A chemotherapeutic or other cytotoxic agent may be administered as a prodrug. By "Prodrug" as used herein is meant a precursor or derivative form of a pharmaceutically active substance that is less cytotoxic to tumor cells compared to the parent drug and is capable of being enzymatically activated or converted into the more active parent form. See, for example Wilman, 1986, Biochemical Society Transactions, 615th Meeting Belfast, 14:375-382; and Stella et al., "Prodrugs: A Chemical Approach to Targeted Drug Delivery," Directed Drug Delivery, Borchardt et al., (ed.): 247-267, Humana Press, 1985, each incorporated by reference in its entirety. The prodrugs that may find use with the present invention include but are not limited to phosphate-containing prodrugs, thiophosphate-containing prodrugs, sulfate-containing prodrugs, peptide-containing prodrugs, D-amino acid-modified prodrugs, glycosylated prodrugs, beta-lactam-containing prodrugs, optionally substituted phenoxyacetamide-containing prodrugs or optionally substituted phenylacetamide-containing prodrugs, 5-fluorocytosine and other 5-fluorouridine prodrugs which can be

converted into the more active cytotoxic free drug. Examples of cytotoxic drugs that can be derivatized into a prodrug form for use with the conjugates of the present invention include but are not limited to any of the aforementioned chemotherapeutic agents.

Surgery and Additional Therapeutic Techniques

[0168] It is of course contemplated that the antibodies and Fc fusions of the invention may employ in combination with still other therapeutic techniques such as surgery or phototherapy.

Combination Therapy with Cytokines

[0169] In an alternate embodiment, the conjugates of the present invention are administered with a cytokine. By "cytokine" as used herein is meant a generic term for proteins released by one cell population that act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor- α and - β ; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF- β ; platelet-growth factor; transforming growth factors (TGFs) such as TGF- α and TGF- β ; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon- α , β , and - γ ; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1 α , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-15, a tumor necrosis factor such as TNF- α or TNF- β ; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture, and biologically active equivalents of the native sequence cytokines.

[0170] In a preferred embodiment, cytokines or other agents that stimulate cells of the immune system are co-administered with the conjugate of the present invention. Such a mode of treatment may enhance desired effector function. For example, agents that stimulate NK cells, including but not limited to IL-2 may be co-administered. In another embodiment, agents that stimulate macrophages, including but not limited to C5a, formyl peptides such as N-formyl-methionyl-leucyl-phenylalanine (Beigier-Bompadre et. al. (2003) *Scand. J. Immunol.* 57: 221-8), incorporated by reference in its entirety, may be co-administered. Also, agents that stimulate neutrophils, including but not limited to G-CSF, GM-CSF, and the like may be administered. Furthermore, agents that promote migration of such immunostimulatory cytokines may be used. Also additional agents including but not limited to interferon gamma, IL-3 and IL-7 may promote one or more effector functions.

[0171] In an alternate embodiment, cytokines or other agents that inhibit effector cell function are co-administered

with the conjugate of the present invention. Such a mode of treatment may limit unwanted effector function.

Antibody-Chemoattractant Combinations

[0172] Examples of antibody-chemoattractant combinations include any combination of an antibody and one or more molecules selected from the group consisting of C5a, fMLP, C3a and C4a. Examples include, but are not limited to: a Fab conjugated to fMLP on the N-terminus of the heavy chain; a Fc fused to a C5a fragment on the C-terminus of the heavy chain a G(SG)_n linker, a Fab'2 conjugated to fMLP on Lys site changes; etc.

EXAMPLES

[0173] Examples are provided below to illustrate the present invention. These examples are not meant to constrain the present invention to any particular application or theory of operation.

Example 1

[0174] Shown in **FIG. 1A** is a model of an antibody-C5a fusion. The antibody is an intact IgG1. The C5a is attached to the C-terminus of the heavy chain with a G(SG)₅ linker. (SEQ ID NO:22)

[0175] The amino acid sequences of the light chain and heavy chain of an antibody-C5a fusion are listed in **FIGS. 1B and 1C**, respectively.

Example 2

[0176] Shown in **FIG. 2A** is a model of an antibody-C5a fusion. The antibody is a Fab fragment with the heavy chain truncated at 227. The C5a is attached to the C-terminus of the light chain with a G(SG)₅ linker. (SEQ ID NO:22)

[0177] The amino acid sequences of the light chain and heavy chain of an antibody-C5a fusion are listed in **FIGS. 2B and 2C**, respectively.

Example 3

[0178] Shown in **FIG. 3A** is a model of an antibody-C5a fusion. The antibody is a F(ab')₂ fragment with the heavy chain truncated at 240. The C5a is attached to the C-terminus of the light chain with a G(SG)₅ linker. (SEQ ID NO:22)

[0179] The amino acid sequences of the light chain and heavy chain of an antibody-C5a fusion are listed in **FIGS. 3B and 3C**, respectively.

Example 4

[0180] Shown in **FIG. 4A** is a model of an antibody-C5a fusion. The antibody is an intact IgG1. The C5a is attached to the C-terminus of the light chain with a G(SG)₅ linker. (SEQ ID NO:22)

[0181] The amino acid sequences of the light chain and heavy chain of an antibody-C5a fusion are listed in **FIGS. 4B and 4C**, respectively.

Example 5

[0182] Shown in **FIG. 5A** is a model of an antibody-C5a fusion. The antibody is an intact IgG1 (with only the Fc region shown). The C5a is a fragment consisting of residues

58-74, (i.e., last 17 amino acids on the C-terminus.) The C5a is directly attached to the C-terminus of the heavy chain.

[0183] The amino acid sequences of the light chain and heavy chain of an antibody-C5a fusion are listed in **FIGS. 5B and 5C**, respectively.

Example 6

[0184] Shown in **FIG. 6A** is a model of an antibody-(f)MLP fusion. The antibody is a Fab fragment with the heavy chain truncated at 227. The (f)MLP is directly attached to the N-terminus of the heavy chain.

[0185] The amino acid sequences of the light chain and heavy chain of an antibody-(f)MLP fusion are listed in **FIGS. 6B and 6C**, respectively.

Example 7

[0186] Shown in **FIG. 7A** is a model of an antibody-C5a-(f)MLP fusion. The antibody is a Fab fragment with the heavy chain truncated at 227. The C5a is a fragment consisting of residues 58-74, (i.e., last 17 amino acids on the C-terminus.) The C5a is attached directly to the C-terminus of the light chain. The (f)MLP is directly attached to the N-terminus of the heavy chain.

[0187] The amino acid sequences of the light chain and heavy chain of an antibody-C5a fusion are listed in **FIGS. 7B and 7C**, respectively.

Example 8

[0188] Shown in **FIG. 8A** is a model of an EGF-antibody-C5a fusion. The EFG is directly connected to the hinge region of the antibody. The antibody is an Fc region. The C5a is attached to the C-terminus of the Fc region with a G(SG)₅ linker. (SEQ ID NO:22)

[0189] The amino acid sequences of the light chain and heavy chain of an antibody-C5a fusion are listed in **FIGS. 8B and 8C**, respectively.

[0190] **FIGS. 9A, 9B and 9C** list the amino acid sequences of C3a, C4a and C5a, respectively.

[0191] All references are herein expressly incorporated by reference in their entirety.

[0192] Whereas particular embodiments of the invention have been described above for purposes of illustration, it will be appreciated by those skilled in the art that numerous variations of the details may be made without departing from the invention as described in the appended claims.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 22

<210> SEQ ID NO 1

<211> LENGTH: 213

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<213> ORGANISM: Artificial

<220> FEATURE:

<223> OTHER INFORMATION: Synthetic

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1 5 10 15

Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Ile
20 25 30

His Trp Phe Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr
35 40 45

Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Val Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu Ala Glu
65 70 75 80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Thr Ser Asn Pro Pro Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro
100 105 110

Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr
115 120 125

Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys
130 135 140

Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu
145 150 155 160

-continued

Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser
 165 170 175

Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala
 180 185 190

Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe
 195 200 205

Asn Arg Gly Glu Cys
 210

<210> SEQ ID NO 2
 <211> LENGTH: 536
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 2

Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys Pro Gly Ala
 1 5 10 15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
 20 25 30

Asn Met His Trp Val Lys Gln Thr Pro Gly Arg Gly Leu Glu Trp Ile
 35 40 45

Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe
 50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
 65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg Ser Thr Tyr Tyr Gly Gly Asp Trp Tyr Phe Asn Val Trp Gly
 100 105 110

Ala Gly Thr Thr Val Thr Val Ser Ala Ala Ser Thr Lys Gly Pro Ser
 115 120 125

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
 130 135 140

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
 145 150 155 160

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
 165 170 175

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
 180 185 190

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
 195 200 205

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Ala Glu Pro Lys Ser Cys
 210 215 220

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
 225 230 235 240

Gly Pro Asp Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
 245 250 255

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
 260 265 270

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
 275 280 285

-continued

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
 290 295 300
 Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
 305 310 315 320
 Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Leu Pro Glu
 325 330 335
 Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
 340 345 350
 Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
 355 360 365
 Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
 370 375 380
 Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
 385 390 395 400
 Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
 405 410 415
 Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
 420 425 430
 His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
 435 440 445
 Pro Gly Lys Gly Ser Gly Ser Gly Ser Gly Ser Gly Met Leu
 450 455 460
 Gln Lys Lys Ile Glu Glu Ile Ala Ala Lys Tyr Lys His Ser Val Val
 465 470 475 480
 Lys Lys Cys Cys Tyr Asp Gly Ala Cys Val Asn Asn Asp Glu Thr Cys
 485 490 495
 Glu Gln Arg Ala Ala Arg Ile Ser Leu Gly Pro Arg Cys Ile Lys Ala
 500 505 510
 Phe Thr Glu Cys Cys Val Val Ala Ser Gln Leu Arg Ala Asn Ile Ser
 515 520 525
 His Lys Asp Met Gln Leu Gly Arg
 530 535

<210> SEQ ID NO 3
 <211> LENGTH: 296
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 3

Glu Leu Val Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15
 Asp Arg Val Asn Ile Ala Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
 20 25 30
 Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Arg Leu Leu Ile
 35 40 45
 Tyr Asp Ala Ser Asn Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65 70 75 80
 Glu Asp Phe Ala Ile Tyr Tyr Cys Gln Gln Phe Asn Ser Tyr Pro Leu
 85 90 95

-continued

Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
 100 105 110
 Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
 115 120 125
 Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
 130 135 140
 Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
 145 150 155 160
 Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
 165 170 175
 Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
 180 185 190
 Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
 195 200 205
 Phe Asn Arg Gly Glu Cys Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser
 210 215 220
 Gly Met Leu Gln Lys Lys Ile Glu Glu Ile Ala Lys Tyr His Ser Val
 225 230 235 240
 Val Lys Cys Cys Tyr Asp Gly Ala Cys Val Asn Asn Asp Glu Thr Cys
 245 250 255
 Glu Gln Arg Ala Ala Arg Ile Ser Leu Gly Pro Arg Cys Ile Lys Ala
 260 265 270
 Phe Thr Glu Cys Cys Val Val Ala Ser Gln Leu Arg Ala Asn Ile Ser
 275 280 285
 His Lys Asp Met Gln Leu Gly Arg
 290 295

<210> SEQ ID NO 4
 <211> LENGTH: 227
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 4

Gln Val Lys Leu Leu Glu Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
 1 5 10 15
 Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Ser
 20 25 30
 Tyr Gly Leu His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp
 35 40 45
 Met Gly Trp Ile Ser Ala Gly Thr Gly Asn Thr Lys Tyr Ser Gln Lys
 50 55 60
 Phe Arg Gly Arg Val Thr Phe Thr Arg Asp Thr Ser Ala Thr Thr Ala
 65 70 75 80
 Tyr Met Gly Leu Ser Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr
 85 90 95
 Cys Ala Arg Asp Pro Tyr Gly Gly Gly Lys Ser Glu Phe Asp Tyr Trp
 100 105 110
 Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
 115 120 125
 Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
 130 135 140

-continued

Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
 145 150 155 160
 Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
 165 170 175
 Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
 180 185 190
 Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
 195 200 205
 His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
 210 215 220
 Cys Asp Lys
 225

<210> SEQ ID NO 5
 <211> LENGTH: 296
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 5

Glu Leu Val Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15
 Asp Arg Val Asn Ile Ala Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
 20 25 30
 Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Arg Leu Leu Ile
 35 40 45
 Tyr Asp Ala Ser Asn Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65 70 75 80
 Glu Asp Phe Ala Ile Tyr Tyr Cys Gln Gln Phe Asn Ser Tyr Pro Leu
 85 90 95
 Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
 100 105 110
 Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
 115 120 125
 Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
 130 135 140
 Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
 145 150 155 160
 Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
 165 170 175
 Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
 180 185 190
 Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
 195 200 205
 Phe Asn Arg Gly Glu Cys Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser
 210 215 220
 Gly Met Leu Gln Lys Lys Ile Glu Glu Ile Ala Lys Tyr His Ser Val
 225 230 235 240
 Val Lys Cys Cys Tyr Asp Gly Ala Cys Val Asn Asn Asp Glu Thr Cys
 245 250 255

-continued

Glu Gln Arg Ala Ala Arg Ile Ser Leu Gly Pro Arg Cys Ile Lys Ala
 260 265 270
 Phe Thr Glu Cys Cys Val Val Ala Ser Gln Leu Arg Ala Asn Ile Ser
 275 280 285
 His Lys Asp Met Gln Leu Gly Arg
 290 295

<210> SEQ ID NO 6
 <211> LENGTH: 240
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 6

Gln Val Lys Leu Leu Glu Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
 1 5 10 15
 Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Ser
 20 25 30
 Tyr Gly Leu His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp
 35 40 45
 Met Gly Trp Ile Ser Ala Gly Thr Gly Asn Thr Lys Tyr Ser Gln Lys
 50 55 60
 Phe Arg Gly Arg Val Thr Phe Thr Arg Asp Thr Ser Ala Thr Thr Ala
 65 70 75 80
 Tyr Met Gly Leu Ser Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr
 85 90 95
 Cys Ala Arg Asp Pro Tyr Gly Gly Gly Lys Ser Glu Phe Asp Tyr Trp
 100 105 110
 Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
 115 120 125
 Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
 130 135 140
 Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
 145 150 155 160
 Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
 165 170 175
 Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
 180 185 190
 Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
 195 200 205
 His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser
 210 215 220
 Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
 225 230 235 240

<210> SEQ ID NO 7
 <211> LENGTH: 295
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 7

Gln Ile Val Leu Ser Gln Ser Pro Ala Ile Leu Ser Ala Ser Pro Gly

-continued

1	5	10	15
Glu Lys Val Thr Met Thr Cys Arg Ala Ser Ser Ser Val Ser Tyr Ile	20	25	30
His Trp Phe Gln Gln Lys Pro Gly Ser Ser Pro Lys Pro Trp Ile Tyr	35	40	45
Ala Thr Ser Asn Leu Ala Ser Gly Val Pro Val Arg Phe Ser Gly Ser	50	55	60
Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Val Glu Ala Glu	65	70	75
Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Thr Ser Asn Pro Pro Thr	85	90	95
Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro	100	105	110
Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr	115	120	125
Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys	130	135	140
Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu	145	150	155
Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser	165	170	175
Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala	180	185	190
Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe	195	200	205
Asn Arg Gly Glu Cys Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser Gly	210	215	220
Met Leu Gln Lys Lys Ile Glu Glu Ile Ala Lys Tyr His Ser Val Val	225	230	235
Lys Cys Cys Tyr Asp Gly Ala Cys Val Asn Asn Asp Glu Thr Cys Glu	245	250	255
Gln Arg Ala Ala Arg Ile Ser Leu Gly Pro Arg Cys Ile Lys Ala Phe	260	265	270
Thr Glu Cys Cys Val Val Ala Ser Gln Leu Arg Ala Asn Ile Ser His	275	280	285
Lys Asp Met Gln Leu Gly Arg	290	295	

<210> SEQ ID NO 8
 <211> LENGTH: 451
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 8

Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys Pro Gly Ala	1	5	10	15
Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr	20	25	30	
Asn Met His Trp Val Lys Gln Thr Pro Gly Arg Gly Leu Glu Trp Ile	35	40	45	
Gly Ala Ile Tyr Pro Gly Asn Gly Asp Thr Ser Tyr Asn Gln Lys Phe				

-continued

50					55					60					
Lys 65	Gly	Lys	Ala	Thr	Leu 70	Thr	Ala	Asp	Lys	Ser 75	Ser	Thr	Ala	Tyr 80	
Met	Gln	Leu	Ser	Ser 85	Leu	Thr	Ser	Glu	Asp 90	Ser	Ala	Val	Tyr	Tyr 95	Cys
Ala	Arg	Ser	Thr 100	Tyr	Tyr	Gly	Gly	Asp 105	Trp	Tyr	Phe	Asn 110	Val	Trp	Gly
Ala	Gly	Thr 115	Thr	Val	Thr	Val	Ser 120	Ala	Ala	Ser	Thr	Lys 125	Gly	Pro	Ser
Val	Phe 130	Pro	Leu	Ala	Pro	Ser 135	Ser	Lys	Ser	Thr	Ser 140	Gly	Gly	Thr	Ala
Ala 145	Leu	Gly	Cys	Leu	Val 150	Lys	Asp	Tyr	Phe	Pro 155	Glu	Pro	Val	Thr	Val 160
Ser	Trp	Asn	Ser	Gly 165	Ala	Leu	Thr	Ser	Gly 170	Val	His	Thr	Phe	Pro 175	Ala
Val	Leu	Gln 180	Ser	Ser	Gly	Leu	Tyr 185	Ser	Leu	Ser	Ser	Val 190	Val	Thr	Val
Pro	Ser	Ser 195	Ser	Leu	Gly	Thr	Gln 200	Thr	Tyr	Ile	Cys	Asn 205	Val	Asn	His
Lys 210	Pro	Ser	Asn	Thr	Lys 215	Val	Asp	Lys	Lys	Ala	Glu 220	Pro	Lys	Ser	Cys
Asp 225	Lys	Thr	His	Thr	Cys 230	Pro	Pro	Cys	Pro	Ala 235	Pro	Glu	Leu	Leu	Gly 240
Gly	Pro	Asp	Val	Phe 245	Leu	Phe	Pro	Pro	Lys 250	Pro	Lys	Asp	Thr	Leu 255	Met
Ile	Ser	Arg 260	Thr	Pro	Glu	Val	Thr	Cys 265	Val	Val	Val	Asp 270	Val	Ser	His
Glu	Asp 275	Pro	Glu	Val	Lys	Phe	Asn 280	Trp	Tyr	Val	Asp	Gly 285	Val	Glu	Val
His 290	Asn	Ala	Lys	Thr	Lys 295	Pro	Arg	Glu	Glu	Gln 300	Tyr	Asn	Ser	Thr	Tyr
Arg 305	Val	Val	Ser	Val	Leu 310	Thr	Val	Leu	His	Gln 315	Asp	Trp	Leu	Asn	Gly 320
Lys	Glu	Tyr	Lys 325	Cys	Lys	Val	Ser	Asn	Lys 330	Ala	Leu	Pro	Ala	Pro 335	Glu
Glu	Lys	Thr	Ile 340	Ser	Lys	Ala	Lys	Gly 345	Gln	Pro	Arg	Glu 350	Pro	Gln	Val
Tyr	Thr 355	Leu	Pro	Pro	Ser	Arg	Asp 360	Glu	Leu	Thr	Lys	Asn 365	Gln	Val	Ser
Leu 370	Thr	Cys	Leu	Val	Lys 375	Gly	Phe	Tyr	Pro	Ser	Asp 380	Ile	Ala	Val	Glu
Trp 385	Glu	Ser	Asn	Gly 390	Gln	Pro	Glu	Asn	Asn	Tyr 395	Lys	Thr	Thr	Pro	Pro 400
Val	Leu	Asp	Ser	Asp 405	Gly	Ser	Phe	Phe	Leu 410	Tyr	Ser	Lys	Leu	Thr 415	Val
Asp	Lys	Ser	Arg 420	Trp	Gln	Gln	Gly	Asn 425	Val	Phe	Ser	Cys	Ser 430	Val	Met
His	Glu 435	Ala	Leu	His	Asn	His	Tyr 440	Thr	Gln	Lys	Ser	Leu 445	Ser	Leu	Ser
Pro	Gly 450	Lys													

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<210> SEQ ID NO 9
 <211> LENGTH: 214
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 9

Glu Leu Val Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15
 Asp Arg Val Asn Ile Ala Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
 20 25 30
 Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Arg Leu Leu Ile
 35 40 45
 Tyr Asp Ala Ser Asn Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65 70 75 80
 Glu Asp Phe Ala Ile Tyr Tyr Cys Gln Gln Phe Asn Ser Tyr Pro Leu
 85 90 95
 Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
 100 105 110
 Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
 115 120 125
 Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
 130 135 140
 Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
 145 150 155 160
 Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
 165 170 175
 Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
 180 185 190
 Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
 195 200 205
 Phe Asn Arg Gly Glu Cys
 210

<210> SEQ ID NO 10
 <211> LENGTH: 465
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 10

Gln Val Lys Leu Leu Glu Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
 1 5 10 15
 Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser Phe Thr Ser
 20 25 30
 Tyr Gly Leu His Trp Val Arg Gln Ala Pro Gly Gln Arg Leu Glu Trp
 35 40 45
 Met Gly Trp Ile Ser Ala Gly Thr Gly Asn Thr Lys Tyr Ser Gln Lys
 50 55 60
 Phe Arg Gly Arg Val Thr Phe Thr Arg Asp Thr Ser Ala Thr Thr Ala

-continued

65	70	75	80
Tyr Met Gly Leu Ser Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr	85	90	95
Cys Ala Arg Asp Pro Tyr Gly Gly Gly Lys Ser Glu Phe Asp Tyr Trp	100	105	110
Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro	115	120	125
Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr	130	135	140
Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr	145	150	155
Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro	165	170	175
Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr	180	185	190
Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn	195	200	205
His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser	210	215	220
Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu	225	230	235
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu	245	250	255
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser	260	265	270
His Glu Asn Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu	275	280	285
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr	290	295	300
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn	305	310	315
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro	325	330	335
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln	340	345	350
Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val	355	360	365
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val	370	375	380
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro	385	390	395
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr	405	410	415
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val	420	425	430
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu	435	440	445
Ala Ser Gln Leu Arg Ala Asn Ile Ser His Lys Asp Met Gln Leu Gly	450	455	460
Arg			
465			

-continued

<210> SEQ ID NO 11
 <211> LENGTH: 214
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 11

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Glu Leu Val Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1           5           10           15
Asp Arg Val Asn Ile Ala Cys Arg Ala Ser Gln Gly Ile Ser Ser Ala
20          25          30
Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Arg Leu Leu Ile
35          40          45
Tyr Asp Ala Ser Asn Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50          55          60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65          70          75          80
Glu Asp Phe Ala Ile Tyr Tyr Cys Gln Gln Phe Asn Ser Tyr Pro Leu
85          90          95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100         105         110
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115         120         125
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130         135         140
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145         150         155         160
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165         170         175
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180         185         190
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195         200         205
Phe Asn Arg Gly Glu Cys
210

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<210> SEQ ID NO 12
 <211> LENGTH: 230
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 12

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Met Leu Phe Gln Val Lys Leu Leu Glu Gln Ser Gly Ala Glu Val Lys
1           5           10           15
Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser
20          25          30
Phe Thr Ser Tyr Gly Leu His Trp Val Arg Gln Ala Pro Gly Gln Arg
35          40          45
Leu Glu Trp Met Gly Trp Ile Ser Ala Gly Thr Gly Asn Thr Lys Tyr
50          55          60
Ser Gln Lys Phe Arg Gly Arg Val Thr Phe Thr Arg Asp Thr Ser Ala

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<210> SEQ ID NO 13
<211> LENGTH: 231
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic
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<400> SEQUENCE: 13

Glu 1	Leu	Val	Met	Thr 5	Gln	Ser	Pro	Ser	Ser 10	Leu	Ser	Ala	Ser	Val 15	Gly
Asp	Arg	Val	Asn 20	Ile	Ala	Cys	Arg	Ala 25	Ser	Gln	Gly	Ile	Ser 30	Ser	Ala
Leu	Ala	Trp 35	Tyr	Gln	Gln	Lys	Pro 40	Gly	Lys	Ala	Pro	Arg 45	Leu	Leu	Ile
Tyr	Asp 50	Ala	Ser	Asn	Leu	Glu 55	Ser	Gly	Val	Pro	Ser 60	Arg	Phe	Ser	Gly
Ser 65	Gly	Ser	Gly	Thr 70	Asp	Phe	Thr	Leu	Thr	Ile 75	Ser	Ser	Leu	Gln 80	Pro
Glu	Asp	Phe	Ala 85	Ile	Tyr	Tyr	Cys	Gln	Gln 90	Phe	Asn	Ser	Tyr	Pro 95	Leu
Thr	Phe	Gly 100	Gly	Gly	Thr	Lys	Val	Glu 105	Ile	Lys	Arg	Thr 110	Val	Ala	Ala
Pro	Ser	Val 115	Phe	Ile	Phe	Pro	Pro 120	Ser	Asp	Glu	Gln	Leu 125	Lys	Ser	Gly
Thr 130	Ala	Ser	Val	Val	Cys	Leu 135	Leu	Asn	Asn	Phe	Tyr 140	Pro	Arg	Glu	Ala
Lys 145	Val	Gln	Trp	Lys 150	Val	Asp	Asn	Ala	Leu	Gln 155	Ser	Gly	Asn	Ser	Gln 160
Glu	Ser	Val	Thr 165	Glu	Gln	Asp	Ser	Lys	Asp 170	Ser	Thr	Tyr	Ser 175	Leu	Ser
Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	Tyr

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180	185	190
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser		
195	200	205
Phe Asn Arg Gly Glu Cys Ala Ser Gln Leu Arg Ala Asn Ile Ser His		
210	215	220
Lys Asp Met Gln Leu Gly Arg		
225	230	

<210> SEQ ID NO 14
 <211> LENGTH: 230
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 14

Met Leu Phe Gln Val Lys Leu Leu Glu Gln Ser Gly Ala Glu Val Lys		
1	5	10
Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Ser		
20	25	30
Phe Thr Ser Tyr Gly Leu His Trp Val Arg Gln Ala Pro Gly Gln Arg		
35	40	45
Leu Glu Trp Met Gly Trp Ile Ser Ala Gly Thr Gly Asn Thr Lys Tyr		
50	55	60
Ser Gln Lys Phe Arg Gly Arg Val Thr Phe Thr Arg Asp Thr Ser Ala		
65	70	75
Thr Thr Ala Tyr Met Gly Leu Ser Ser Leu Arg Pro Glu Asp Thr Ala		
85	90	95
Val Tyr Tyr Cys Ala Arg Asp Pro Tyr Gly Gly Gly Lys Ser Glu Phe		
100	105	110
Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr		
115	120	125
Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser		
130	135	140
Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu		
145	150	155
Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His		
165	170	175
Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser		
180	185	190
Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys		
195	200	205
Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu		
210	215	220
Pro Lys Ser Cys Asp Lys		
225	230	

<210> SEQ ID NO 15
 <211> LENGTH: 361
 <212> TYPE: PRT
 <213> ORGANISM: Artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic

<400> SEQUENCE: 15

-continued

Asn	Ser	Asp	Ser	Glu	Cys	Pro	Leu	Ser	His	Asp	Gly	Tyr	Cys	Leu	His
1				5					10					15	
Asp	Gly	Val	Cys	Met	Tyr	Ile	Glu	Ala	Leu	Asp	Lys	Tyr	Ala	Cys	Asn
			20					25					30		
Cys	Val	Val	Gly	Tyr	Ile	Gly	Glu	Arg	Cys	Gln	Tyr	Arg	Asp	Leu	Lys
		35					40					45			
Trp	Trp	Glu	Leu	Arg	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala
	50					55					60				
Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro
65					70					75				80	
Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val
				85					90					95	
Val	Asp	Val	Ser	His	Glu	Asn	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val
			100					105					110		
Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln
		115					120					125			
Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln
	130					135					140				
Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala
145					150					155				160	
Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro
				165					170					175	
Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr
			180					185					190		
Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser
		195					200					205			
Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr
	210					215					220				
Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr
225					230					235				240	
Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe
				245					250					255	
Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys
			260					265					270		
Ser	Leu	Ser	Leu	Gly	Ser	Gly	Ser	Gly	Ser	Gly	Ser	Gly	Ser	Gly	Met
		275				280						285			
Leu	Gln	Lys	Lys	Ile	Glu	Glu	Ile	Ala	Ala	Lys	Tyr	Lys	His	Ser	Val
	290					295					300				
Val	Lys	Lys	Cys	Cys	Tyr	Asp	Gly	Ala	Cys	Val	Asn	Asn	Asp	Glu	Thr
305					310					315				320	
Cys	Glu	Gln	Arg	Ala	Ala	Arg	Ile	Ser	Leu	Gly	Pro	Arg	Cys	Ile	Lys
				325					330					335	
Ala	Phe	Thr	Glu	Cys	Cys	Val	Val	Ala	Ser	Gln	Leu	Arg	Ala	Asn	Ile
			340					345					350		
Ser	His	Lys	Asp	Met	Gln	Leu	Gly	Arg							
		355					360								

<210> SEQ ID NO 16

<211> LENGTH: 77

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 16

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Ser Val Gln Leu Thr Glu Lys Arg Met Asp Lys Val Gly Lys Tyr Pro
1           5           10           15
Lys Glu Leu Arg Lys Cys Cys Glu Asp Gly Met Arg Glu Asn Pro Met
20          25          30
Arg Phe Ser Cys Gln Arg Arg Thr Arg Phe Ile Ser Leu Gly Glu Ala
35          40          45
Cys Lys Lys Val Phe Leu Asp Cys Cys Asn Tyr Ile Thr Glu Leu Arg
50          55          60
Arg Gln His Ala Arg Ala Ser His Leu Gly Leu Ala Arg
65          70          75

```

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<210> SEQ ID NO 17
<211> LENGTH: 77
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 17

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Asn Val Asn Phe Gln Lys Ala Ile Asn Glu Lys Leu Gly Gln Tyr Ala
1           5           10           15
Ser Pro Thr Ala Lys Arg Cys Cys Gln Asp Gly Val Thr Arg Leu Pro
20          25          30
Met Met Arg Ser Cys Glu Gln Arg Ala Ala Arg Val Gln Gln Pro Asp
35          40          45
Cys Arg Glu Pro Phe Leu Ser Cys Cys Gln Phe Ala Glu Ser Leu Arg
50          55          60
Lys Lys Ser Arg Asp Lys Gly Gln Ala Gly Leu Gln Arg
65          70          75

```

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<210> SEQ ID NO 18
<211> LENGTH: 74
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 18

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Met Leu Gln Lys Lys Ile Glu Glu Ile Ala Ala Lys Tyr Lys His Ser
1           5           10           15
Val Val Lys Lys Cys Cys Tyr Asp Gly Ala Cys Val Asn Asn Asp Glu
20          25          30
Thr Cys Glu Gln Arg Ala Ala Arg Ile Ser Leu Gly Pro Arg Cys Ile
35          40          45
Lys Ala Phe Thr Glu Cys Cys Val Val Ala Ser Gln Leu Arg Ala Asn
50          55          60
Ile Ser His Lys Asp Met Gln Leu Gly Arg
65          70

```

```

<210> SEQ ID NO 19
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

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<400> SEQUENCE: 19

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```

Gly Ser Gly Gly Ser
1           5

```

-continued

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<210> SEQ ID NO 20
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

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<400> SEQUENCE: 20

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```

Gly Gly Gly Gly Ser
1             5

```

```

<210> SEQ ID NO 21
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

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<400> SEQUENCE: 21

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```

Gly Gly Gly Ser
1

```

```

<210> SEQ ID NO 22
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic

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<400> SEQUENCE: 22

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Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser Gly
1             5             10

```

What is claimed is:

1. A chemoattractant-antibody conjugate comprising an antibody operably linked to a chemoattractant or fragment thereof, the chemoattractant selected from the group consisting of C5a, C4a, and C3a.

2. The chemoattractant-antibody conjugate of claim 1 wherein said chemoattractant is C5a or fragment thereof.

3. The chemoattractant-antibody conjugate of claim 2 wherein said chemoattractant comprises residues 58-74 of said C5a chemoattractant.

4. The chemoattractant-antibody conjugate of claim 2 wherein said C5a is operably linked to the antibody by a glycine-serine linker.

5. The chemoattractant-antibody conjugate of claim 4 wherein said C5a is operably linked to a light chain of said antibody.

6. The chemoattractant-antibody conjugate of claim 1 wherein said chemoattractant is C4a or fragment thereof.

7. The chemoattractant-antibody conjugate of claim 6 wherein said C4a is operably linked to the antibody by a glycine-serine linker.

8. The chemoattractant-antibody conjugate of claim 6 wherein said C4a is operably linked to a light chain of said antibody.

9. The chemoattractant-antibody conjugate of claim 1 wherein said chemoattractant is C3a or fragment thereof.

10. The chemoattractant-antibody conjugate of claim 9 wherein said C3a is operably linked to the antibody by a Gly-Ser linker.

11. The chemoattractant-antibody conjugate of claim 9 wherein said C3a is operably linked to a light chain of said antibody.

12. The chemoattractant-antibody conjugate of claim 1 wherein said antibody comprises a Fab fragment.

13. The chemoattractant-antibody conjugate of claim 1 wherein said antibody comprises an Fc fragment.

14. The chemoattractant-antibody conjugate of claim 16 wherein said chemoattractant is operably linked by a glycine-serine linker.

15. The chemoattractant-antibody conjugate of claim 16 wherein said chemoattractant is directly connected to the C-terminal amino acid of an antibody heavy chain.

* * * * *