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(54) **Title:** LEUKOTOXIN E/D AS A NEW ANTI-INFLAMMATORY AGENT AND MICROBICIDE

(57) **Abstract:** The present invention relates to methods for preventing or treating Human Immunodeficiency Virus (HIV) infection, inflammatory conditions, and graft-versus-host- disease (GVHD) in a subject. Therapeutic compositions of the present invention comprise Leukocidin E (LukE) and/or D proteins or polypeptides. The invention further relates to methods of treating *Staphylococcus aureus* infection by administering a composition comprising a CCR5 antagonist or any molecule that blocks LukE/D interaction with CCR5⁺ cells in an amount effective to treat the *S. aureus* infection in the subject.

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LEUKOTOXIN E/D AS A NEW ANTI-INFLAMMATORY AGENT AND MICROBICIDE

[0001] This application claims the priority benefit of U.S. Provisional Patent
5 Application Serial No. 61/498,606, filed June 19, 2011, which is hereby incorporated by
reference in its entirety.

FIELD OF THE INVENTION

[0002] This invention relates to methods of treating and preventing HIV infections.
10 This invention further relates to method of treating inflammatory conditions and
Staphylococcus aureus infections.

BACKGROUND OF THE INVENTION

Staphylococcus aureus

15 [0003] *Staphylococcus aureus* (“*S. aureus*”) is a bacterium that commensally
colonizes more than 25% of the human population. Importantly, this organism is capable
of breaching its initial site of colonization, resulting in bacterial dissemination and disease.
S. aureus is the leading cause of nosocomial infections, is the most common etiological
agent of infectious endocarditis as well as skin and soft tissue infections, and is one of the
20 four leading causes of food-borne illness. Altogether, *S. aureus* infects more than 1.2
million patients per year in U.S. hospitals. The threat of *S. aureus* to human health is
further highlighted by the emergence of antibiotic-resistant strains (i.e., methicillin-
resistant *S. aureus* (MRSA) strains), including strains that are resistant to vancomycin, an
antibiotic considered the last line of defense against *S. aureus* infection. These facts
25 highlight the importance of developing novel therapeutics against this important pathogen.

[0004] *S. aureus* produces a diverse array of virulence factors and toxins that
enable this bacterium to neutralize and withstand attack by different kinds of immune
cells, specifically subpopulations of white blood cells that make up the body’s primary
defense system. The production of these virulence factors and toxins allow *S. aureus* to
30 maintain an infectious state (Nizet, “Understanding How Leading Bacterial Pathogens
Subvert Innate Immunity to Reveal Novel Therapeutic Targets,” *J. Allergy Clin. Immunol.*

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120(1):13 22 (2007)). Among these virulence factors, *S. aureus* produces several bi-component leukotoxins, which damage membranes of host defense cells and erythrocytes by the synergistic action of two non-associated proteins or subunits (see Menestrina et al., “Mode of Action of Beta-Barrel Pore-Forming Toxins of the Staphylococcal Alpha-Hemolysin Family,” *Toxicol.* 39(11):1661-1672 (2001)). Among these bi-component leukotoxins, gamma-hemolysin (HlgAB and HlgCB) and the Pantone-Valentine Leukocidin (PVL) are the best characterized.

[0005] The toxicity of the leukocidins towards mammalian cells involves the action of two components. The first subunit is named class S-subunit (i.e., “slow-eluted”), and the second subunit is named class F-subunit (i.e., “fast-eluted”). The S-and F-subunits act synergistically to form pores on white blood cells including monocytes, macrophages, dendritic cells and neutrophils (collectively known as phagocytes) (Menestrina et al., “Mode of Action of Beta-Barrel Pore-Forming Toxins of the Staphylococcal Alpha-Hemolysin Family,” *Toxicol.* 39(11):1661 1672 (2001)). The mechanism by which the bi-component toxins form pores in target cell membranes is not entirely understood. The proposed mechanism of action of these toxins involves binding of the S-subunit to the target cell membrane, most likely through a receptor, followed by binding of the F-subunit to the S-subunit, thereby forming an oligomer which in turn forms a pre-pore that inserts into the target cell membrane (Jayasinghe et al., “The Leukocidin Pore: Evidence for an Octamer With Four LukF Subunits and Four LukS Subunits Alternating Around a Central Axis,” *Protein. Sci.* 14(10):2550 2561 (2005)). The pores formed by the bi-component leukotoxins are typically cation-selective. Pore formation causes cell death via lysis, which in the cases of the target white blood cells, has been reported to result from an osmotic imbalance due to the influx of cations (Miles et al., “The Staphylococcal Leukocidin Bicomponent Toxin Forms Large Ionic Channels,” *Biochemistry* 40(29):8514 8522 (2001)).

[0006] Designing effective therapy to treat MRSA infection has been especially challenging. In addition to the resistance to methicillin and related antibiotics, MRSA has also been found to have significant levels of resistance to macrolides (e.g., erythromycin), beta-lactamase inhibitor combinations (e.g., Unasyn, Augmentin) and fluoroquinolones (e.g. ciprofloxacin), as well as to clindamycin, trimethoprim/sulfamethoxisol (Bactrim), and rifampin. In the case of serious *S. aureus* infection, clinicians have resorted to

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intravenous vancomycin. However, there have been reports of *S. aureus* resistance to vancomycin. Thus, there is a need to develop new antibiotic drugs that effectively combat *S. aureus* infection.

C-C Chemokine Receptor Type 5

5 [0007] C-C chemokine receptor type 5 (CCR5) is a member of the beta chemokine receptors family (Samson M et al., "Molecular Cloning and Functional Expression of a New Human CC-Chemokine Receptor Gene" *Biochemistry* 35:3362 (1996)). The normal ligands for this receptor are RANTES, Mip1b, and Mip1a (*see* Samson, *supra* and Gon W et al "Monocyte Chemotactic Protein-2 Activates CCR5 and Blocks CD4/CCR5 Mediated

10 HIV-1 Entry/Replication," *J. Biol. Chem.* 273:4289 (1998)). CCR5 is expressed on a subset of T cells, macrophages, dendritic cells, natural killer cells, and microglia. CCR5⁺ T cells secrete pro-inflammatory cytokines and are recruited to sites of inflammation. Thus, it is likely that CCR5 plays a role in inflammatory responses to infection and in pathological conditions such as autoimmune diseases. CCR5 is also the receptor for major

15 strain of HIV (Deng H et al., "Identification of a Major Co-Receptor for Primary Isolates of HIV-1," *Nature* 381:661-666 (1996)). In individuals infected with HIV, CCR5-using viruses are the predominant species isolated during the early stages of viral infection, suggesting that these viruses may have a selective advantage during transmission or the acute phase of disease. Moreover, at least half of all infected individuals harbor only

20 CCR5-using viruses throughout the course of infection. Around 1% of Northern Europeans lack functional CCR5 expression, due to a 32 base pair deletion in this gene. Individuals with the $\Delta 32$ allele of CCR5 are healthy, suggesting that CCR5 is largely dispensable. However, these individuals have very strong resistance to HIV infection (Liu R et al., "Homozygous Defect in HIV-1 Coreceptor Accounts for Resistance of Some

25 Multiply-Exposed Individuals to HIV-1 Infection," *Cell* 86:367-377 (1996)). Indeed, an AIDS patient who had myeloid leukemia was treated with chemotherapy to suppress the cancer, which killed all of his T cells. The patient was then transplanted with a donor blood that had the 32 bp CCR5 deletion mutant to restore the immune system. After 600 days, the patient was healthy and had undetectable levels of HIV in the blood and in

30 examined brain and rectal tissues (Hütter G et al., "Long-Term Control of HIV by CCR5 Delta32/Delta32 Stem-Cell Transplantation," *N. Engl. J. Med.* 360:692-698 (2009)). A number of new experimental HIV drugs, called entry inhibitors have been designed to

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interfere with the interaction between CCR5 and HIV, including PRO140, Vicriviroc, Aploviroc, and Maraviroc (Pfizer), of which the latter is currently an approved drug for HIV infection.

[0008] CCR5 is also involved in uncontrolled inflammation (Charo et al., “The
5 Many Roles of Chemokine Receptors in Inflammation,” *N. Engl. J. Med.* 354:610-621
(2006)). This association is based on the role of this chemokine receptor in the
recruitment of inflammatory leukocytes. In particular, CCR5 is expressed in a subset of
effector T cells that produce proinflammatory cytokines such as interferon gamma (IFNg)
and interleukin-17 (IL-17), which are enriched locally during inflammation. Thus, CCR5
10 is being considered as a target to dampen inflammatory disorders, such as rheumatoid
arthritis (RA), Crohn’s Disease (CD), atherosclerosis, and psoriasis among others.

[0009] The present invention is directed to overcoming these and other limitations
in the art.

15

SUMMARY OF THE INVENTION

[0010] A first aspect of the present invention relates to a method of preventing or
treating Human Immunodeficiency Virus (HIV) infection in a subject. This method
involves administering a composition comprising an isolated Leukocidin E (LukE)
protein, or polypeptide thereof, and an isolated Leukocidin D (LukD) protein, or
20 polypeptide thereof in an amount effective to prevent or treat HIV infection in the subject.

[0011] Another aspect of the present invention relates to a method of preventing
HIV infection in a subject. This method involves providing a composition comprising an
isolated LukE protein, or polypeptide thereof, and an isolated LukD protein, or
polypeptide thereof, and contacting the tissue of the subject with the composition under
25 conditions effective to block HIV infectivity of cells in the tissue, thereby inhibiting HIV
infection of the subject.

[0012] Another aspect of the present invention relates to a composition comprising
a therapeutically effective amount of an isolated LukE protein or polypeptide thereof, an
isolated LukD protein or polypeptide thereof, or a combination thereof, and
30 one or more additional agents selected from the group consisting of a lubricant, an
antimicrobial agent, a humectant, an emulsifier, and a mixture of two or more thereof.

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[0013] Another aspect of the invention relates to a method of treating an inflammatory condition in a subject. This method involves administering a composition comprising an isolated LukE protein, or polypeptide thereof, and an isolated LukD protein, or polypeptide thereof, in an amount effective to treat an inflammatory condition in the
5 subject.

[0014] Another aspect of the present invention relates to a method of preventing graft-versus-host-disease (GVHD) in a subject. This method involves administering a composition comprising an isolated LukE protein, or polypeptide thereof, and an isolated LukD protein, or polypeptide thereof, in an amount effective to prevent graft-versus-host-
10 disease (GVHD) in the subject.

[0015] Another aspect of the present invention relates to a method of treating a *Staphylococcus aureus* infection in a subject. This method involves selecting a subject having a *S. aureus* infection and administering a composition comprising a CCR5 antagonist to the subject in an amount effective to treat the *S. aureus* infection in the
15 subject.

[0016] As demonstrated herein, applicants have found that the bi-component leukotoxin of *Staphylococcus aureus*, leukocidin E/D, mediates its cytotoxicity via the CCR5 receptor on the surface of leukocytes. Exploitation of this toxin-receptor interaction has a number of therapeutic implications. Firstly, since LukE/D significantly contributes
20 to the pathogenesis of *S. aureus* infections, CCR5 receptor antagonists offer a novel therapeutic approach to treat *S. aureus* infections, especially infections caused by MRSA strains. Secondly, due to its role in mediating HIV infectivity, a variety of CCR5 antagonists are being tested in clinical trials as anti-HIV drugs. Use of composition containing LukE and LukD to target latently infected cells in HIV-infected individuals
25 represents a superior therapeutic strategy compared to CCR5-antagonism, because use of this toxin will deplete all CCR5 positive cells, thereby eliminating HIV positive cells. A composition containing LukE and LukD can also be administered prophylactically to prevent the transmission of HIV by killing CCR5-positive cells that are required for HIV transmission. These therapeutic approaches are novel because they will eradicate HIV
30 cells or cells susceptible to HIV infection in a subject. Finally, since CCR5 is also involved in uncontrolled inflammation, use of a LukE/D composition to target and deplete CCR5 positive cells offers a new treatment modality to combat localized inflammatory

conditions. This treatment approach is highly targeted to the source of inflammation, thereby avoiding side effects often encountered with current anti-inflammatory strategies.

BRIEF DESCRIPTION OF THE DRAWINGS

5 [0017] Figures 1A–1B illustrate that Luke/D contributes to *S. aureus* infection in a mouse model of systemic infection. Figure 1A demonstrates that Luke/D is critical for the death of mice infected systemically with *S. aureus*. The survival of mice was monitored after intravenous injection with $\sim 1 \times 10^7$ CFU with *S. aureus* strain Newman wild type, a $\Delta lukE/D$ mutant, and the complemented $\Delta lukE/D::plukE/D$ strain. Total number of mice
10 per group were N=6. Statistical significance between survival curves was determined using the Log-rank (Mantel-Cox) test ($p \leq 0.0005$). Figure 1B demonstrates that Luke/D is required for *S. aureus* proliferation *in vivo*. The bacterial burden was determined by enumeration of bacterial CFU from kidneys 96 hours post-infection as described for Figure 1A. Statistical significance was determined using 1-Way ANOVA with Tukey's
15 multiple comparisons posttest (***, $p \leq 0.0005$).

[0018] Figures 2A–2B show that Luke/D is toxic to select human immune cell lines. Figure 2A demonstrates that Luke/D is selectively toxic to the monocyte-like cell line THP-1 and the T lymphocyte-like cell line Hut cells. Cytotoxicity was determined by a cell viability assay where indicated human immune cells lines were intoxicated with
20 different concentrations of an equimolar mixture of Luke + LukD (Luke/D). Cell viability was monitored 1 hour post-intoxication using CellTiter, where cells treated with medium were set at 100% viable. Results represent the average of triplicate samples \pm S.D. Figure 2B depicts that Luke/D kills Hut cell but not other human T lymphocyte-like cell lines. Indicated cell lines were intoxicated with different concentrations of an
25 equimolar mixture of Luke+LukD (Luke/D) and cell viability monitored as in Figure 2A. Results represent the average of triplicate samples \pm S.D.

[0019] Figure 3 illustrates that the chemokine receptor CCR5 is necessary and sufficient to renders mammalian cells susceptible to Luke/D mediated cytotoxicity. Parental Jurkat (top, left) and GHOST cells (bottom, left) or these cells transduced with a
30 CCR5 cDNA (Jurkat CCR5⁺, top/right; GHOST CCR5⁺, bottom/right), were intoxicated with Luke, LukD, or equimolar mixture of Luke+LukD (Luke/D). One hour post-

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intoxication cell viability was monitored with CellTiter, where cells treated with medium were set at 100% viable. Results represent the average of triplicate samples \pm S.D.

[0020] Figures 4A–4C show that LukeE/D cytotoxicity towards host cells is blocked by CCR5 inhibitors. Figure 4A demonstrates that CCR5-specific antagonist
5 potently block LukeE/D cytotoxicity towards CCR5⁺ cells. CCR5⁺ Jurkats were preincubated with different concentrations of Maraviroc (MVC), Vicriviroc (VVC), or TAK-779 (TAK) for 30 minutes followed by intoxication with an equimolar mixture of LukeE + LukD (LukeE/D). One hour post-intoxication, the percent death was determined by CellTiter where cells treated with media + LukeE/D was set to 100% cell death. Results
10 represent the average of triplicate samples \pm S.D. Figure 4B demonstrates that monoclonal antibodies directed towards CCR5 inhibit LukeE/D cytotoxicity towards CCR5⁺ cells. CCR5⁺ Jurkats were preincubated with indicated monoclonal antibodies for 30 minutes followed by intoxication with an equimolar mixture of LukeE + LukD (LukeE/D). One hour post-intoxication, the viability of the cells was determined by
15 CellTiter. Results represent the average of triplicate samples \pm S.D. Figure 4C demonstrates that CCR5 ligands inhibit LukeE/D cytotoxicity towards CCR5⁺ cells. CCR5⁺ Jurkats were preincubated with buffer (PBS; negative control) or different concentrations of the indicated ligands for 30 minutes followed by intoxication with an equimolar mixture of LukeE + LukD (LukeE/D). One hour post-intoxication, the viability of the cells was
20 determined by CellTiter. Results represent the average of triplicate samples \pm S.D.

[0021] Figures 5A-5C illustrate that blocking LukeE/D binding to the plasma membrane of target cells protects the cells from LukeE/D mediated cytotoxicity. Figure 5A demonstrates that LukeE/D binds to host cells in a CCR5-dependent manner and that this binding is potently inhibited by Maraviroc. Jurkat (CCR5⁻) and CCR5⁺ Jurkat (CCR5⁺)
25 cells were preincubated with buffer or with Maraviroc (CCR5⁺ + MVC) followed by incubation of an equimolar mixture of a green fluorescent protein (GFP) fused LukeE with LukD toxin (^{GFP}LukeE/D). Binding of the toxin to the plasma membrane of the cells was monitored via flow cytometry. Figures 5B demonstrates that LukeE/D forms pores in the plasma membrane of CCR5⁺ cells, which are potently blocked by Maraviroc. CCR5⁺
30 Jurkat cells were pre-incubated with Maraviroc (MVC) and subsequently intoxicated with an equimolar mixture of LukeE + LukD (LukeE/D) in the presence of ethidium bromide. Pore formation was measured over-time by monitoring ethidium bromide incorporation.

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Results represent the average of triplicate samples \pm S.D. Figure 5C show that pore formation by LukeE/D is associated with cell swelling, a cytophatic effect potentially inhibited by Maraviroc. CCR5⁺ Jurkat cells were pre-incubated with buffer (NO MVC) or with Maraviroc (MVC) and subsequently intoxicated with an equimolar mixture of LukeE + LukD (LukeE/D) in the presence of ethidium bromide. Intoxicated cells were monitored by light (top panels) and by fluorescence microscopy to determine ethidium bromide uptake. Representative images are shown.

[0022] Figures 6A–C show that LukeE/D potentially kills CCR5⁺ primary human immune cells. Figure 6A demonstrates that LukeE/D targets primary human T lymphocytes in a CCR5-dependent manner. T cells from human peripheral blood mononuclear cells (PBMC) from wild type *CCR5* and a $\Delta 32$ *CCR5* donor were expanded *in vitro* and subsequently incubated with media (negative control), an equimolar mixture of LukeE + LukD (LukeE/D), or with Maraviroc (MVC) followed by intoxication with an equimolar mixture of LukeE + LukD (LukeE/D). Cells were then stained with an anti-CCR5 antibody and a viability dye prior analysis by flow cytometry. Figures 6B–6C demonstrate that LukeE/D is cytotoxic towards primary human macrophages (Figure 6B) and primary human dendritic cells (Figure 6C) and that Maraviroc potentially protects these cells from LukeE/D mediated cytotoxicity. Macrophages and dendritic cells were incubated with media (negative control), an equimolar mixture of LukeE + LukD (LukeE/D), or with Maraviroc (MVC) followed by intoxication with an equimolar mixture of LukeE + LukD (LukeE/D). One hour post-intoxication, the percent death was determined by flow cytometry.

DETAILED DESCRIPTION OF THE INVENTION

[0023] A first aspect of the present invention relates to a composition comprising a therapeutically effective amount of an isolated LukeE protein or polypeptide thereof, an isolated LukD protein or polypeptide thereof, and a pharmaceutically acceptable carrier.

[0024] In accordance with this aspect of the invention, suitable isolated LukeE proteins include those derived from any strain of *S. aureus*. The amino acid sequence of LukeE proteins from various strains of *S. aureus* that are suitable for the composition of the present invention are shown in the Table 1 below (*i.e.*, SEQ ID Nos: 1–10). SEQ ID NO: 11 of Table 1 is a LukeE consensus sequence demonstrating the high level of sequence

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identity across LukE proteins of various *S. aureus* strains. Accordingly, in one embodiment of the present invention, the isolated LukE protein comprises an amino acid sequence of SEQ ID NO:11. In another embodiment of the present invention, the isolated LukE protein comprises an amino acid sequence having about 70–80% sequence similarity to SEQ ID NO:11, more preferably, about 80–90% sequence similarity to SEQ ID NO:11, and more preferably 90–95% sequence similarity to SEQ ID NO:11, and most preferably about 95–99% sequence similarity to SEQ ID NO:11.

[0025] In another embodiment of the present invention, the composition comprises an isolated polypeptide of LukE. Suitable LukE polypeptides are about 50 to about 100 amino acids in length. More preferably LukE polypeptides are between about 100–200 amino acids in length, more preferably between about 200–250 amino acids in length, and most preferably between 250–300 amino acids in length. The N-terminal amino acid residues of the full-length LukE represent the native secretion/signal sequence. Thus, the “mature” secreted form of LukE is represented by amino acid residues 29–311 in each of SEQ ID NOs: 1–10 and SEQ ID NO:11. Correspondingly, amino acid residues 1–311 in each of SEQ ID NOs: 1–10 and SEQ ID NO:11 are referred to as the “immature” form of LukE. Accordingly, in one embodiment of the present invention, the LukE polypeptide comprises amino acid residues 29–311 of SEQ ID NO:11., amino acid residues 48–291 of SEQ ID NO:11, amino acid residues 29–301 of SEQ ID NO:11, and amino acids 48–301 of SEQ ID NO:11. In either case, suitable LukE polypeptides also include those polypeptides comprising an amino acid sequence having about 70–80% sequence similarity, preferably 80–90% sequence similarity, more preferably 90–95% sequence similarity, and most preferably 95–99% sequence similarity to amino acid residues 29–311 of SEQ ID NO:11 or 48–291 of SEQ ID NO: 11.

[0026] In accordance with this aspect of the invention, suitable isolated LukD proteins include those proteins derived from any strain of *S. aureus*. The amino acid sequence of LukD proteins from various strains of *S. aureus* that are suitable for the composition of the present invention are shown in the Table 2 below (*i.e.*, SEQ ID Nos: 12–21). SEQ ID NO: 22 of Table 2 is a LukD consensus sequence demonstrating the high level of sequence identity across LukD proteins of various *S. aureus* strains. Accordingly, in one embodiment of the present invention, the isolated LukD protein comprises an amino acid sequence of SEQ ID NO:22. In another embodiment of the present invention,

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the isolated LukD protein comprises an amino acid sequence having about 70–80% sequence similarity to SEQ ID NO:22, preferably, about 80–90% sequence similarity to SEQ ID NO:22, and more preferably 90–95% sequence similarity to SEQ ID NO:22, and most preferably about 95–99% sequence similarity to SEQ ID NO:22.

5 [0027] In another embodiment of the present invention, the composition comprises an isolated polypeptide of LukD. Suitable LukD polypeptides are about 50 to about 100 amino acids in length. More preferably LukD polypeptides are between about 100–200 amino acids in length, more preferably between about 200–250 amino acids in length, and most preferably between 250–300 amino acids in length. The N-terminal amino acid
10 residues of the full length LukD represent the native secretion/signal sequence. Thus, the mature secreted form of LukD is represented by amino acid residues 27–327 in each of SEQ ID NOs: 12-21 and SEQ ID NO: 22. Correspondingly, amino acid residues 1–327 of SEQ ID NOs: 12-21 and SEQ ID NO: 22 are referred to as the “immature” form of LukD. Accordingly, in one embodiment of the present invention, the LukD polypeptide
15 comprises amino acid residues 27–327 of SEQ ID NO:22. Alternatively, the LukD polypeptide of the present invention comprises amino acid residues 46–307, amino acid residues 27-312, and amino acid residues 46-312 of SEQ ID NO:22. In either case, suitable polypeptides also include those polypeptide comprising an amino acid sequence having about 70–80% sequence similarity, preferably 80–90% sequence similarity, more
20 preferably 90–95% sequence similarity, and most preferably 95–99% sequence similarity to amino acid residues 27-327 of SEQ ID NO:22, amino acid residues of 46-307 of SEQ ID NO:22, amino acid residues of 46-312 of SEQ ID NO:22, or amino acid residues of 27-312 of SEQ ID NO:22.

USA_300_TCH1516 SAHDRYL FVQSPNGPTGSAREYFAPDNQLPPLVQSGFNPSFITLTSHEKG 250
 N315 SAHDRYL FVQSPNGPTGSAREYFAPDNQLPPLVQSGFNPSFITLTSHEKG 250
 D30 SAHDRYL FVQSPNGPTGSAREYFAPDNQLPPLVQSGFNPSFITLTSHEKG 250
 Mu50 SAHDRYL FVQSPNGPTGSAREYFAPDNQLPPLVQSGFNPSFITLTSHEKG 250
 TCH_70 SAHDRYL FVQSPNGPTGSAREYFAPDNQLPPLVQSGFNPSFITLTSHEKG 250
 MRSA131 SAHDRYL FVQSPNGPTGSAREYFAPDNQLPPLVQSGFNPSFITLTSHEKG 250

5

Luke Consensus Sequence

10

SUBSTITUTE SHEET (RULE 26)

Newman SS DTSEFEISYGRNLDI TYATLFPRTGIYAERKHNAFVNRNFVRYEVNW 300
 MW2 SS DTSEFEISYGRNLDI TYATLFPRTGIYAERKHNAFVNRNFVRYEVNW 300
 USA_300_FPR3757 SS DTSEFEISYGRNLDI TYATLFPRTGIYAERKHNAFVNRNFVRYEVNW 300
 COL SS DTSEFEISYGRNLDI TYATLFPRTGIYAERKHNAFVNRNFVRYEVNW 300
 USA_300_TCH1516 SS DTSEFEISYGRNLDI TYATLFPRTGIYAERKHNAFVNRNFVRYEVNW 300
 N315 SS DTSEFEISYGRNLDI TYATLFPRTGIYAERKHNAFVNRNFVRYEVNW 300
 D30 SS DTSEFEISYGRNLDI TYATLFPRTGIYAERKHNAFVNRNFVRYEVNW 300
 Mu50 SS DTSEFEISYGRNLDI TYATLFPRTGIYAERKHNAFVNRNFVRYEVNW 300
 TCH_70 SS DTSEFEISYGRNLDI TYATLFPRTGIYAERKHNAFVNRNFVRYEVNW 300
 MRSA131 SS DTSEFEISYGRNLDI TYATLFPRTGIYAERKHNAFVNRNFVRYEVNW 300

20

Luke Consensus Sequence

25

KTHEIKVKGHN 311
 KTHEIKVKGHN 311
 KTHEIKVKGHN 311
 KTHEIKVKGHN 311
 KTHEIKVKGHN 311
 KTHEIKVKGHN 311
 KTHEIKVKGHN 311
 KTHEIKVKGHN 311
 KTHEIKVKGHN 311

30

TCH_70
 MRSA131
 KTHEIKVKGHN 311
 KTHEIKVKGHN 311

 Luke Consensus Sequence KTHEIKVKGHN

5

→ Depicts the start of the secreted Luke protein

10 **Table 2 – LukD Amino Acid Sequence Alignment**



15	Newman	MKMKKLVKSSVASSIALLLLSNTVDAAQHI	TPVSEKKVDDKI	TLYKTTAT	50	SEQ	ID	NO:12
	MW2	MKMKKLVKSSVASSIALLLLSNTVDAAQHI	TPVSEKKVDDKI	TLYKTTAT	50	SEQ	ID	NO:13
	USA_300_FPR3757	MKMKKLVKSSVASSIALLLLSNTVDAAQHI	TPVSEKKVDDKI	TLYKTTAT	50	SEQ	ID	NO:14
	COL	MKMKKLVKSSVASSIALLLLSNTVDAAQHI	TPVSEKKVDDKI	TLYKTTAT	50	SEQ	ID	NO:15
	USA_300_TCH1516	MKMKKLVKSSVASSIALLLLSNTVDAAQHI	TPVSEKKVDDKI	TLYKTTAT	50	SEQ	ID	NO:16
	MRSA131	MKMKKLVKSSVASSIALLLLSNTVDAAQHI	TPVSEKKVDDKI	TLYKTTAT	50	SEQ	ID	NO:17
20	TCH_70	MKMKKLVKSSVASSIALLLLSNTVDAAQHI	TPVSEKKVDDKI	TLYKTTAT	50	SEQ	ID	NO:18
	D30	MKMKKLVKSSVASSIALLLLSNTVDAAQHI	TPVSEKKVDDKI	TLYKTTAT	50	SEQ	ID	NO:19
	N315	MKMKKLVKSSVASSIALLLLSNTVDAAQHI	TPVSEKKVDDKI	TLYKTTAT	50	SEQ	ID	NO:20
	Mu50	MKMKKLVKSSVASSIALLLLSNTVDAAQHI	TPVSEKKVDDKI	TLYKTTAT	50	SEQ	ID	NO:21
		*****	*****	*****	50	SEQ	ID	NO:22
25	LukD Consensus Sequence	MKMKKLVKSSVASSIALLLLSNTVDAAQHI	TPVSEKKVDDKI	TLYKTTAT	50	SEQ	ID	NO:22
	Newman	SDNDKLNISQILTFNFIKDKSYDKDTLVLKAAGNINSGYKKPNPKDYNYS			100			
	MW2	SDNDKLNISQILTFNFIKDKSYDKDTLVLKAAGNINSGYKKPNPKDYNYS			100			
	USA_300_FPR3757	SDNDKLNISQILTFNFIKDKSYDKDTLVLKAAGNINSGYKKPNPKDYNYS			100			
	COL	SDNDKLNISQILTFNFIKDKSYDKDTLVLKAAGNINSGYKKPNPKDYNYS			100			
30	USA_300_TCH1516	SDNDKLNISQILTFNFIKDKSYDKDTLVLKAAGNINSGYKKPNPKDYNYS			100			
	MRSA131	SDNDKLNISQILTFNFIKDKSYDKDTLVLKAAGNINSGYKKPNPKDYNYS			100			

TCH_70 SDNDKLNISQILTFNFIKDKSYDKDTLVLKAAGNINSGYKKNPKDYNYS 100
 D30 SDNDKLNISQILTFNFIKDKSYDKDTLVLKAAGNINSGYKKNPKDYNYS 100
 N315 SDNDKLNISQILTFNFIKDKSYDKDTLVLKAAGNINSGYKKNPKDYNYS 100
 Mu50 SDNDKLNISQILTFNFIKDKSYDKDTLVLKAAGNINSGYKKNPKDYNYS 100

 LukD Consensus Sequence SDNDKLNISQILTFNFIKDKSYDKDTLVLKAAGNINSGYKKNPKDYNYS

5

Newman QFYWGGKYNVSVSSESNDAVNVDYAPKNQNEEFQVQQTLGYSYGGDINI 150
 MW2 QFYWGGKYNVSVSSESNDAVNVDYAPKNQNEEFQVQQTLGYSYGGDINI 150
 USA_300_FPR3757 QFYWGGKYNVSVSSESNDAVNVDYAPKNQNEEFQVQQTLGYSYGGDINI 150
 COL QFYWGGKYNVSVSSESNDAVNVDYAPKNQNEEFQVQQTLGYSYGGDINI 150
 USA_300_TCH1516 QFYWGGKYNVSVSSESNDAVNVDYAPKNQNEEFQVQQTLGYSYGGDINI 150
 MRSA131 QFYWGGKYNVSVSSESNDAVNVDYAPKNQNEEFQVQQTLGYSYGGDINI 150
 TCH_70 QFYWGGKYNVSVSSESNDAVNVDYAPKNQNEEFQVQQTLGYSYGGDINI 150
 D30 QFYWGGKYNVSVSSESNDAVNVDYAPKNQNEEFQVQQTLGYSYGGDINI 150
 N315 QFYWGGKYNVSVSSESNDAVNVDYAPKNQNEEFQVQQTLGYSYGGDINI 150
 Mu50 QFYWGGKYNVSVSSESNDAVNVDYAPKNQNEEFQVQQTLGYSYGGDINI 150

 LukD Consensus Sequence QFYWGGKYNVSVSSESNDAVNVDYAPKNQNEEFQVQQTLGYSYGGDINI

20

Newman SNLSSGGLNGSKSFSETINYPKQESYRTTIDRKTNHKSIWGVEAHKIMNN 200
 MW2 SNLSSGGLNGSKSFSETINYPKQESYRTTIDRKTNHKSIWGVEAHKIMNN 200
 USA_300_FPR3757 SNLSSGGLNGSKSFSETINYPKQESYRTTIDRKTNHKSIWGVEAHKIMNN 200
 COL SNLSSGGLNGSKSFSETINYPKQESYRTTIDRKTNHKSIWGVEAHKIMNN 200
 USA_300_TCH1516 SNLSSGGLNGSKSFSETINYPKQESYRTTIDRKTNHKSIWGVEAHKIMNN 200
 MRSA131 SNLSSGGLNGSKSFSETINYPKQESYRTTIDRKTNHKSIWGVEAHKIMNN 200
 TCH_70 SNLSSGGLNGSKSFSETINYPKQESYRTTIDRKTNHKSIWGVEAHKIMNN 200
 D30 SNLSSGGLNGSKSFSETINYPKQESYRTTIDRKTNHKSIWGVEAHKIMNN 200
 N315 SNLSSGGLNGSKSFSETINYPKQESYRTTIDRKTNHKSIWGVEAHKIMNN 200

30

Mu50 SNGLSGLNGSKSFSETIN YKQESYRTTIDRKTNHKSIGWGEA HKIMNN 200

LukD Consensus Sequence SNGLSGLNGSKSFSETIN YKQESYRTTIDRKTNHKSIGWGEA HKIMNN

5

Newman GWGPGRDSYDPTYGNE LFLGGRQSSSNAGQNF LPTHQMP LLLARGNFNPE 250
MW2 GWGPGRDSYDPTYGNE LFLGGRQSSSNAGQNF LPTHQMP LLLARGNFNPE 250
USA_300_FPR3757 GWGPGRDSYDPTYGNE LFLGGRQSSSNAGQNF LPTHQMP LLLARGNFNPE 250
COL GWGPGRDSYDPTYGNE LFLGGRQSSSNAGQNF LPTHQMP LLLARGNFNPE 250
USA_300_TCH1516 GWGPGRDSYDPTYGNE LFLGGRQSSSNAGQNF LPTHQMP LLLARGNFNPE 250
MRSA131 GWGPGRDSYDPTYGNE LFLGGRQSSSNAGQNF LPTHQMP LLLARGNFNPE 250
TCH_70 GWGPGRDSYDPTYGNE LFLGGRQSSSNAGQNF LPTHQMP LLLARGNFNPE 250
D30 GWGPGRDSYDPTYGNE LFLGGRQSSSNAGQNF LPTHQMP LLLARGNFNPE 250
N315 GWGPGRDSYDPTYGNE LFLGGRQSSSNAGQNF LPTHQMP LLLARGNFNPE 250
Mu50 GWGPGRDSYDPTYGNE LFLGGRQSSSNAGQNF LPTHQMP LLLARGNFNPE 250

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15

LukD Consensus Sequence GWGPGRDSYDPTYGNE LFLGGRQSSSNAGQNF LPTHQMP LLLARGNFNPE

Newman FISVL SHKQNDTKKSKI KVTYQREMDRY TNQWNRLLHWVGN NYKNQNTVTF 300
MW2 FISVL SHKQNDTKKSKI KVTYQREMDRY TNQWNRLLHWVGN NYKNQNTVTF 300
USA_300_FPR3757 FISVL SHKQNDTKKSKI KVTYQREMDRY TNQWNRLLHWVGN NYKNQNTVTF 300
COL FISVL SHKQNDTKKSKI KVTYQREMDRY TNQWNRLLHWVGN NYKNQNTVTF 300
USA_300_TCH1516 FISVL SHKQNDTKKSKI KVTYQREMDRY TNQWNRLLHWVGN NYKNQNTVTF 300
MRSA131 FISVL SHKQNDTKKSKI KVTYQREMDRY TNQWNRLLHWVGN NYKNQNTVTF 300
TCH_70 FISVL SHKQNDTKKSKI KVTYQREMDRY TNQWNRLLHWVGN NYKNQNTVTF 300
D30 FISVL SHKQNDTKKSKI KVTYQREMDRY TNQWNRLLHWVGN NYKNQNTVTF 300
N315 FISVL SHKQNDTKKSKI KVTYQREMDRY TNQWNRLLHWVGN NYKNQNTVTF 300
Mu50 FISVL SHKQNDTKKSKI KVTYQREMDRY TNQWNRLLHWVGN NYKNQNTVTF 300

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25

LukD Consensus Sequence FISVL SHKQNDTKKSKI KVTYQREMDRY TNQWNRLLHWVGN NYKNQNTVTF

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[0028] Thus, unless indicated to the contrary, both the immature and the mature forms of native LukE and LukD, and the sequences having less than 100% similarity with native LukE (i.e., native sequences and analogs alike, collectively referred to herein as “LukE” and “LukD”) may be used in the methods of the present invention.

5 [0029] LukE and LukD proteins and polypeptides of the invention may differ from the native polypeptides designated as SEQ ID NOS:1-11 and 12-22 respectively, in terms of one or more additional amino acid insertions, substitutions or deletions, *e.g.*, one or more amino acid residues within SEQ ID NOS:1-22 may be substituted by another amino acid of a similar polarity, which acts as a functional equivalent, resulting in a silent
10 alteration. That is to say, the change relative to the native sequence would not appreciably diminish the basic properties of native LukE or LukD. Any such analog of LukE or LukD may be screened in accordance with the protocols disclosed herein (*e.g.*, the cell toxicity assay and the membrane damage assay) to determine if it maintains native LukE or LukD activity. Substitutions within these leukocidins may be selected from other members of
15 the class to which the amino acid belongs. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. Polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. Positively charged (basic) amino acids include arginine, lysine and histidine. Negatively charged (acidic) amino acids include aspartic
20 acid and glutamic acid.

[0030] In other embodiments, non-conservative alterations (*e.g.*, one or amino acid substitutions, deletions and/or additions) can be made for purposes of increasing the selectivity and/or activity of LukE and/or LukD. The modified LukE and LukD may be used in the therapeutic compositions described herein. Molecular alterations can be
25 accomplished by methods well known in the art, including primer extension on a plasmid template using single stranded templates (Kunkel et al., *Proc. Acad. Sci., USA* 82:488-492 (1985), which is hereby incorporated by reference in its entirety), double stranded DNA templates (Papworth et al., *Strategies* 9(3):3-4 (1996), which is hereby incorporated by reference in its entirety), and by PCR cloning (Braman, J. (ed.), *IN VITRO*
30 MUTAGENESIS PROTOCOLS, 2nd ed. Humana Press, Totowa, N.J. (2002), which is hereby incorporated by reference in its entirety). Methods of determining whether a given molecular alteration in LukE and LukD alters LukE/D cytotoxicity are described herein.

[0031] In a preferred embodiment of the present invention, a highly purified LukE/LukD preparation is utilized. Methods of purifying LukE and LukD toxins are known in the art (Gravet et al., "Characterization of a Novel Structural Member, LukE-LukD, of the Bi-Component Staphylococcal Leucotoxins Family," *FEBS* 436: 202–208 (1998), which is hereby incorporated by reference in its entirety). As used herein, "isolated" protein or polypeptide refers to a protein or polypeptide that has been separated from other proteins, lipids, and nucleic acids with which it is naturally associated with. Purity can be measured by any appropriate standard method, for example, by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis. An isolated protein or polypeptide of the invention can be purified from a natural source, produced by recombinant DNA techniques, or by chemical methods.

[0032] The therapeutic compositions of the present invention are prepared by formulating LukE and LukD with a pharmaceutically acceptable carrier and optionally a pharmaceutically acceptable excipient. As used herein, the terms "pharmaceutically acceptable carrier" and "pharmaceutically acceptable excipient" (e.g., additives such as diluents, immunostimulants, adjuvants, antioxidants, preservatives and solubilizing agents) are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Examples of pharmaceutically acceptable carriers include water, e.g., buffered with phosphate, citrate and another organic acid. Representative examples of pharmaceutically acceptable excipients that may be useful in the present invention include antioxidants such as ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; adjuvants (selected so as to avoid adjuvant-induced toxicity, such as a β -glucan as described in U.S. Patent 6,355,625 to Pavliak et al., which is hereby incorporated by reference in its entirety, or a granulocyte colony stimulating factor (GCSF)); hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt forming counterions such as sodium; and/or nonionic surfactants such as TWEEN[®], polyethylene glycol (PEG), and PLURONICS[®].

[0033] Therapeutic compositions of the present invention may be prepared for storage by mixing the active ingredient(s) having the desired degree of purity with the

pharmaceutically acceptable carrier and optional excipient and/or additional active agent, in the form of lyophilized formulations or aqueous solutions.

[0034] Another aspect of the present invention relates to a method of preventing or treating Human Immunodeficiency Virus (HIV) infection in a subject. This method
5 involves administering a composition comprising an isolated LukE protein, or polypeptide thereof, and an isolated LukD protein, or polypeptide thereof in an amount effective to prevent or treat HIV infection in the subject.

[0035] In accordance with this aspect of the invention a suitable composition for administration to a subject to treat HIV infection contains both LukE and LukD proteins or
10 polypeptides that retain receptor binding and the cytotoxic function of the full-length LukE or LukD proteins. A suitable composition for administration to a subject to prevent HIV infection contains both LukE and LukD proteins or polypeptides that retain receptor binding functionality and retain cytotoxicity. In another embodiment of the present invention, LukE and LukD proteins retain receptor binding function but are not cytotoxic
15 or have reduced cytotoxicity.

[0036] In accordance with this aspect of the invention, suitable LukE and LukD proteins and polypeptides include those described *supra*. This aspect of the invention is based on the applicants' discovery that LukE/D binds to the CCR5 receptor of leukocytes, which mediates HIV cell entry and infectivity. LukE/D binding to CCR5 mediates
20 LukE/D cytotoxicity. Therefore, when treating a subject having HIV, LukE and LukD proteins or polypeptides of the composition bind to the CCR5 receptor and cause cell death of all HIV positive cells. This method of treatment is superior to current HIV therapeutic strategies because LukE/D treatment will selectively and specifically deplete all CCR5 positive, and therefore, all HIV positive cells in a subject.

[0037] When administering the LukE/D composition of the invention to prevent
25 HIV infection in a subject, the LukE and LukD proteins or polypeptides are preferably modified to reduce cytotoxicity as described *supra* and/or to enhance LukE/LukD receptor binding. Accordingly, the composition may comprise a modified LukE or LukD protein or polypeptide that retains at least 70% sequence similarity to SEQ ID NOs: 11 and 22,
30 respectively. Preferably, the LukE and LukD proteins or polypeptides of the invention retain at least 80% sequence similarity to SEQ ID NOs: 11 and 22, respectively. More preferably, the LukE and LukD proteins or polypeptides of the invention retain at least 90% sequence similarity to SEQ ID NOs: 11 and 22, respectively. Most preferably, the

LukE and LukD proteins or polypeptides of the invention retain at least 95% sequence similarity to SEQ ID NOs: 11 and 22, respectively.

[0038] The therapeutic compositions of the present invention can be administered as part of a combination therapy in conjunction with another anti-HIV agent. Accordingly, 5 the composition comprising an isolated LukE protein, or polypeptide thereof, and an isolated LukD protein, or polypeptide thereof may further comprise or be administered in combination with one or more antiviral or other agents useful in the treatment of HIV. Suitable antiviral agents include nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors and protease inhibitors. More specifically, 10 suitable antiviral agents include, without limitation, zidovudine, lamivudine, zalcitabine, didanosine, stavudine, abacavir, adefovir dipivoxil, lobucavir, BC H-10652, emtricitabine, beta-L-FD4, DAPD, lodenosine, nevirapine, delaviridine, efavirenz, PNU-142721, AG-1549, MKC-442, (+)-calanolide A and B, saquinavir, indinavir, ritonavir, nelfinavir, lasinavir, DMP-450, BMS-2322623, ABT-378, amprenavir, hydroxyurea, 15 ribavirin, IL-2, IL-12, pentafuside, Yissum No. 1 1607 and AG-1549.

[0039] For purposes of this and other aspects of the invention, the target "subject" encompasses any animal, preferably a mammal, more preferably a human. In the context of administering a composition of the invention for purposes of preventing HIV infection in a subject, the target subject encompasses any subject that is at risk for being infected by 20 HIV. In the context of administering a composition of the invention for purposes of treating HIV infection in a subject, the target subject encompasses any subject infected with HIV.

[0040] In the context of using therapeutic compositions of the present invention to treat an HIV infection, a therapeutically effective amount of LukE and LukD is that 25 amount capable of achieving a reduction in symptoms associated with infection, a decrease in the severity of at least one symptom, a decrease in the viral load of the subject, and preferably a complete eradication of the virus from the subject.

[0041] Therapeutically effective amounts of a LukE and LukD composition can be determined in accordance with standard procedures, which take numerous factors into 30 account, including, for example, the concentrations of these active agents in the composition, the mode and frequency of administration, the severity of the HIV infection to be treated (or prevented), and subject details, such as age, weight and overall health and immune condition. General guidance can be found, for example, in the publications of the

International Conference on Harmonization and in REMINGTON'S PHARMACEUTICAL SCIENCES (Mack Publishing Company 1990), which is hereby incorporated by reference in its entirety. A clinician may administer a composition containing LukE and LukD proteins or polypeptides, until a dosage is reached that provides the desired or required prophylactic or therapeutic effect. The progress of this therapy can be easily monitored by conventional assays.

[0042] Therapeutic compositions of the present invention may be administered in a single dose, or in accordance with a multi-dosing protocol. For example, in a multi-dosing protocol, the therapeutic composition may be administered once or twice daily, weekly, or monthly depending on the use and severity of the condition being treated. Different dosages, timing of dosages, and relative amounts of the therapeutic composition can be selected and adjusted by one of ordinary skill in the art. Modes of administration of the therapeutic compositions of the present invention are described *infra*.

[0043] Another aspect of the present invention relates to a method of preventing HIV infection of a subject. This method involves providing a composition comprising an isolated LukE protein, or polypeptide thereof, and an isolated LukD protein, or polypeptide thereof, and contacting the tissue of the subject with the composition under conditions effective to block HIV infectivity of cells in the tissue, thereby inhibiting HIV infection of the subject.

[0044] In accordance with this aspect of the invention, the composition comprising LukE and LukD serves as an anti-HIV microbicide, killing cells that are susceptible to HIV infection before infection occurs. The composition can be administered to any female or a male subject that is at risk for exposure to HIV as a prophylactic means of preventing HIV infection.

[0045] In accordance with this aspect of the invention, the LukE and LukD containing compositions of the present invention may further comprise one or more one or more additional agents. The one or more additional agents include, for example, and without limitation, a lubricant, an anti-microbial agent, an antioxidant, a humectant, an emulsifier, a spermicidal agent, or a mixture of two or more thereof.

[0046] Suitable lubricants include, without limitation, cetyl esters wax, hydrogenated vegetable oil, magnesium stearate, methyl stearate, mineral oil, polyoxyethylene-polyoxypropylene copolymer, polyethylene glycol, polyvinyl alcohol, sodium lauryl sulfate or white wax, or a mixture of two or more thereof. Suitable

antimicrobial agents include, without limitation, propylene glycol, methyl paraben or propyl paraben, or a mixture of two or more thereof. Suitable antioxidants include, without limitation, butylated hydroxyanisole, butylated hydroxytoluene, or edetate disodium, or a mixture of two or more thereof. Suitable humectants include, without
5 limitation, ethylene glycol, glycerin, or sorbitol, or a mixture of two or more thereof. Suitable emulsifiers include, without limitation, carbomer, polyoxyethylene-10-stearyl ether, polyoxyethylene-20-stearyl ether, cetostearyl alcohol, cetyl alcohol, cholesterol, diglycol stearate, glyceryl monostearate, glyceryl stearate, hydroxypropyl cellulose, hydroxypropylmethyl cellulose, lanolin, polyoxyethylene lauryl ether, methyl cellulose,
10 polyoxyethylene stearate, polysorbate, propylene glycol monostearate, sorbitan esters or stearic acid, or a mixture of two or more thereof.

[0047] In one embodiment of this aspect of the invention, the composition is formulated for topical application. Compositions for topical administration according to the present invention can be formulated as solutions, ointments, creams, foams,
15 suspensions, lotions, powders, pastes, gels, sprays, aerosols, or oils for vaginal, anal, or buccal administration. In another embodiment of the invention, the composition is formulated for vaginal and/or rectal administration. In another embodiment of the invention, the composition is formulated for slow release from a vaginal device, such as a vaginal ring, an IUD, or a sponge, or other contraceptive device (*e.g.*, condom). In yet
20 another embodiment of the present invention, the composition is formulated for application as an oral rinse. In a preferred embodiment of the invention, the composition is applied or contacted directly with the skin or a mucous membrane of the subject.

[0048] Another aspect of the invention relates to a method of treating an inflammatory condition in a subject. This method involves administering a composition of
25 the present invention comprising an isolated LukE protein, or polypeptide thereof, and an isolated LukD protein, or polypeptide thereof, in an amount effective to treat an inflammatory condition in the subject.

[0049] Applicants have discovered that LukE/D targets and kills human CCR5-positive leukocytes and that this LukE/D mediated cytotoxicity is substantially specific to
30 these cells but not other nucleated mammalian cells. Since CCR5 is expressed in a subset of effector T cells that produce proinflammatory cytokines that are enriched locally during inflammation, compositions of the present invention comprising LukE and LukD proteins and polypeptides are useful in treating inflammatory conditions by depleting the CCR5

positive cell populations. Any subject, preferably a mammal, more preferably a human, can be treated in accordance with this aspect of the invention, regardless of the cause of the inflammation, *e.g.*, any bacterial or viral infection. Suitable compositions containing LukE and LukD proteins and/or polypeptides are described *supra*.

5 [0050] The therapeutic compositions of the present invention may be used to treat a number of inflammatory conditions, including but not limited to acute inflammatory conditions, rheumatoid arthritis, Crohn's disease, atherosclerosis, psoriasis, ulcerative colitis, psoriatic arthritis, multiple sclerosis, lupus, type I diabetes, primary biliary cirrhosis, inflammatory bowel disease, tuberculosis, skin wounds and infections, tissue
10 abscesses, folliculitis, osteomyelitis, pneumonia, scalded skin syndrome, septicemia, septic arthritis, myocarditis, endocarditis, toxic shock syndrome, allergic contact dermatitis, acute hypersensitivity, and acute neurological inflammatory injury (*e.g.*, caused by acute infection).

[0051] Acute inflammatory conditions encompass the initial response of the body
15 to invading stimuli, and involve the recruitment of plasma and white blood cells (leukocytes) to the localized area of the injured or infected tissues. Acute inflammatory conditions have a rapid onset and severe symptoms. The duration of the onset, from a normal condition of the patient to one in which symptoms of inflammation are seriously manifested, generally lasts up to about 72 hours. Acute inflammatory conditions that are
20 amenable to treatment with the therapeutic compositions of the present invention include conjunctivitis, iritis, uveitis, central retinitis, external otitis, acute suppurative otitis media, mastoiditis, labyrinthitis, chronic rhinitis, acute rhinitis, sinusitis, pharyngitis, tonsillitis, contact dermatitis, dermonecrosis, diabetic polyneuritis, polymyositis, myositis ossificans, degenerative arthritis, rheumatoid arthritis, peri-arthritis scapulothoracalis, and osteitis
25 deformans. In one embodiment of the present invention, the acute inflammatory condition is an infected wound in the skin or soft tissue.

[0052] In the context of treatment of an inflammatory condition, an effective amount of a LukE and LukD composition is the amount that is therapeutically effective in the sense that treatment is capable of achieving a reduction in the inflammation, a decrease
30 in the severity of the inflammation, or even a total alleviation of the inflammatory condition.

[0053] The anti-inflammatory compositions of the present invention may be administered by any route of administration as described *infra*. In the case of treatment of

acute inflammatory conditions that are localized, non-systemic administration may be preferred in which case the administration of the therapeutic composition is at or around the site of the acute inflammation. In this regard, compositions for topical administration are preferred. In addition to the topical formulations described *supra*, the topical
5 formulation can also be in the form of patches or dressings impregnated with active ingredient(s), which can optionally comprise one or more excipients or diluents. In some embodiments, the topical formulation includes a material that enhances absorption or penetration of the active agent(s) through the skin or other affected areas.

[0054] A therapeutically effective amount of a Luke/LukD composition in
10 accordance with this and other aspects of the invention is the amount necessary to obtain beneficial or desired results. A therapeutically effective amount can be administered in one or more administrations, applications or dosages and is not intended to be limited to a particular formulation or administration route.

[0055] Also in accordance with this aspect of the invention, the Luke/LukD
15 composition can be administered in combination with other anti-inflammatory compositions, a TNF α inhibitor, or a combination thereof. Exemplary anti-inflammatory medications include, but are not limited to, non-steroidal anti-inflammatory drugs (NSAID), analgesics, glucocorticoids, disease-modifying anti-rheumatic drugs, dihydrofolate reductase inhibitors (e.g., methotrexate), biologic response modifiers, and
20 any combination thereof.

[0056] A suitable NSAID is a selective cyclooxygenase-2 (COX-2) inhibitor. Exemplary COX-2 inhibitors include, without limitation, nimesulide, 4-hydroxynimesulide, flosulide, meloxicam, celecoxib, and Rofecoxib (Vioxx). Alternatively, a non-selective NSAID inhibitor is administered in combination with the
25 Luke/D composition of the present invention. Exemplary non-selective NSAIDS inhibitors include, without limitation, diclofenac, diflunisal, etodolac, fenoprofen, flurbiprofen, ibuprofen, indomethacin, ketoprofen, ketorolac, mefenamic acid, meloxicam, nabumetone, naproxen, oxaprozin, piroxicam, salsalate, sulindac and tolmetin.

[0057] Preferred analgesics include, without limitation, acetaminophen,
30 oxycodone, tramadol, and propoxyphene hydrochloride.

[0058] Preferred glucocorticoids include, without limitation, cortisone, dexamethosone, hydrocortisone, methylpredisolone, prednisolone, and prednisone.

[0059] Preferred biological response modifiers include a B-cell inhibitor, such as Rituximab, or a T cell activation inhibitor, such as, Leflunomide, Etanercept (Enbrel), or Infliximab (Remicade).

[0060] Suitable TNF α inhibitors include a TNF- α antibody, a matrix metalloproteinase inhibitor, a corticosteroid, a tetracycline TNF α antagonist, a fluoroquinolone TNF α antagonist, and a quinolone TNF α antagonist. Exemplary TNF α antagonist antibodies include, without limitation, infliximab, etanercept, CytoFAB, AGT-1, afelimomab, PassTNF, and CDP-870. Exemplary corticosteroids include, without limitation, mometasone, fluticasone, ciclesonide, budesonide, beclomethasone, beconase, flunisolide, deflazacort, betamethasone, methyl-prednisolone, dexamethasone, prednisolone, hydrocortisone, cortisol, triamcinolone, cortisone, corticosterone, dihydrocortisone, beclomethasone dipropionate, and prednisone. Exemplary tetracycline TNF- α antagonists include, without limitation, doxycycline, minocycline, oxytetracycline, tetracycline, lymecycline, and 4-hydroxy-4-dimethylaminotetracycline.

[0061] Another aspect of the present invention relates to a method of preventing graft-versus-host-disease (GVHD) in a subject. This method involves administering a composition comprising an isolated LukE protein, or polypeptide thereof, and an isolated LukD protein, or polypeptide thereof, in an amount effective to prevent graft-versus-host-disease (GVHD) in the subject.

[0062] Graft-versus-host disease (GVHD) remains the primary complication of clinical bone marrow transplantation (BMT) and a major impediment to widespread application of this important therapeutic modality. The hallmark of GVHD is infiltration of donor T lymphocytes into host epithelial compartments of the skin, intestine, and biliary tract. GVHD occurs when mature T cells, contained in the bone marrow of the graft, are transplanted into immuno-suppressed hosts. After transplantation, host antigen presenting cells (APCs) activate T cells of the graft (donor T cells) by presenting host histocompatibility antigens to the graft T-cells. Donor-derived APCs may also activate donor T cells by cross-presenting host alloantigens. The newly generated host-specific T effector (hsTeff) populations then migrate to peripheral host organs and effect target organ damage

[0063] GVHD generally occurs in an acute and chronic form. Acute GVHD will be observed within about the first 100 days post BMT, whereas chronic GVHD occurs after this initial 100 days. In addition to chronology, different clinical symptoms are also

manifest in acute GVHD versus chronic GVHD. Acute GVHD is generally characterized by damage to host liver, skin, mucosa and intestinal epithelium in the host subject, although some forms of idiopathic pneumonia have also been reported. Chronic GVHD is, on the other hand, associated with damage to connective tissue as well as the organs and tissues damaged during acute GVHD in the host subject. In general, the methods of the present invention relate to therapies for either addressing GVHD that is already present in a host subject or preventing GVHD from arising in a host subject. In one embodiment, the present invention relates to methods of treating or preventing acute GVHD. In particular, the methods of the present invention are suitable for treating acute GVHD where the GVHD is damaging host intestinal epithelium. The methods of the present invention are also suitable for treating acute GVHD where the GVHD is damaging at least one tissue selected from the group consisting of the host liver, the host skin, the host lung and the host mucosa. Of course, the methods may be used to treat acute GVHD where the GVHD is damaging more than one tissue.

[0064] In accordance with this embodiment of the invention, CCR5-positive donor T cells transplanted into the recipient host during allogenic transplantation mediate GVHD. Accordingly, in one embodiment of the present invention, donor bone marrow cells are treated with a composition containing LukeE and Luke D prior to transplantation to effectuate cell death of all CCR5⁺ cells, thereby preventing GVDH.

[0065] In another embodiment of the present invention, treatment of the donor bone marrow cells is achieved by treating the graft. "Treating the graft" is intended to mean administering a composition or performing a procedure to the graft material, where the treatment is not intended to directly affect the host organism. Of course, successful treatment of the graft will indirectly affect the host organism in that the severity of GVHD may be reduced, or even removed entirely. The methods of the invention are not limited to the location of the graft at the time the graft is treated. Thus, in one embodiment, the graft is treated prior to removal from the donor organism. In another embodiment, the graft is treated after removal from the donor organism. In yet another embodiment, the graft is treated after removal from the donor organism, but prior to transplantation into the host subject. In still another embodiment, the graft is treated after transplantation into the host organism.

[0066] In accordance with this aspect of the invention, the composition comprising LukeE and LukD may be administered as part of a combination therapy. For example, the

Luke/D composition may be co-administered with another pharmaceutically active substance, such as but not limited to, methotrexate and cyclosporine. Additional agents that may be co-administered include but are not limited to, antibodies directed to various targets, tacrolimus, sirolimus, interferons, opioids, TNF α (tumor necrosis factor- α),
5 binding proteins, Mycophenolate mofetil and other inhibitors of inosine monophosphate dehydrogenase (IMPDH), glucocorticoids, azathioprine and other cytostatic agents such as, but not limited to, antimetabolites and alkylating agents. In one embodiment, the graft or donor may be pretreated by administration of immunosuppressive drugs such as cyclosporine (alone or in combination with steroids) and methotrexate prior to
10 transplantation. For prevention, immunosuppressive therapy typically consists of combined regimens of methotrexate (MTX), cyclosporin (CsA), tacrolimus (FK 506), and/or a corticosteroid. Intravenous gamma-globulin preparations administered prophylactically have also been shown to be beneficial for the prevention of GVHD. In addition, pentoxifylline, a xanthine derivative capable of down-regulating TNF α
15 production, may be administered with cyclosporin plus either methotrexate or methylprednisolone to further decrease incidence of GVHD. Chronic GVHD may be treated with steroids such as prednisone, ozathioprine and cyclosporine. Also, antithymocyte globulin (ATG) and/or Ursodiol may be used. Thalidomide with immunosuppressive properties has shown promising results in the treatment of chronic
20 GVHD. Similar to thalidomide, clofazimine may also be coadministered with the composition of the present invention comprising Luke and LukD. Antibody targets for co-administered antibodies include, but are not limited to, T cell receptor (TCR), interleukin-2 (IL-2) and IL-2 receptors. Additionally, a CD(25) monoclonal antibody, anti-CD8 monoclonal antibody, or an anti-CD103 antibody may be co-administered for
25 GVHD prophylaxis.

[0067] In accordance with this and all aspects of the present invention, composition of the present invention can be formulated for pharmaceutical use and administered by parenteral, topical, intravenous, oral, subcutaneous, intraperitoneal, intranasal, intramuscular, intra-arterial, intracranial, intradermal injection for prophylactic
30 and/or therapeutic treatment.

[0068] When it is desirable to deliver the pharmaceutical compositions of the present invention systemically, they may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may

be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing, and/or dispersing agents. Solutions or suspensions of the agent
5 can be prepared in water suitably mixed with a surfactant such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof in oils. Illustrative oils are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, or mineral oil. In general, water, saline, aqueous dextrose and related sugar solution, and glycols, such as propylene glycol or
10 polyethylene glycol, are preferred liquid carriers, particularly for injectable solutions. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

[0069] Pharmaceutical formulations suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation
15 of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (*e.g.*, glycerol,
20 propylene glycol, and liquid polyethylene glycol), suitable mixtures thereof, and vegetable oils.

[0070] Intraperitoneal or intrathecal administration of the agents of the present invention can also be achieved using infusion pump devices such as those described by Medtronic, Northridge, CA. Such devices allow continuous infusion of desired compounds
25 avoiding multiple injections and multiple manipulations.

[0071] In addition to the formulations described previously, the pharmaceutical compositions may also be formulated as a depot preparation. Such long acting formulations may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly
30 soluble derivatives, for example, as a sparingly soluble salt.

[0072] The required dosage of the composition comprising Luke and LukD of the present invention depends on the choice of the route of administration; the nature of the formulation; the nature of the subject's illness; the subject's size, weight, surface area, age,

and sex; other drugs being administered; and the judgment of the attending physician. Suitable dosages are in the range of 0.01-100 mg/kg. Variations in the needed dosage are to be expected in view of the variety of compounds available and the different efficiencies of various routes of administration. Variations in these dosage levels can be adjusted
5 using standard empirical routines for optimization as is well understood in the art.

Encapsulation of the compound in a suitable delivery vehicle (e.g., polymeric microparticles or implantable devices) may increase the efficiency of delivery.

[0073] Another aspect of the present invention relates to a method of treating a *Staphylococcus aureus* infection in a subject. This method involves selecting a subject
10 having a *S. aureus* infection and administering a composition comprising a CCR5 antagonist to the subject in an amount effective to treat the *S. aureus* infection in the subject.

[0074] For purposes of this aspect of the invention, the target subject encompasses any animal, preferably a mammal, more preferably a human that is infected and/or at risk
15 to be infected with *S. aureus* or is at risk of *S. aureus* infection. Particularly suitable subjects include infants, juveniles, adults, and elderly adults, as well as immunocompromised individual. Additionally, suitable subjects include those subjects infected with methicillin-resistant *S. aureus* (MRSA) infection or methicillin sensitive *S. aureus* (MSSA) infection.

[0075] In accordance with this aspect of the invention, suitable CCR5 antagonists for inhibiting *S. aureus* LukE/D mediated cytotoxicity, thereby treating or preventing *S. aureus* infection are known in the art, and include, without limitation, maraviroc, vicriviroc, NCB-9471, PRO-140, CCR5 mAb004, 8-[4-(2-butoxyethoxy)phenyl]-1-isobutyl-N-[4-[[[(1-propyl-1H-imidazol-5-yl-)methyl]sulphinyl]phenyl]-1,2,3,4-
25 tetrahydro-1-benzacocine-5-carboxamide, methyl 1-endo-{8-[(3S)-3-(acetylamino)-3-(3-fluorophenyl)propyl]-8-azabicyclo[3.2.1]oct-3-yl}-2-methyl-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-5-carboxylate, methyl 3-endo-{8-[(3S)-3-(acetamido)-3-(3-fluorophenyl)propyl]-8-azabicyclo[3.2.-1]oct-3-yl}-2-methyl-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridine-5-carboxylate, ethyl 1-endo-{8-[(3S)-3-(acetylamino)-3-(3-fluorophenyl)propyl]-8-azabicyclo[3.-2.1]oct-3-yl}-2-methyl-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-5-carboxylate, and N-[(1S)-3-[3-endo-(5-isobutyryl-2-methyl-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridin-1-yl)-8-azabicyclo[3.2.1]oct-8-yl]-1-(3-fluorophenyl)propyl]acetamide).

[0076] Additional CCR5 antagonists and compositions containing the same are further described in U.S. Patent Publication No. 2007/0010509 to Shiota et al., and U.S. Patent No. 7,625,905 to Lemoine et al., U.S. Patent No. 6,476,062 to Chu et al., U.S. Patent No. 7,728,135 to Shi et al., and U.S. Patent No. 7,220,856 to Dunning et al., which
5 are all hereby incorporated by reference in their entirety.

[0077] The CCR-5 antagonist can be administered as part of a combination therapy in conjunction with another active agent depending upon the nature of the *S. aureus* infection that is being treated. Such additional active agents include anti-infective agents, antibiotic agents, and antimicrobial agents. Representative anti-infective agents that may
10 be useful in the present invention include vancomycin and lysostaphin. Other suitable anti-infective agents include agents that inhibit LukeE/D mediated cytotoxicity (e.g., anti-LukeE antibody, anti-LukD antibody, anti-LukeE/D antibody).

[0078] Representative antibiotic agents and antimicrobial agents that may be useful in the present invention include penicillinase-resistant penicillins, cephalosporins
15 and carbapenems, including vancomycin, lysostaphin, penicillin G, ampicillin, oxacillin, nafcillin, cloxacillin, dicloxacillin, cephalothin, cefazolin, cephalexin, cephradine, cefamandole, cefoxitin, imipenem, meropenem, gentamycin, teicoplanin, lincomycin and clindamycin. Dosages of these antibiotics are well known in the art. See, e.g., MERCK
20 MANUAL OF DIAGNOSIS AND THERAPY, Section 13, Ch. 157, 100th Ed. (Beers & Berkow, eds., 2004), which is hereby incorporated by reference in its entirety. The anti-inflammatory, anti-infective, antibiotic and/or antimicrobial agents may be combined prior to administration, or administered concurrently (as part of the same composition or by way of a different composition) or sequentially with the CCR5 antagonist composition of the present invention. In certain embodiments, the administering is repeated.

[0079] Compositions containing CCR-5 antagonists can be administered by
25 parenteral, topical, intravenous, oral, subcutaneous, intraperitoneal, intranasal, intramuscular, intra-arterial, intracranial, or intradermal injections, for prophylactic and/or therapeutic treatment.

[0080] Another aspect of the present invention relates to a method of identifying a
30 suitable treatment for a subject having a *S. aureus* infection. This method involves obtaining a sample from the subject and detecting or quantifying the level of *CCR5* expression and CCR5 surface level in the sample. The method further involves comparing the detected level of *CCR5* expression and CCR5 surface level in the sample to a control

sample having a known or baseline *CCR5* expression level and *CCR5* surface level and determining a suitable treatment for the subject based on this comparison. The method further involves administering the determined suitable treatment to the subject.

[0081] In accordance with this aspect of the invention, individuals lacking *CCR5* or having lower levels of *CCR5* expression will be more resistant to infection with *lukE/D*⁺ *S. aureus* compared to individuals with higher levels of *CCR5*. Individuals having higher levels of *CCR5* are more suitable candidates for treatment using a *CCR5* receptor antagonist as described herein.

[0082] A further aspect of the present invention relates to a method of predicting severity of an *S. aureus* infection in a subject by monitoring *CCR5* levels in the subject. This method involves isolating PBMCs from whole blood of the subject and performing flow cytometry analysis to determine *CCR5* surface expression. The quantified amounts of surface *CCR5* expression in the cells from the subject are compared to the amount of *CCR5* in a control sample which produces little or undetectable amounts of *CCR5* and control sample which produces high levels of *CCR5* (e.g., Jurkat *CCR5*⁺) and the severity of the *S. aureus* infection is predicted based on *CCR5* levels. High levels of *CCR5* in the subject predict a more severe *S. aureus* infection, while lower levels of *CCR5* in the subject predict a less severe infection. Methods of isolating and/or labeling PBMCs from a whole blood sample for FACs analysis are readily known in the art.

20

EXAMPLES

[0083] The following examples are provided to illustrate embodiments of the present invention but are by no means intended to limit its scope.

Example 1 - *LukE/D* Significantly Contributes to *S. aureus* Pathogenesis

[0084] To test whether *LukE/D* plays a major role in the pathogenesis of *S. aureus* septicemic infection, a Δ *lukE/D* mutant in the MSSA strain Newman was constructed and the impact of the *lukE/D* deletion on virulence examined. Survival over time dramatically increased for mice infected with 10^7 CFU of the Δ *lukE/D* mutant compared to that of mice infected with wild type (WT) *S. aureus*. All mice infected with WT *S. aureus* succumbed to infection by 250 hours. In contrast, nearly 100% of mice infected with Δ *lukE/D* mutant survived until at least 300 hours post infection, a phenotype fully complemented by

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introducing *luke/D* into the Δ *luke/D* mutant strain (Δ *luke/D::pluke/D*; Figure 1A). In addition, bacterial burden to the kidney was reduced by 10-fold compared to the WT or complemented strain (Figure 1B). These results show that LukeE/D is a critical virulence factor for *S. aureus* systemic infection. Thus LukeE/D is an attractive novel target for
5 development of new therapeutics to counter *S. aureus* infection.

Example 2 – LukeE/D Selectively Kills Human Immune Cell Lines

[0085] As described *supra*, LukeE/D contributes to the pathogenesis of *S. aureus* mediated sepsis and systemic infection (Figures 1A–1B), indicating that inhibiting LukeE/D could prove to be a novel mean by which to treat *S. aureus* infections.

10 [0086] One mechanism by which LukeE/D could be blocked is by inhibiting the interaction of the toxin with its receptor. As an initial strategy to understand how LukeE/D interact with host cells, a collection of human immune cell lines were incubated (“intoxicated”) with different concentrations of either individual subunits (*i.e.*, Luke or LukD) or an equimolar mixture of Luke + LukD (LukeE/D). These experiments revealed
15 that LukeE/D exhibits cytotoxicity toward THP1 cells (human monocytes) and Hut cells (T lymphocyte-like cells) (Figure 2A). Interestingly, LukeE/D was cytotoxic towards Hut cells but not towards Jurkat cells, both commonly used T lymphocyte-like cells. This surprising result prompted investigation into what rendered the Hut cells sensitive to LukeE/D. Intoxication of additional lymphocyte cell lines (PM1 and H9) revealed that only
20 the Hut cells were susceptible to LukeE/D mediated toxicity (Figure 2B). Upon further investigation, it was discovered that the Hut cells employed the experiments described above have been engineered to over-express the CC-chemokine receptor 5 (CCR5), a receptor for the chemokines MIP-1 α , MIP-1 β , and RANTES.

Example 3 – LukeE/D Targets and Kills Cells in a CCR5-Dependent Manner

25 [0087] To directly determine the contribution of CCR5 for the ability of LukeE/D to target and kill host cells, CCR5 was introduced into Jurkat cells by viral transduction of the *CCR5* cDNA resulting in CCR5⁺ Jurkat. Jurkat and CCR5⁺ Jurkat cells were subsequently intoxicated with different concentrations of either individual subunits (*i.e.*, Luke or LukD) or equimolar mixtures of Luke + LukD (LukeE/D). This experiment
30 revealed that production of CCR5 was sufficient to render Jurkat cells susceptible to LukeE/D mediated toxicity (Figure 3, top panel). Importantly, similar results were

observed when the human osteosarcoma cell line “GHOST” cells engineered to produce CCR5 on their surface were examined (Figure 3 bottom panel). Altogether, these data indicate that CCR5 renders mammalian cells susceptible to Luke/D mediated cytotoxicity.

5 **Example 4 – Luke/D Mediated Targeting of CCR5⁺ Cells is Blocked With Agonist, Antibodies and CCR5 Ligands**

[0088] CCR5 is a protein that has been highly studied because of its critical role in HIV-1 infection. Together with CD4, CCR5 is used by the virus to gain entry into cells. The importance of CCR5 to HIV pathogenesis in humans is best highlighted by the identification of subjects that have a mutation in the *CCR5* gene (*i.e.*, $\Delta 32$ *CCR5*) that prevent the surface exposure of CCR5. Patients with this mutation are highly refractory to HIV infection. Currently, a variety of CCR5 antagonist (*e.g.*, peptide mimetics, antibodies, small molecules) are being tested in clinical trials to be used as anti-HIV drugs as well as anti-inflammatory agents.

[0089] To determine if targeting CCR5 blocks Luke/D, the effect of several CCR5 antagonist and ligands on the ability of Luke/D to kill CCR5⁺ cells was evaluated. Among the CCR5 antagonist, the drugs Selzentry/Celsentri/Maraviroc (MVC), Vicriviroc (VVC) and TAK-779 (TAK) were tested for inhibition of Luke/D activity. CCR5⁺ Jurkat cells were pre-incubated with different concentrations of the antagonists, followed by intoxication with an equimolar mixture of Luke + LukD (Luke/D). These experiments indicated that all three CCR5 antagonists potently blocked Luke/D mediated cytotoxicity (Figure 4A). In addition, the potential of monoclonal antibodies directed against CCR5 to protect cells from Luke/D cytotoxicity was also evaluated following the experimental protocol described for the CCR5 antagonist. These experiments also revealed that several of the tested monoclonal antibodies were indeed able to block Luke/D (Figure 4B). Lastly, the potential inhibitory effect of natural ligands of CCR5 was also evaluated. CCR5⁺ Jurkat cells were pre-incubated with different concentrations of RANTES, MIP-1 β , or a combination of equimolar mixture of RANTES+MIP-1 β followed by intoxication with an equimolar mixture of Luke + LukD (Luke/D). These experiments also revealed that CCR5 ligands potently inhibit Luke/D cytotoxic effect (Figure 4C). Collectively, these findings indicate that the potent cytotoxic activity of Luke/D could be blocked by employing CCR5 antagonist and/or ligands.

Example 5 – Maraviroc Blocks LukeE/D Binding To CCR5⁺ Cells Preventing the Formation of LukeE/D Pores

[0090] To elucidate the mechanism by which LukeE/D utilizes CCR5 to target and kill host cells, Jurkat (CCR5⁻) and CCR5⁺ Jurkat (CCR5⁺) cells were incubated with a GFP-fused LukeE/D toxin (GFP^LLukeE/D) and binding of the fluorescent toxin to the plasma membrane of the cells monitored by flow cytometry. These experiments revealed that LukeE/D binds to CCR5⁺ Jurkat cells but not to the parental CCR5⁻ Jurkat cells (Figure 5A). To elucidate the mechanism by which Maraviroc inhibits LukeE/D mediated cytotoxicity, CCR5⁺ Jurkat cells were pre-incubated with Maraviroc (MVC) followed by incubation with the GFP-labeled LukeE/D toxin and toxin binding to the cells evaluated by flow cytometry. These experiments indicated that Maraviroc potently inhibited LukeE/D binding to CCR5⁺ cells (Figure 5A).

[0091] To examine the mechanism by which LukeE/D is toxic to CCR5⁺ cells, cells were incubated in the presence or absence of Maraviroc and subsequently intoxicated with LukeE/D in the presence of ethidium bromide, a small cationic dye that is normally impermeable to host cell membranes, but can gain access to host cells via the toxin pores. These experiments revealed that LukeE/D forms pores in the plasma membrane of CCR5⁺ cells in a time-dependent manner. Importantly, Maraviroc (MVC) potently blocked LukeE/D mediated pore formation (Figure 5B). In addition, LukeE/D pores were associated with cell swelling, a characteristic of cells intoxicated with leukotoxins, a phenotype fully blocked by Maraviroc (MVC) (Figure 5C). Altogether, these findings indicate that LukeE/D binds to host cells in a CCR5-dependent manner resulting in the formation of toxin mediated pores at the plasma membrane of target cells, leading to the observed LukeE/D mediated cytotoxicity. Importantly, the CCR5 antagonist Maraviroc, potently inhibits LukeE/D by blocking the interaction of LukeE/D with the surface of CCR5⁺ cells, thus preventing pore formation and cell death.

Example 6 – LukeE/D Targets CCR5 to Kill Primary Human Lymphocytes, Macrophages, and Dendritic Cells

[0092] If CCR5 is the receptor of LukeE/D, then primary host cells that their surfaces are decorated with CCR5 (*e.g.*, T lymphocytes, macrophages, natural killer cells, dendritic cells, etc.) will be susceptible to LukeE/D mediated cell death. To investigate this in more detail, primary human peripheral blood mononuclear cells (PBMC) were isolated

from a wild type *CCR5* ($CCR5^+$) donor and a $\Delta 32CCR5$ ($CCR5^-$) donor and the T lymphocytes expanded followed by intoxication with LukE/D and the viability of the cells determined by flow cytometry. Primary human T lymphocytes from $CCR5^+$ donor were highly susceptible to LukE/D (5.4% cell death in the media treated cells vs. 34% in
5 LukE/D intoxicated cells; Figure 6A, top panel), an effect potentially neutralized by Maraviroc (LukE/D vs. LukE/D + MVC; Figure 6A, top panel). In contrast, T lymphocytes from the $\Delta 32CCR5$ donor were highly refractory to LukE/D mediated cytotoxicity (Figure 6A, bottom panel).

[0093] In addition to T lymphocytes, the cytotoxic activity of LukE/D towards
10 primary human macrophages and dendritic cells was also evaluated. Macrophages and dendritic cells were incubated with LukD (negative control), intoxicated with an equimolar mixture of LukE + LukD (LukE/D), or incubated with Maraviroc (MVC) followed by intoxication with an equimolar mixture of LukE + LukD (LukE/D). LukE/D but not LukD potentially killed both macrophages (Figure 6B) and dendritic cells (Figure
15 6C). Importantly, the cytotoxic effect of LukE/D towards these phagocytes was potentially neutralized by Maraviroc (LukE/D vs. LukE/D + MVC; Figures 6B and 6C). Collectively, these data indicate that LukE/D targets and kills primary human leukocytes that harbor CCR5 at their surfaces, and that the CCR5 antagonist Maraviroc potentially block LukE/D cytotoxic effects. Thus, blockade of LukE/D with CCR5 antagonist and/or inhibitors will
20 offer a new therapeutic option to prevent and treat *S. aureus* infection.

[0094] Although the invention has been described in detail for the purposes of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

WHAT IS CLAIMED:

1. A method of preventing or treating Human Immunodeficiency Virus (HIV) infection in a subject comprising:
5 administering a composition comprising an isolated Leukocidin E (LukE) protein, or polypeptide thereof, and an isolated Leukocidin D (LukD) protein, or polypeptide thereof, in an amount effective to prevent or treat HIV infection in the subject.
2. The method of claim 1, wherein the composition comprises an
10 isolated LukE protein comprising an amino acid sequence of SEQ ID NO:11 or an amino acid sequence having at least 70% sequence similarity to SEQ ID NO:11.
3. The method of claim 1, wherein the composition comprises an
15 isolated LukE polypeptide comprising amino acid residues 48–291 of SEQ ID NO:11.
4. The method of claim 1, wherein the composition comprises an
isolated LukD protein comprising an amino acid sequence of SEQ ID NO:22 or an amino acid sequence having at least 70% sequence similarity to SEQ ID NO:22.
- 20 5. The method of claim 1, wherein the composition comprises an isolated LukD polypeptide comprising amino acid residues 46–307 of SEQ ID NO:22.
6. The method of claim 1 further comprising:
administering one or more antiviral or other agent useful in the treatment
25 of HIV in combination with the composition comprising an isolated LukE protein, or polypeptide thereof, and an isolated LukD protein, or polypeptide thereof.
7. The method of claim 6, wherein the antiviral agent is selected from
the group consisting of a nucleoside reverse transcriptase inhibitor, a non-nucleoside
30 reverse transcriptase inhibitor, and a protease inhibitor.
8. The method of claim 6, wherein the antiviral agent is selected from
the group consisting of zidovudine, lamivudine, zalcitabine, didanosine, stavudine,

abacavir, adefovir dipivoxil, lobucavir, BC H-10652, emtricitabine, beta-L-FD4, DAPD, lodenosine, nevirapine, delaviridine, efavirenz, PNU-142721, AG-1549, MKC-442, (+)-calanolide A and B, saquinavir, indinavir, ritonavir, nelfinavir, lasinavir, DMP-450, BMS-2322623, ABT-378, amprenavir, hydroxyurea, ribavirin, IL-2, IL-12, pentafuside, Yissum
5 No. 1 1607, and AG-1549.

9. A composition comprising:

a therapeutically effective amount of an isolated LukE protein or polypeptide thereof, an isolated LukD protein or polypeptide thereof, or a combination thereof, and

10 one or more additional agents selected from the group consisting of a lubricant, an antimicrobial agent, a humectant, an emulsifier, and a mixture of two or more thereof.

10. The composition of claim 9, wherein the composition comprises an
15 isolated LukE protein comprising an amino acid sequence of SEQ ID NO:11 or an amino acid sequence having at least 70% sequence similarity to SEQ ID NO:11.

11. The composition of claim 9, wherein the composition comprises an
isolated LukE polypeptide comprising amino acid residues 48–291 of SEQ ID NO:11.

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12. The composition of claim 9, wherein the composition comprises an isolated LukD protein comprising an amino acid sequence of SEQ ID NO:22 or an amino acid sequence having at least 70% sequence similarity to SEQ ID NO:22.

13. The composition of claim 9, wherein the composition comprises an
25 isolated LukD polypeptide comprising amino acid residues 46–307 of SEQ ID NO:22.

14. The composition of claim 9, wherein the lubricant is selected from the group consisting of cetyl esters wax, hydrogenated vegetable oil, magnesium stearate,
30 methyl stearate, mineral oil, polyoxyethylene-polyoxypropylene copolymer, polyethylene glycol, polyvinyl alcohol, sodium lauryl sulfate or white wax, and a mixture of two or more thereof.

15. The composition of claim 9, wherein the antimicrobial agent is selected from the group consisting of propylene glycol, methyl paraben, propyl paraben, and a mixture of two or more thereof.

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16. The composition of claim 9, wherein the antioxidant is selected from the group consisting of butylated hydroxyanisole, butylated hydroxytoluene, edetate disodium, and a mixture of two or more thereof.

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17. The composition of claim 9, wherein the humectant is selected from the group consisting of ethylene glycol, glycerin, sorbitol, or a mixture of two or more thereof.

18. The composition of claim 9, wherein the emulsifier is selected from the group consisting of carbomer, polyoxyethylene-10-stearyl ether, polyoxyethylene-20-stearyl ether, cetostearyl alcohol, cetyl alcohol, cholesterol, diglycol stearate, glyceryl monostearate, glyceryl stearate, hydroxypropyl cellulose, hydroxypropylmethyl cellulose, lanolin, polyoxyethylene lauryl ether, methyl cellulose, polyoxyethylene stearate, polysorbate, propylene glycol monostearate, sorbitan esters, stearic acid, and a mixture of two or more thereof.

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19. The composition of claim 9, wherein said composition is formulated for topical administration.

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20. The composition of claim 9, wherein said composition is formulated for vaginal and/or rectal administration

21. The composition of claim 9, wherein said composition is formulated as an oral rinse.

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22. A method of preventing HIV infection in a subject, said method comprising:

providing a composition of claim 9, and

contacting tissue of the subject with the composition under conditions effective to block HIV infectivity of cells in the tissue, thereby inhibiting HIV infection in the subject.

5 23. The method of claim 22, wherein the contacted tissue is skin or a mucous membrane.

 24. A method of treating an inflammatory condition in a subject comprising:

10 administering a composition comprising an isolated LukE protein, or polypeptide thereof, and an isolated LukD protein, or polypeptide thereof, in an amount effective to treat an inflammatory condition in the subject.

 25. The method of claim 24, wherein the composition comprises an isolated LukE protein comprising an amino acid sequence of SEQ ID NO:11 or an amino acid sequence having at least 70% sequence similarity to SEQ ID NO:11.

 26. The method of claim 24, wherein the composition comprises an isolated LukD protein comprising an amino acid sequence of SEQ ID NO:22 or an amino acid sequence having at least 70% sequence similarity to SEQ ID NO:22.

 27. The method of claim 24, wherein the subject has an acute inflammatory condition.

25 28. The method of claim 27, wherein the acute inflammatory condition is localized.

 29. The method of claim 27, wherein the acute inflammatory condition is an infected wound in the skin or soft tissue.

30 30. The method of claim 24, wherein the inflammatory condition is selected from the group consisting of rheumatoid arthritis, Crohn's disease, atherosclerosis, psoriasis, ulcerative colitis, psoriatic arthritis, multiple sclerosis, lupus,

type I diabetes, primary biliary cirrhosis, inflammatory bowel disease, tuberculosis, skin wounds and infections, tissue abscesses, folliculitis, osteomyelitis, pneumonia, scalded skin syndrome, septicemia, septic arthritis, myocarditis, endocarditis, toxic shock syndrome, allergic contact dermatitis, acute hypersensitivity, and acute neurological
5 inflammatory injury.

31. A method of preventing graft-versus-host-disease (GVHD) in a subject comprising:

administering a composition comprising an isolated Leukocidin E (LukE)
10 protein, or polypeptide thereof, and an isolated Leukocidin D (LukD) protein, or polypeptide thereof, in an amount effective to prevent graft-versus-host-disease (GVHD) in the subject.

32. The method of claim 31, wherein the composition comprises an
15 isolated LukE protein comprising an amino acid sequence of SEQ ID NO:11 or an amino acid sequence having at least 70% sequence similarity to SEQ ID NO:11.

33. The method of claim 31, wherein the composition comprises an
20 isolated LukD protein comprising an amino acid sequence of SEQ ID NO:22 or an amino acid sequence having at least 70% sequence similarity to SEQ ID NO:22.

34. A method of treating a *Staphylococcus aureus* infection in a subject comprising:

selecting a subject having a *S. aureus* infection and
25 administering a composition comprising a CCR5 antagonist in an amount effective to treat the *S. aureus* infection in the subject.

35. The method of claim 34, wherein the CCR5 antagonist is selected from the group consisting of maraviroc, vicriviroc, NCB-9471, PRO-140, CCR5 mAb004,
30 8-[4-(2-butoxyethoxy)phenyl]-1-isobutyl-N-[4-[[1-propyl-1H-imadazol-5-yl-
)methyl]sulphinyl]phenyl]-1,2,3,4-tetrahydro-1-benzacocine-5-carboxamide, methyl 1-
endo-{8-[(3S)-3-(acetylamino)-3-(3-fluorophenyl)propyl]-8-azabicyclo[3.2.1]oct-3-yl}-
2-methyl-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-- 5-carboxylate, methyl 3-endo-

{8-[(3S)-3-(acetamido)-3-(3-fluorophenyl)propyl]-8-azabicyclo[3.2.- 1]oct-3-yl}-2-methyl-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridine-5-carbox- ylate, ethyl 1-endo-{8-[(3S)-3-(acetylamino)-3-(3-fluorophenyl)propyl]-8-azabicyclo[3.- 2.1]oct-3-yl}-2-methyl-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-5-carb- oxylate, and N-{(1S)-3-[3-endo-(5-
5 isobutyryl-2-methyl-4,5,6,7-tetrahydro-1H-imidazo[4,- 5-c]pyridin-1-yl)-8-azabicyclo[3.2.1]oct-8-yl]-1-(3-fluorophenyl)propyl} ac- etamide).

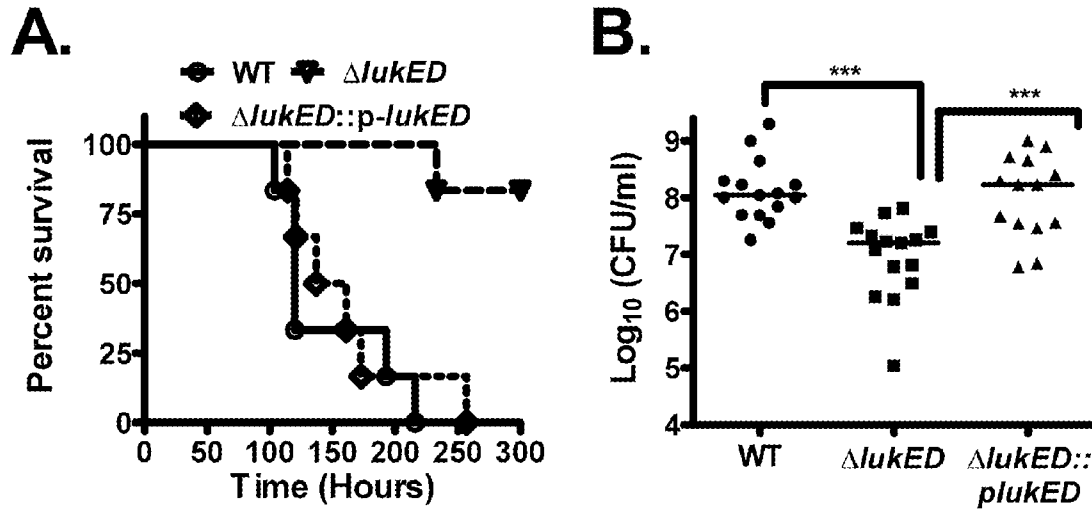
36. The method of claim 34 further comprising
administering an agent selected from the group consisting of an anti-
10 infective agent, an antibiotic agent, and an antimicrobial agent.

37. The method of claim 34, wherein the *Staphylococcus aureus*
infection is a MRSA infection or MSSA infection.

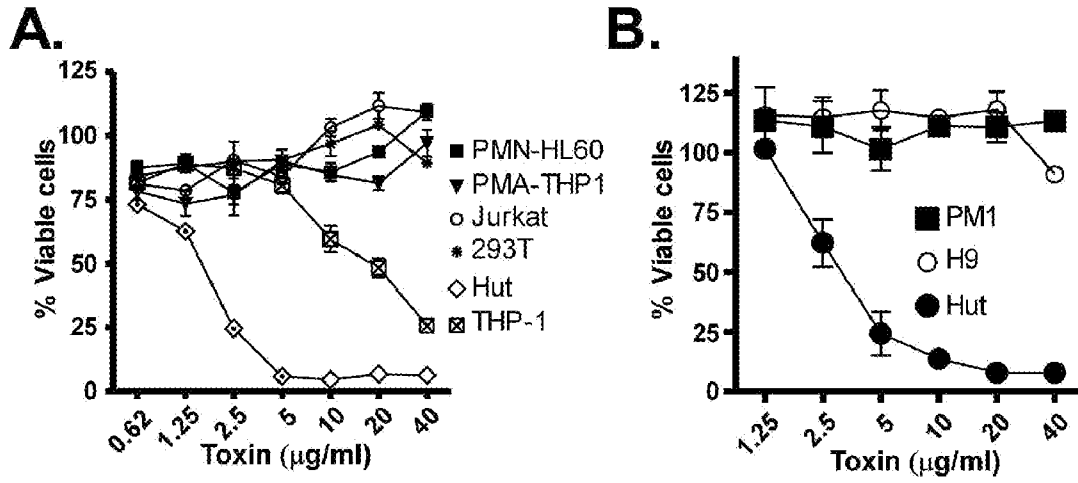
15 38. The method of claim 34 further comprising repeating said
administering.

20

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FIGURES 1A-1B



FIGURES 2A-2B

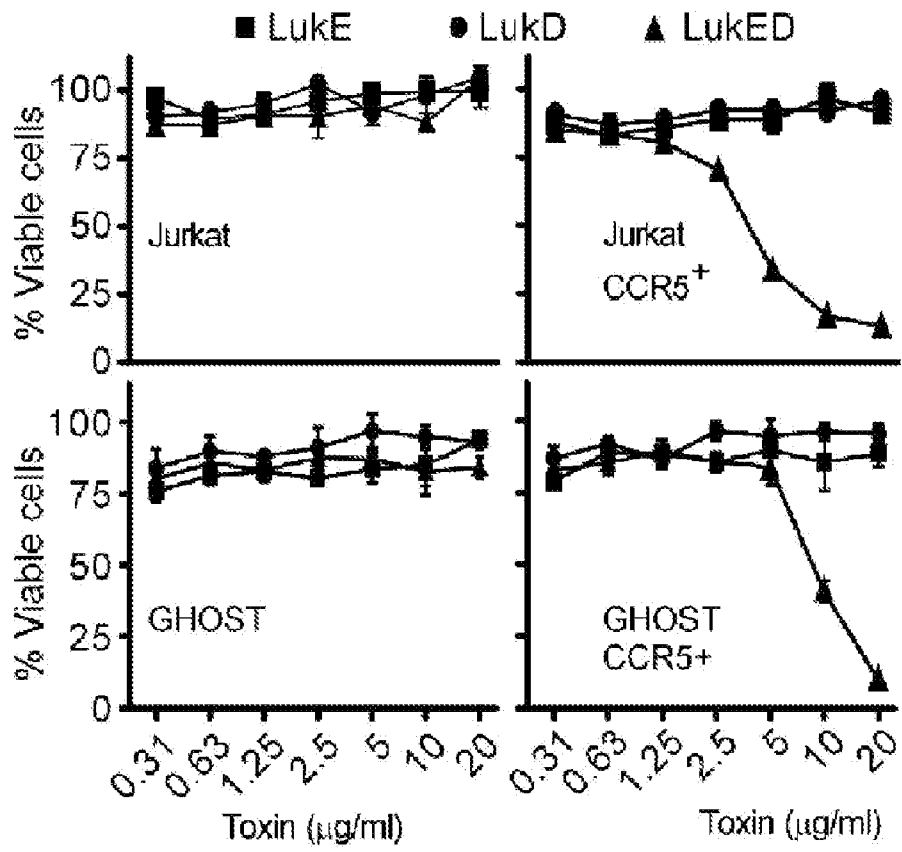
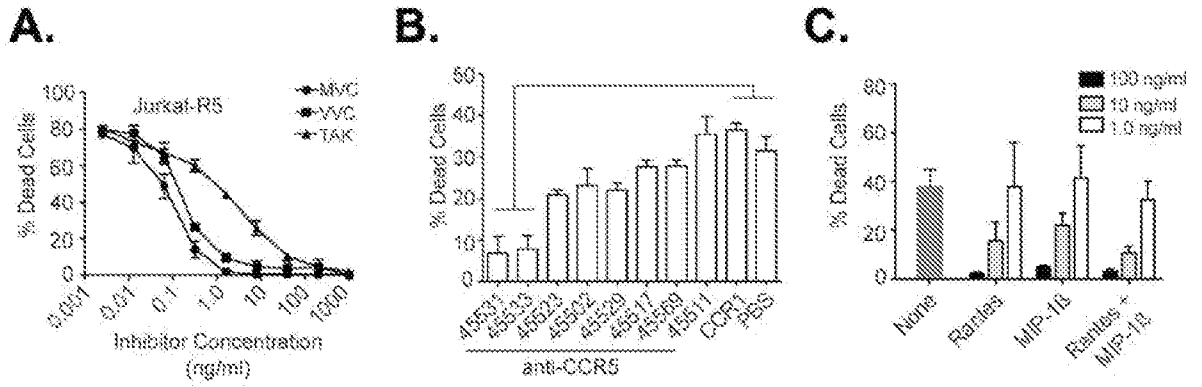
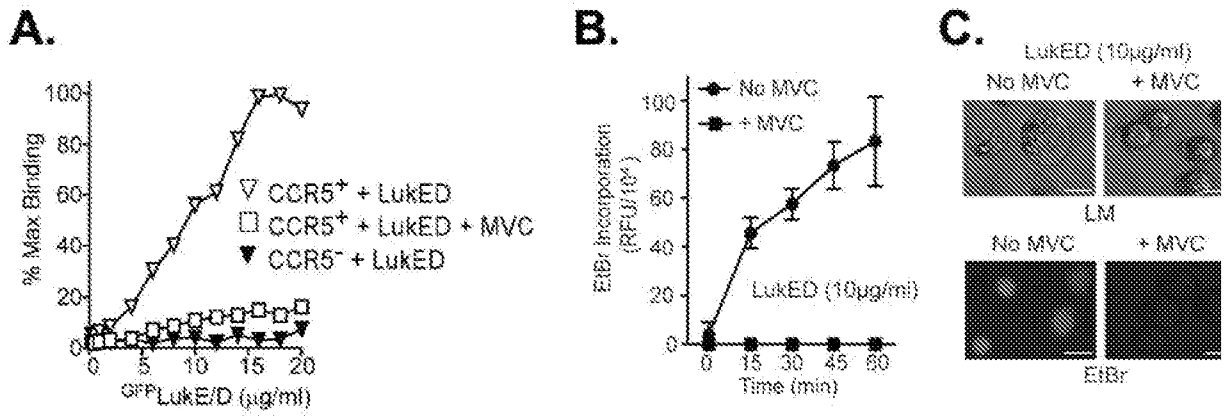


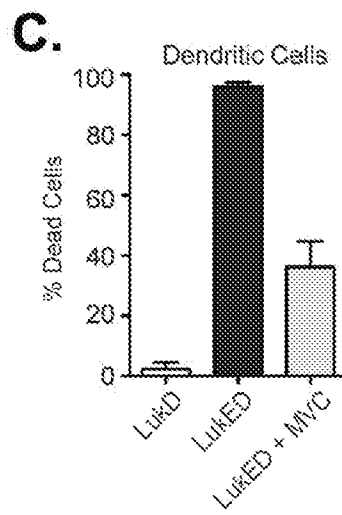
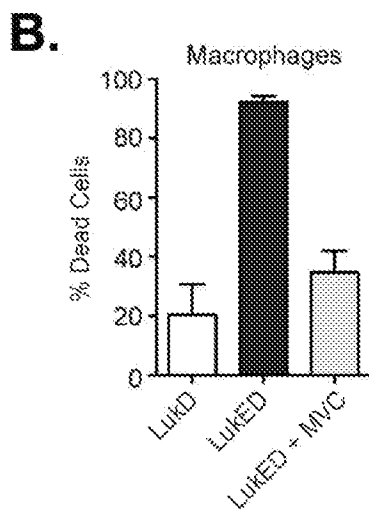
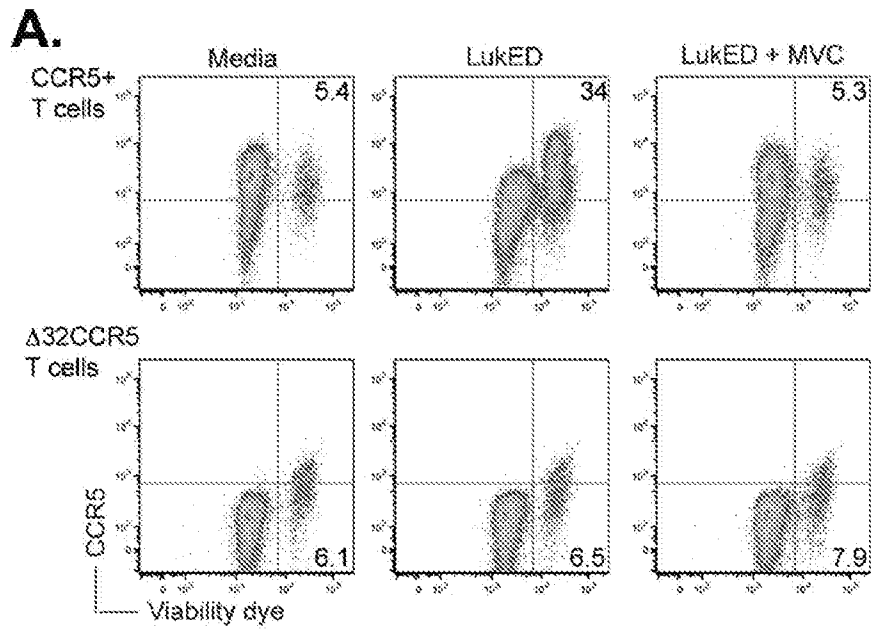
FIGURE 3



FIGURES 4A-4C



FIGURES 5A-5C



FIGURES 6A-6C