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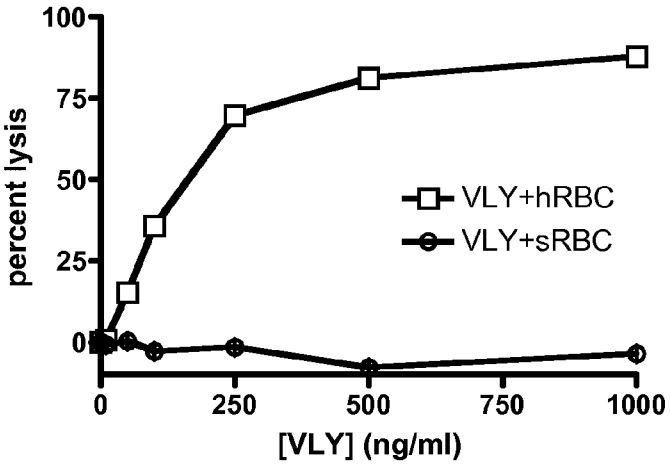
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(54) **Title**: TREATMENT AND PREVENTION OF *GARDNERELLA VAGINALIS* INFECTIONS

FIG. 2A



(57) **Abstract**: The present invention is drawn to the nucleic and amino acid sequences encoding vaginolysin (VLY) toxin from *Gardnerella vaginalis*, and biologically active fragments and variants thereof. The invention is also directed to anti-VLY antibodies and to their use therapeutically and in a new ELISA assay of VLY toxin. Other embodiments of the invention are directed to VLY toxoids and to vaccines that use the new VLY toxoids as immunogens.

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TREATMENT AND PREVENTION OF *GARDNERELLA VAGINALIS* INFECTIONS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of Provisional Application. No. 61/036,943 filed March 15, 2008 and Provisional Application. No. 61/057,190 filed May 29, 2008, the entire contents of which are hereby incorporated by reference as if fully set forth herein, under 35 U.S.C. §119(e).

STATEMENT OF GOVERNMENTAL INTEREST

[0002] This invention was made with government support under National Institutes of Health Grant AI065450. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0003] The present invention relates to the discovery of the nucleic acid sequence and amino acid sequence encoding a newly identified *G. vaginalis* pore-forming toxin called vaginolysin (VLY), and to methods for diagnosing, preventing and treating *G. vaginalis* and bacterial vaginosis (BV). Other aspects are related to vaccines that include a toxoid form of VLY as an immunogen.

2. Description of the Related Art

[0004] Bacterial vaginosis (BV) is the most common vaginal infection worldwide and is associated with significant adverse consequences including and preterm labor and delivery (40, 41), post-partum endometritis, (42) and an increased risk of HIV acquisition. (43-45) Reported prevalence rates range from 10-40% depending upon the population studied. (46) However, suboptimal methods of diagnosis and a high percentage of asymptomatic patients make the true prevalence of BV difficult to ascertain. *Gardnerella vaginalis* is a bacterial species associated with bacterial vaginosis (BV).

[0005] The pathogenesis of BV remains poorly understood. It is most commonly defined as a pathological state characterized by the loss of normal vaginal flora, particularly Lactobacillus species, and overgrowth of other microbes including *Gardnerella vaginalis*, Bactericides species, Mobiluncus species, and Mycoplasma hominis. Recent data however,

suggest a primary role for *G. vaginalis* as a specific and sexually transmitted etiological agent in BV, as was initially postulated by Gardner and Dukes in 1955. (47-49)

[0006] Alterations of both local host immunity and the genital tract microflora appear to contribute to the pathogenesis of BV (39), which can be difficult to eradicate even using targeted antimicrobial therapy (4). In addition, randomized trials of antibiotics for the prevention of BV-associated preterm birth have not shown consistently beneficial effects, suggesting that host inflammatory responses set in motion early in the course of disease may contribute significantly to the consequences of infection (26, 27).

[0007] In the 1950s, Leopold (25) and then Gardner and Dukes (14) observed abundant small, pleomorphic gram-variable rods in the genital tract of women with BV. This organism, first called *Haemophilus vaginalis* (13) and repeatedly renamed as more information about its characteristics became available (reviewed in (5)), is now classified as *Gardnerella vaginalis*, the sole member of the genus *Gardnerella* (16, 30). Phylogenetic analysis based on 16S rRNA places *Gardnerella* in the gram-positive family *Bifidobacteriales*. An abundance of *G. vaginalis* and a paucity of *Lactobacillus* species are characteristic of a BV-associated microflora, but the relative contribution of *G. vaginalis* to the pathogenesis of BV is not clear. *G. vaginalis* is present in essentially all cases of BV but can also be detected in a minority of asymptomatic women (1). Likewise, several groups have demonstrated that the vaginal microflora is exceedingly complex in BV where the vaginal mucosa is host to many non-*Gardnerella* organisms (12, 18, 20). Mechanistic studies of BV and its adverse consequences have been limited by the absence of definitive diagnostic testing and a suitable animal model (22, 23, 26). Existing methods of diagnosis for BV are suboptimal and frequently underutilized by practitioners. A recent study by Hogan et al. reports that the prevalence of BV among pregnant women varies greatly depending on the diagnostic criteria used. (51) Established in 1983, Amsel's criteria are widely accepted as the best available means for diagnosing BV in the clinical setting, however, these criteria are complex, somewhat subjective, and necessitate that microscopy equipment be present on site. (52, 53) The Nugent scoring system for interpretation of Gram-stained vaginal smears was put forth in an attempt to standardize diagnosis of BV and increase inter-rater reliability. (54) While the Nugent scoring system exhibits superior sensitivity and specificity compared to the Amsel criteria, (55) its use remains largely restricted to research protocols. Furthermore, questions regarding the risk of potential morbidities and the need for antimicrobial therapy in those women found to have "intermediate flora" remain unanswered. (56, 57)

[0008] Several alternative diagnostic methodologies focusing upon the detection of microbial virulence factors produced by the various BV-associated organisms have been proposed in recent years. These include detection of bacterial sialidases, determination of amine levels, and measurement of proline aminopeptidase activity. (58-60) While these techniques are relatively simple, rapid and inexpensive, they fail to identify the specific microbial pathogens present. A potential role for novel, molecular-based techniques for diagnosing BV has recently emerged. Importantly, preliminary studies evaluating these PCR-based strategies have provided additional evidence for *G. vaginalis* as the primary etiologic agent of BV. (61-63) Menard et al. analyzed 213 vaginal samples from pregnant women using molecular probes targeting 8 BV–related organisms. (64) These authors report that an increased load of *G. vaginalis* ($> 10^9$ copies of *G. vaginalis* DNA per ml) had both high negative and positive predictive values for the diagnosis of BV. While these molecular based diagnostic strategies are promising, the required expertise, laboratory resources and expense limits their use in the primary care setting.

[0009] *G. vaginalis* produces a cholesterol-dependent cytolysin (CDC) (protein pore-forming toxin) called vaginolysin ("VLY"), that acts as a hemolysin (8, 35, 50). IgA-mediated immune responses to the hemolysin occur during BV and are useful as a marker of disease (8, 35). However, complete characterization of the VLY has been limited by the absence of genetic information and an inability to produce recombinant toxin. Therefore there is a great need to sequence and characterize the human-specific VLY toxin, and for methods for treating or preventing *G. vaginalis* and BV.

[0010] The global impact of the HIV epidemic cannot be overstated. Even as antiretroviral drugs prolong and improve life for HIV-infected people in wealthy nations, millions of people suffer and ultimately perish from the ravages of the disease worldwide. Despite this, and despite decades of research, prevention and cure of HIV remain elusive goals. Thus, there is a need for novel and creative approaches to preventing HIV acquisition. BV is exceedingly common, especially in Africa where more than 50% of women in numerous trials, including the recent trial of acyclovir for HSV suppression in Tanzania1) were infected with BV. BV has been repeatedly associated with both increased risk of HIV acquisition and increased viral shedding among those already infected with HIV2. *In vitro,* treatment of HIV-infected cells with *Gardnerella* leads to increased production of viral transcripts. Comparatively little attention has been paid to targeting BV as a means of affecting the progression of the HIV epidemic due to several factors: (1) BV is not a

"traditional" sexually transmitted infection (STI) and is often omitted from analyses of STI-HIV interactions; (2) *Gardnerella* is enigmatic: difficult to culture, without an available genome sequence, lacking known virulence factors, and (perhaps most importantly) without an animal model; (3) BV and *Gardnerella* colonization are extremely difficult to eradicate even with targeted antibiotic therapy. A randomized, controlled trial of mass antibiotic treatment targeting STI in Uganda did not affect the prevalence of BV in either the control or the treatment group, emphasizing the challenges of BV prevention and treatment (and failing to address the impact of an efficacious BV therapy) (4).

[0011] Therefore there is a great need for a new methods and compositions to *G. vaginalis* and BV to minimize the risk of transmitting HIV from person to person, particularly from an HIV-positive mother who has *G. vaginalis* to a fetus or an infant during delivery.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] The present invention is illustrated by way of example, and not by way of limitation, in the FIGs.

[0013] FIG. 1 Phylogenetic relationship between VLY and other members of the cholesterol-dependent cytolysin (CDC) family. (A) Phylogram of full-length CDC protein sequences predicted by the neighbor joining algorithm. Numbers represent calculated relative phylogenetic distances. (B) Multiple alignments of undecapeptide regions from known CDC. The predicted amino acid sequence of VLY contains a variant undecapeptide region most similar to the undecapeptide from intermedilysin (ILY). The sequence labeled "consensus" corresponds to the undecapeptide from MLY, PLY, SUI, IVN, ALV, SPH, THU, SLO, ALO, LLO, PFO, CER, and TET. (C) Western blot of lysed *G. vaginalis* bacteria (*G. vaginalis*) and purified, recombinant VLY, probed with anti-PLY monoclonal antibody. Abbreviations for CDC proteins: PLY, pneumolysin; LLO, listeriolysin O; IVN, ivanolysin; SLG, seeligeriolysin; SPH, sphaericolysin; ALO, anthrolysin O; CER, cereolysin; PFO, perfringolysin O; ALV, alveolysin; TET, tetanolysin O; PYO, pyolysin; MLY, mitilysin; SLO, streptolysin O; SUI, suilysin; THU, thuringensolysin.

[0014] FIG. 2 Human-specific, cholesterol-dependent hemolytic activity of vaginolysin (VLY). (A) Washed human (hRBC) or sheep (sRBC) erythrocytes (1% solution in PBS) were exposed to the indicated concentrations of purified recombinant VLY for 30 min, followed by pelleting of cells. Hemoglobin release was measured by OD_{415} of the supernatant and normalized to 100% lysis for each species tested ($P<0.01$, ANOVA). (B) Erythrocytes from various species were exposed to VLY or the non-species-specific toxin pneumolysin (PLY; both toxins at 5 μg/ml) and lysis measured. (C) Addition of cholesterol (Ch) at 1 μg/ml or 10 μg/ml inhibits human erythrocyte lysis by VLY (5 μg/ml) ($P<0.001$, ANOVA).

[0015] FIG. 3 Host specificity of VLY depends on the complement regulatory molecule CD59. (A) VLY-induced lysis of human erythrocytes was inhibited by monoclonal antibody to human CD59 ($P<0.0001$) but not antibody to another GPI-anchored cell surface antigen (CD55) or mock treatment (PBS). (B) Antibody to CD59 does not inhibit PLY-mediated

lysis of human erythrocytes. (C) LDH release from Chinese hamster ovary (CHO) cells transfected with empty vector (IRES) or human CD59 (IRES-hCD59) and exposed to VLY (10 μg/ml) for 30 min. Transfection of human CD59 increases VLY-mediated lysis ($P<0.0001$). (D) Transfection of human CD59 into CHO cells does not affect PLY (1 μg/ml)-mediated lysis ($P>0.05$).

[0016] FIG. 4 VLY-mediated epithelial cell activation and erythrocyte lysis require P480. (A) Human cervical epithelial cell line HeLa was treated for 30 min with media alone (-), VLY or VLY (P480W) (1-10 μg/ml) prior to lysis and Western blotting with antibodies specific for total (p38) and phospho-p38 (pp38) MAPK. (B) HeLa cells were treated with VLY or VLY (P480W) (10 μg/ml) for 2 hr prior to RNA extraction and assay of relative quantity of interleukin-8 (IL-8) message by real-time PCR. (C) Human (hRBC) and sheep (sRBC) erythrocytes were treated with the indicated concentrations of VLY or VLY (P480W) and hemolysis assessed as above. (D) Human (hRBC) erythrocytes were treated with the indicated concentrations of pneumolysin (PLY) or PLY (W435P) and hemolysis assessed as above.

[0017] FIG. 5 Novel antibody techniques for the detection of VLY. (A) Western blot of *G. vaginalis* 14018 lysate probed with rabbit polyclonal antiserum (1:500,000 dilution). Numbers represent approximate MW in kD (B) Immunofluorescent detection of VLY production by *G.vaginalis* using pre-immune rabbit serum (left panel) or anti-VLY antiserum (right panel). Anti-rabbit IgG-AF488 was the secondary antibody (green). DNA staining with DAPI demonstrates bacteria in both panels (blue). Scale bar: 10 μm.

[0018] FIG. 6 Quantification of VLY production in vivo. (A) Detection of VLY in *G. vaginalis* supernatants by ELISA at various time points following inoculation of broth culture. (B) Bacterial growth (OD600) over the course of the experiment.

[0019] FIG. 7 Polyclonal immune serum inhibits VLY-mediated hemolysis. (A) Human erythrocytes were exposed to varying concentrations of purified recombinant VLY for 30 min. Cells were pelleted, and hemoglobin release was determined by OD415 of the supernatant. Values were normalized to 100% lysis. When indicated, VLY was preincubated with pre-bleed (VLY + PB) or immune serum (VLY + IS) diluted 1:50 for 30 min prior to

use in the assay. (B) Erythrocytes were exposed to VLY (500 ng/ml), VLY + PB, or serial dilutions of VLY + IS. ($P < 0.001$)

[0020] FIG. 8 Immune serum inhibits VLY-mediated lysis of human cervical and vaginal cells. Human cervical (A, HeLa) or vaginal (B, VK2) epithelial cells were exposed to VLY (10 μg/ml), VLY + PB, or VLY + IS. Lysis was measured by LDH release assay following 30 min of incubation with toxin. Values were normalized to 100% lysis for each cell line ($P < 0.005$).

SUMMARY OF THE INVENTION

[0021] Certain embodiments of the present invention are directed to an isolated nucleic acid encoding VLY toxin protein from *G. vaginalis*, identified as the nucleotide sequence SEQ ID No: 1 from strains 14018 or 14019, SEQ ID NO: 3 from strain 49145, or SEQ ID NO: 10 from strain ARG3, and degenerate variants or fragments thereof. Other embodiments are directed to an isolated nucleic acid encoding domain 4 of VLY from *G. vaginalis*, identified as nucleotide sequence of SEQ ID No: 4 from strains 14018 or 14019 or SEQ ID NO: 12 from strain 49145, and degenerate variants or fragments thereof. Other embodiments are directed to an isolated nucleic acid encoding the undecapeptide of VLY from *G. vaginalis*, identified as nucleotide sequence of SEQ ID No: 6, from strains 14018 or 14019 or SEQ ID NO: 14 from strain 49145, and degenerate variants or fragments thereof.

[0022] Other embodiments are directed to isolated and purified VLY protein from *G. vaginalis*, identified by the amino acid sequence set forth in SEQ ID NO: 2 from strains 14018 and 14019 and SEQ ID NO: 11 from strain ARG3, and biologically active fragments or variants thereof. Other embodiments are directed to the isolated and purified conserved undecapeptide region of VLY of *G. vaginalis*, the amino acid sequence (EKTGLVWEPWR) of which is set forth as SEQ ID NO: 7, or a biologically active fragment or variant thereof, and the undecapeptide region for toxoid 480P (EKTGLVWEWWR) set forth in SEQ ID NO: 8. Also claimed are isolated and purified domain 4 peptides of VLY of *G. vaginalis*, the amino acid sequences of which are set forth in SEQ ID NO: 5 from strains 14018 and 14019, or SEQ ID NO: 13 from strain 49145, and biologically active fragments or variants thereof. Another embodiment is directed to isolated and purified immunogenic *G. vaginalis* VLY polypeptide fragments, that include at least ten consecutive amino acid residues of SEQ ID NO: 2 from strains 14018 or 14019, and SEQ ID NO: 3 from strain 49145, and variants thereof.

[0023] Other embodiments are directed to various isolated and purified pore-forming toxoids of VLY from *G. vaginalis*, as defined herein, and to their use in a VLY vaccine.

[0024] Other embodiments are directed to a method for diagnosing and treating a *G. vaginalis* infection or *bacterial vaginosis* in a patient, the steps of which include a.) obtaining a biological sample from the patient, b.) detecting the presence of VLY protein having the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 3, or a biologically active fragment or variant tereof in the biological sample taken from the patient, and c.) if the VLY

protein is detected then administering to the patient a therapeutically effective amount of an antibiotic known to treat *G. vaginalis* or a protective agent that is a member selected from the group comprising anti-VLY antibody, anti-PLY antibody, anti-CD59 antibody, anti-pneumolysin antibody, soluble CD59, *G. vaginalis* VLY toxoid, or a biologically active fragment or variant thereof.

[0025] Other embodiments are directed to methods for treating or preventing a *G. vaginalis infection* or bacterial vaginosis in a patient, by administering to the patient a therapeutically effective amount of a protective agent that is a member selected from the group comprising anti-VLY antibodies including newly discovered rabbit polyAnti-VLY, anti-PLY antibodies, anti-CD59 antibodies, anti-pneumolysin antibodies, soluble CD59, *G. vaginalis* VLY toxoid, or biologically active fragments or variants thereof.

[0026] Other embodiments are directed to compositions for therapeutic use that include anti-VLY antibodies, preferably polyAnti-VLY, and to compositions that include two compounds selected from the group comprising soluble CD59, anti CD59, anti-VLY, anti-PLY or anti-pneumolysin monoclonal or polyclonal antibodies or immunologically active fragments or variants thereof including polyAnti-VLY, VLY toxoids or immunologically active fragments or variants thereof, and antibiotics known to treat bacterial vaginosis or *G. vaginalis.*

[0027] Other embodiments are directed to an ELISA kit for diagnosing *G. vaginalis* infection in a patient, comprising a detection antibody that specifically binds to VLY protein identified by SEQ ID NO: 2 or SEQ ID NO: 11, or a biologically active fragment thereof, including a kit wherein the detection antibody is rabbit polyAnti-VLY. In some embodiments the detection antibody is covalently bound to an enzyme. The kit optionally provides a substrate for the enzyme. Other embodiments are directed to an ELISA kit that also contains a secondary antibody covalently bound to an enzyme, which secondary antibody specifically binds to the detection antibody, and a substrate for the enzyme. Still other embodiments are directed to an Elisa kit including a capture molecule that specifically binds to VLY, including soluble CD59, anti-VLY antibody, anti-PLY antibody, and anti-pneumolysin.

[0028] Other embodiments are directed to methods for reducing or preventing the transmission of *HIV* to another human by a woman infected with HIV and bacterial vaginosis, by administering to the woman before the woman engages in a sexual activity, a therapeutically effective amount of a composition comprising a protective agent selected

from the group comprising soluble CD59, anti CD59 or anti-VLY, anti-PLY, polyAntiPLY, or anti-pneumolysin monoclonal or polyclonal antibodies, VLY toxoids or biologically active fragments or variants thereof. Other embodiments are directed to methods of reducing or preventing maternal to fetal transmission of HIV from a pregnant woman diagnosed as being infected with both HIV and bacterial vaginosis, by administering to the woman prior to a vaginal birth of a fetus, a therapeutically effective amount of a protective agent selected from the group comprising soluble CD59, anti CD59 or anti-VLY, anti-PLY, polyAntiPLY, or anti-pneumolysin monoclonal or polyclonal antibodies, VLY toxoids or fragments or variants thereof.

[0029] Another embodiment is directed to the polyclonal rabbit anti-VLY antibody poly-Anti-VLY, or an immunologically active fragment or variant thereof, ant to compositions that include this antibody.

DEFINITIONS

[0030] By "protein" or "polypeptide" is meant any chain of amino acids, regardless of length or post-translational modification (e.g., glycosylation or phosphorylation).

[0031] A "pure polypeptide" refers to a polypeptide substantially free from naturally associated molecules, i.e., it is at least 75% (e.g., at least 80, 85, 90, or 95; or 100%) pure by dry weight. Purity can be measured by any appropriate standard method, for example, by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

[0032] "Peptide variants" and "peptide modifications" are used synonymously here and mean polypeptides that may contain one or more substitutions, additions, deletions and/or insertions such that the therapeutic and antigenic properties of the peptide encoded by the variant are not substantially diminished, relative to the corresponding peptide such as VLY. Such modifications may be readily introduced using standard mutagenesis techniques, such as oligonucleotide directed site-specific mutagenesis. Variants also include what are sometimes referred to as "fragments." Peptide variants may contain one or more amino acid substitutions, additions, deletions and/or insertions. When VLY toxin and toxoids are discussed in the context of expression, activity, immunogenicity or binding to receptors on target cells, the terms include biologically active fragments and variants thereof. The amino acids used to make peptides and variants include synthetic amino acids known in the art.

[0033] As used herein, the term VLY means wild type vaginolysin ("VLY") polypeptide that is the human-specific CDC from G. *vaginalis*. VLY is the only known species-specific factor of G. vaginalis. VLY is encoded by DNA SEQ ID NO.1 in *G. vaginalis* 14018 and 14019; and by DNA SEQ ID NO. 3 in strain 49145. The amino acid sequence of VLY is set forth in SEQ ID NO. 2. As used herein VLY includes biologically active fragments and peptide variants thereof, whether naturally-occurring mutants or man-made mutants.

[0034] As used herein, the term VLY toxoid means VLY protein that has reduced pore-forming activity compared to wild type VLY due to the presence of one or more amino acid substitutions, but that retains enough immunologic activity to elicit an immune response in an animal.

[0035] Unless otherwise indicated, a "therapeutically effective amount" of a compound is an amount that provides a therapeutic benefit in the treatment or management of a disease or condition such as Bacterial vaginosa (BV) or G. *vaginalis,* delays or minimizes one or more symptoms associated with the disease or condition, or enhances the therapeutic efficacy of

another therapeutic agent. An agent is said to be administered in a "therapeutically effective amount" if the amount administered results in a desired change in the physiology of a recipient mammal, (e.g. decreases one or more symptoms of the BV or G. *vaginalis,* or decreases the amount of *G. vaginalis* in a biological sample taken from the patient to a level that is at least about 10% less than the level before drug treatment.) A therapeutically effective amount for reducing the risk of transmitting HIV from a *G. vaginalis*-infected woman to a sexual partner or a baby during birth, is an amount that reduces or eliminates viral shedding from HIV-infected cells or that reduces or eliminates the viral load in a biological sample taken from the vagina/birth canal.

[0036] Nucleic acids in the context of this invention include "oligonucleotides", which refers to an oligomer or polymer of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) or mimetics thereof. This term includes oligonucleotides composed of naturally-occurring nucleobases, sugars and covalent internucleoside (backbone) linkages as well as oligonucleotides having non-naturally-occurring portions which function similarly. Such modified or substituted oligonucleotides are often preferred over native forms because of desirable properties such as, for example, enhanced cellular uptake, enhanced affinity for nucleic acid target and increased stability in the presence of nucleases.

[0037] An "isolated nucleic acid" is a nucleic acid the structure of which is not identical to that of any naturally occurring nucleic acid or to that of any fragment of a naturally occurring genomic nucleic acid. The term therefore covers, for example, (a) a DNA which has the sequence of part of a naturally occurring genomic DNA molecule but is not flanked by both of the coding sequences that flank that part of the molecule in the genome of the organism in which it naturally occurs; (b) a nucleic acid incorporated into a vector or into the genomic DNA of a prokaryote or eukaryote in a manner such that the resulting molecule is not identical to any naturally occurring vector or genomic DNA; (c) a separate molecule such as a cDNA, a genomic fragment, a fragment produced by polymerase chain reaction (PCR), or a restriction fragment; and (d) a recombinant nucleotide sequence that is part of a hybrid gene, i.e., a gene encoding a fusion protein.

[0038] As used herein, protective agents are agents that block or reduce the toxic biological activity of VLY. They include agents that bind to or associate with VLY at a site that interferes with the ability of VLY to bind to CD59 receptor on a target host cell, or otherwise reduce the ability of VLY to cause pores in target cells, or reduce the ability of VLY to increase shedding in HIV-infected cells, or reduce *G. vaginalis* infections or BV.

Protective agents include soluble CD59 or fragments or variants thereof that are capable of neutralizing VLY; polyclonal and monoclonal antibodies to CD59, PLY, VLY, pneumolysin, and immunologic fragments or variants thereof, and VLY toxoids that can compete with the more toxic VLY for binding to CD59. Also included is newly discovered polyAnti-VLY and antibiotics that are known to treat bacterial vaginosis and *G. vaginalis* infections.

DETAILED DESCRIPTION

[0039] In the following description, for the purposes of explanation, numerous specific details are set forth in order to provide a thorough understanding of the present invention. It will be apparent, however, to one skilled in the art that the present invention may be practiced without these specific details.

[0040] Bacterial vaginosis, a chronic infectious/inflammatory disease associated with preterm birth, is strongly linked with the mucosal overgrowth of *G. vaginalis* and its attachment to epithelial cells. To summarize, we have cloned, sequenced, characterized, isolated and purified the human-specific cholesterol-dependent cytolysin (CDC) pore-forming toxin vaginolysin ("VLY") from several human strains of *G. vaginalis* (14018, 14019, 49145, and ARG3). VLY, the only known species-specific factor for *G. vaginalis*, is most closely related to intermedilysin (ILY) from *Streptococcus intermedius*. (15, 29). We have kept the name "vaginolysin" (VLY) for consistency with CDC nomenclature.

[0041] VLY lyses target cells in a species-specific manner that is dependent upon the complement regulatory molecule CD59. In addition to causing erythrocyte lysis, VLY activates the conserved epithelial p38 mitogen-activated protein kinase pathway and induces interleukin-8 production by human epithelial cells. Transfection of human CD59 into non-susceptible cells rendered them sensitive to VLY -mediated lysis. In addition, single amino acid substitutions in VLY (including some in the undecapeptide region such as (VLY(P480W)) generated toxoids that did not form pores. Introduction of the analogous proline residue into another CDC, pneumolysin, significantly decreased its cytolytic activity.

[0042] Certain embodiments of the present invention are directed to (1) isolated nucleic acid encoding VLY protein or a degenerate variant or fragment thereof from various strains of *G. vaginalis*, and to isolated and purified VLY protein, or a biologically active fragment or variant thereof, (2) an isolated and purified nucleic acid encoding domain 4 or a degenerate variant or fragment thereof from various strains of *G. vaginalis*, and to isolated and purified domain 4 region of VLY, or a biologically active fragment or variant thereof, and (3) an isolated and purified nucleic acid encoding the undecapeptide or a degenerate variant or fragment thereof from various strains of *G. vaginalis*, and the isolated and purified undecapeptide region of VLY, or a biologically active fragment or variant thereof. So far we have found that the undecapeptide region is conserved.

[0043] Certain other embodiments are directed methods for diagnosing *G. vaginalis* infection by detecting the presence of VLY protein in a biological sample, preferably using a new ELISA with detection antibodies to VLY.

[0044] Certain other embodiments are directed to various newly discovered toxoid forms of human VLY protein or an immunogenic fragment or variant thereof that has reduced pore-forming activity compared to wild-type VLY. Another embodiment is directed to a composition comprising one or more purified VLY toxoid proteins, and to a method of eliciting an immune response in an animal by introducing this toxoid composition into the animal. Other compositions of the present invention include a vaccine comprising one or more VLY toxoids.

[0045] Other embodiments include a method for permeabilizing a cell membrane (such as a cancer cell) to kill the cell or (in the case of cancer) render it more accessible to chemotherapeutic agents, comprising contacting the cell with a composition that includes purified VLY protein or a biologically active fragment or variant thereof. Another embodiment is directed to VLY toxin that is bound to a molecule that is targeted specifically to the cancer cell or VLY toxin that is bound to a chemotherapeutic agent.

[0046] We have discovered that VLY binds to CD59 receptor on epithelial cells, which enables it to permeabilize the vaginal epithelial cell. Therefore certain embodiments are directed to methods for treating or preventing *G. vaginalis* and bacterial vaginosis by administering one or more protective agents (including soluble CD59 itself, and/or Anti CD59 monoclonal or polyclonal antibodies, small molecules or anti-VLY and anti-PLY antibodies), that prevent VLY from binding to its CD59 receptor on target cells including vaginal epithelial confirm cells or otherwise block its pore-forming ability.

[0047] Using purified VLY toxin as an immunogen, we generated polyclonal rabbit immune serum (IS) having a polyclonal rabbit anti-VLY antibody that we named "polyAnti-VLY." PolyAnti-VLY inhibited VLY-mediated lysis of human cervical carcinoma cells and vaginal epithelial cells therefore polyAnti-VLY has therapeutic use and can be used in an ELISA kit for detecting VLY.

[0048] Certain embodiments of the invention are directed to methods that treat BV in order to reduce transmission of HIV from an HIV/BV-infected woman to her sexual partner or to a fetus she may be carrying, or to an infant during childbirth by administering one or more of the described protective agents.

The G. vaginalis genome contains an orthologue of known CDCs

[0049] The CDC family is made up of more than 15 protein toxins produced by several distinct gram-positive genera (reviewed in (38)). The basic local alignment search tool (BLAST) was used to compare raw genomic DNA sequence data from the *Gardnerella vaginalis* 14018 genome project (available at ttp://med.stanford.edu/sgtc/research/gardnerella_vaginalis.html (available at) with a database of known microbial genes (Comprehensive Microbial Resource, J. Craig Venter Institute). We identified a 1551 base pairs (bp) open reading frame with 54% DNA sequence identity to pneumolysin, the *S. pneumoniae* CDC that we proceeded to analyze. This genomic region was amplified from *G. vaginalis* 14018 (ATCC) by PCR, cloned, and sequenced. See Example 1. SEQ ID NO: 1 sets forth the DNA sequence encoding VLY protein from *G. vaginalis* species 14018 and 14019, which are identical. VLY made by *G. vaginalis* strain 49145 is set forth in SEQ ID NO. 3. Certain embodiments are directed to nucleic acids identified by SEQ ID NO: 1 and 3, or a biologically active fragment or variant thereof. Other embodiments are directed to a vector that includes the DNA SEQ ID NO: 1 or 3 encoding VLY protein from strains 14018 and 14019, or 49145, respectively, or a biologically active fragment or variant thereof.

[0050] The predicted amino acid sequence of VLY (from strains 14018 and 14019) set forth in SEQ ID NO. 2, exhibits sequence similarity and identity consistent with reported relationships among members of the CDC family (Appendix). The predicted sequence of VLY from *G. vaginalis* strain (ATCC 49145) is identical with the exception of a single amino acid substitution (R494H-See SEQ ID NO. 3). Certain embodiments are directed to the isolated and purified (naturally occurring and recombinant) VLY proteins described herein and to biologically active variants and fragments thereof, including those having the amino acid sequences set forth in SEQ ID NO: 2 for VLY from strains 14018-19), and the amino acid sequence set forth in SEQ ID NO: 11 for VLY from ARG3. An embodiment of the invention is directed also to Codon optimized VLY gene sequence (that has the same protein sequence as VLY from strain 14018) DNA SEQ ID NO: 9 which greatly improves yield and purity of recombinant toxin made in *E. coli*.

[0051] The phylogram of CDC protein sequences (FIG. 1A) demonstrates three distinct groupings - a *Streptococcus* group (into which VLY falls), a *Listeria* group, and a *Bacillus/Clostridium* group (also containing SLO). The members of the *Streptococcus* clade have the most divergence in the domain 4 undecapeptide, including the presence of a proline

residue as an insertion (pyolysin) or substitution (intermedilysin, VLY). In the case of pyolysin, the unusual undecapeptide has been shown to be required for pore formation (3). Seeligeriolysin, the CDC from *L. seeligeri*, has an alanine to phenylalanine substitution in the undecapeptide that causes a decrease in toxin efficacy compared to listeriolysin 0 (21).

[0052] A phylogram of representative full-length CDC sequences (FIG. 1B) obtained from publicly available databases (Appendix) was constructed using the neighbor-joining algorithm. By this analysis, VLY appears to be most closely related to ILY and it falls within a group consisting of most of the CDC from genus *Streptococcus*, including pneumolysin, mitilysin, and suilysin. Pyolysin, from *Arcanobacterium pyogenes* (2), is the least similar member of this group. VLY is more distantly related to CDC from the *Bacillus, Listeria, and Clostridium* genera, as well as streptolysin O from *Streptococcus pyogenes*, which is divergent from the other streptococcal CDC. Bootstrap analysis indicates a high degree of confidence for the placement of VLY in the streptococcal group (data not shown).

[0053] The undecapeptide is an 11 amino acid sequence in domain 4 of the CDCs that is well conserved and of particular importance for host cell interaction and pore formation (38). The nucleic acid sequences encoding the undecapeptide of *G. vaginalis* are set forth in DNA SEQ ID NO: 6 for strains 14018 and 14019 (nucleic acid residues 1414-1446), and DNA SEQ ID NO: 14 for strain 49145. The VLY undecapeptide is divergent from the CDC consensus sequence at 3 of 11 sites (FIG. 1B), one of which is an alanine to valine substitution. More strikingly, there is a proline substitution at VLY position 480, the site of one of the conserved tryptophan residues that is important for pore formation in other CDC family members (24). The loss of the conserved cysteine residue at the second position of the undecapeptide is seen in two other CDC, ILY and pyolysin, and is consistent with the prior report of insensitivity of the *G. vaginalis* toxin to reducing agents (35). Western blot analysis of lysed *G. vaginalis* bacteria demonstrates a ~57 kD band that cross reacts with a monoclonal antibody directed against the *S. pneumoniae* CDC, pneumolysin.

[0054] Purified, recombinant human-specific VLY (from strains 14018 and 14019) identified by amino acid or a fragment or variant thereof, that also comes within the scope of this invention, migrates at a similar molecular weight to natural VLY and is also detected by anti-pneumolysin antibody (FIG. 1C). One embodiment of the invention is directed to treating a *G. vaginalis* infection by administering anti-pneumolysin antibody and/or anti-VLY antibody, preferably locally in the form of a gel vaginal suppository.

[0055] Our results show that domain 4 stretches from the conserved tyrosine at position 376 (amino acid position) to the end of the protein which is 516 amino acids long. Certain embodiments are directed to the corresponding DNA SEQ ID NO: 4 encoding domain 4 that begins at nucleotide 1126 and continues to the end of the DNA molecule for strains 14018-9 and SEQ ID NO: 12 (for strain 49145), and to amino acid sequences of domain 4 as set forth in SEQ ID NO: 5 (for strains 14018-9) and SEQ ID NO: 13 (for strain 49145).

Species-specific, cholesterol-dependent hemolytic activity of VLY

[0056] Recombinant VLY produced in *E. coli* was used for studies of toxin interaction with target cells. Purified recombinant VLY lysed primary human erythrocytes in a dose-dependent fashion (FIG. 2A). In contrast, sheep (FIG. 2A-B), mouse (FIG. 2B) and horse (data not shown) erythrocytes were resistant to lysis even at substantially higher VLY concentrations. Erythrocytes from all of these species were lysed by pneumolysin, a non-species-restricted member of the CDC family (FIG. 2B). Preincubation of VLY with cholesterol inhibited lysis of human erythrocytes in a dose-dependent manner, consistent with its classification within the CDC family (FIG. 2C). Thus VLY is species specific for lysing human cells.

[0057] We have also noted profound ultrastructural changes in epithelial cells exposed to sublytic quantities of VLY (about 250 ng/ml) using live-cell imaging of HeLa cells. This response occurred rapidly (<1 min) following exposure of cells to VLY, and blebs remained intact and present over the course of hours prior to resolution. These blebs were contiguous with the epithelial cell cytoplasm, as demonstrated with hCD59-IRES-GFP transfected CHO cells, in which cytoplasmic GFP was observed entering the blebs. Most strikingly, these ultrastructural changes were recapitulated by antibody-mediated cross-linking of the hCD59 receptor and by binding of a non-pore-forming GFP-VLY-D4 fusion protein. These findings are consistent with signaling through hCD59 as a potential mechanism for initiating ultrastructural changes. We hypothesize that this bleb formation represents a novel pathway of toxin recognition by epithelial cells' unique response to hCD59-dependent toxins and involves signaling pathways initiated by hCD59 binding.

VLY lytic activity requires binding to complement regulatory molecule CD59 on the surface of epithelial cells

[0058] The human specificity of the *G. vaginalis* hemolysin was noted in earlier studies (8), but no specific mechanism for this was described. The recent characterization of human CD59 as a receptor for intermedilysin (ILY) represented a major step forward in the understanding of the mechanism of action of CDC, which were previously thought to bind cholesterol directly as the sole requirement for pore-formation. The results presented below show that that this model is oversimplified and that at least a subset of the CDCs including VLY require protein receptors on the surface of target cells (15).

[0059] The results in FIG. 3A show that blockade of CD59 on the surface of primary human erythrocytes using monoclonal antibody MEM-43 clone abrogated VLY-induced lysis. By contrast, antibody against another erythrocyte surface marker CD55 was ineffective, anti-CD59 antibodies for use in treating *G. vaginalis* and bacterial vaginosis infections are commercially available for example from- Santa Cruz Biotechnology and Genetex. Thus surface CD59 on epithelial cells is the receptor for VLY and binding of CD59 by VLY is necessary for lytic activity.

[0060] Our results showed that the activity of pneumolysin, a CDC that does not exhibit host specificity, was not inhibited by either anti-CD59 or anti-CD55 (FIG. 3B). However, transfection of human CD59 into Chinese hamster ovary (CHO) cells significantly increased lactate dehydrogenase release in the setting of treatment with VLY (FIG. 3C) but not pneumolysin (FIG. 3D), indicating that CD59 is sufficient to confer susceptibility on at least a subset of VLY resistant cells.

[0061] CD59 is expressed on the surface of human genital tract epithelial cells, the target cell type during *G. vaginalis* colonization and BV, as demonstrated by immunofluorescence staining with monoclonal antibody against hCD59 (MEM-43 clone). Secondary anti-mouse IgG-Alexa Fluor 488 (green) was used. hCD59 has also been shown to be highly expressed in the female genital tract in vivo, along with other complement regulatory molecules (70).

[0062] Certain embodiments of the invention are directed to methods for treating *G. vaginalis* infection by (1) administering soluble CD59 itself (CD59 with the GPI anchor deleted) to saturate or neutralize VLY thereby preventing it from binding to CD59 receptor on vaginal epithelial cells, or (2) administering anti-CD59 or anti-VLY polyclonal or monoclonal antibodies that prevent binding of VLY to the CD59 receptor on vaginal epithelial cells. Monoclonal Anti-CD59 antibodies are commercially available from several sources.

[0063] Anti-VLY antibodies can be directed to the VLY protein or to a biologically active fragment or variant thereof, including domain 4 and the undecapeptide regions of VLY. In a preferred embodiment, the therapeutic anti-CD59 and anti-VLY antibodies and CD59 are locally administered, for example as a vaginal suppository. Such local administration is not only efficient requiring fewer antibodies, but it also enables a high concentration of the therapeutic agent to be delivered to the target cells. Since CD59 is ubiquitous and plays an important role protecting cells from damage by complement, it is also important to specifically target CD59 expressed on the surface of the target epithelial cells, and not administer it systemically.

G. vaginalis is a gram-positive bacterium

[0064] The discovery of the pore-forming toxin VLY in *G. vaginalis* expands the CDC family to another gram-positive genus and to a novel anatomic site. CDCs are produced by organisms that colonize and cause disease at mucosal surfaces including the upper and lower respiratory tracts and the gastrointestinal tract. In many such cases, toxin production has been shown to be essential for maintenance of colonization, pathogenesis of invasive disease, or both (38). CDCs have been described only in gram-positive organisms. Thus, the characterization of VLY and its evolutionary relationship to the other CDC provides further evidence that *G. vaginalis* is most properly grouped with the gram-positives, despite its variable staining characteristics (36). Antibiotics that have been used to treat BV and *G. vaginalis* infections include metronidazole, clindamycin and tinidazole.

[0065] Metronidazole is the most successful therapy. Most comparative studies using multiple divided-dose oral regimens for one week achieved early rates of clinical cure in excess of 90 percent, and cure rates (by Amsel criteria) of approximately 80 percent at four weeks. A randomized trial showed that short-term cure rates were significantly higher when the initial course of metronidazole therapy was 14 days rather than 7 days (Schwebke, JR, Desmond, RA. A randomized trial of the duration of therapy with metronidazole plus or minus azithromycin for treatment of symptomatic bacterial vaginosis. Clin Infect Dis 2007; 44:213.). However, long-term cure rates (21 days after completion of therapy) were similar for both treatment regimens. In one embodiment, the oral regimen of tronidazole is 500 mg twice daily for seven days. Sexually Transmitted Diseases Treatment Guidelines, 2006. MMWR Recomm Rep 006. (RR-11); 55:1-95. Topical vaginal therapy with 0.75 percent

metronidazole gel (5 g once daily for five days) is as effective as oral metronidazole. The choice of oral versus topical therapy depends upon patient preference.

[0066] Clindamycin can be used as a topical vaginal therapy with 2 percent clindamycin cream (5 g of cream containing 100 mg of clindamycin phosphate) as a seven-day regimen. Alternative regimens include oral clindamycin (300 mg twice daily for seven days) or clindamycin ovules (100 mg intravaginally once daily for three days) (Sexually Transmitted Diseases Treatment Guidelines, 2006. MMWR Recomm Rep 2006 (RR-11); 55:1-95; Paavonen, J, Mangioni, C, Martin, MA, Wajszczuk, CP. Vaginal clindamycin and oral metronidazole for bacterial vaginosis: a randomized trial. Obstet Gynecol 2000; 96:256). A one-day or single application of clindamycin as a bioadhesive has also been approved by the FDA (Clindesse). These regimens have not been studied extensively and may have lower efficacy for eradicating BV.

[0067] Tinidazole is a second generation nitroimidazole. It has a longer half-life than metronidazole (12 to 14 hours versus 6 to 7 hours) and fewer side effects (Tinidazole (Tindamax)--a new option for treatment of bacterial vaginosis. Med Lett Drugs Ther 2007; 49:73). In one embodiment, 1 g tindazole is administered orally once daily for five days, as efficacy is slightly higher and side effects are slightly less frequent than with shorter course therapy (tinidazole 2 g orally daily for two days).

VLY Toxoids: The proline residue in the variant undecapeptide of VLY (residue 480) is required for cytolytic and cell stimulatory activity

[0068] Although hemolysis is a useful model for toxin-induced pore formation, erythrocytes are unlikely to be a target cell for *G. vaginalis* under normal physiologic conditions, as *Gardnerella* bacteremia is exceedingly rare (11, 33). Activation of p38 mitogen-activated protein kinase (MAPK) is a conserved element in epithelial detection of bacterial pore-forming toxins (32) and appears to be essential in defense of host cells from toxin attack (19). Exposure of the human cervical epithelial cell line HeLa to VLY led to phosphorylation of p38 MAPK within 30 minutes (FIG. 4A), consistent with epithelial responses to other pore-forming toxins (32). FIG. 4B shows that IL-8 mRNA is upregulated in HeLa cells by VLY and is dependent on there being a proline at position 480 in the undecapeptide. The data showing that VLY activates the p38 MAPK and IL-8 pathways in human epithelial cells, shows that VLY produced by *G. vaginalis* as a major factor in the immunopathology of BV.

[0069] Nucleic acid and amino acid sequence alignments are set forth in the Appendix. At the protein level, the undecapeptide is identical *G. vaginalis* strains 14018 and 14019. DNA sequences encoding VLY are identical for strains 14018 and 14019, and strains 49145 and ARG3 are very close with several single base substitutions (seen in the alignment). For example, there is a single (silent) base pair substitution (G> A at nucleotide position 1428) in 49145 compared to the other two (l40l8 and 14019). One nucleotide substitution leads to a change in VLY protein sequence (R->H at amino acid position 494) in the 49145 strain. This change is in domain 4 but after the undecapeptide region.

[0070] Genetic and structural studies have implicated domain 4 (D4) of the CDC as being crucial for membrane association. Specifically, the undecapeptide appears to have a significant role in CDC function. Because of the importance of the undecapeptide to toxin function, we created a mutant named VLY(P480W) using site-directed mutagenesis to make a single amino acid substitution of tryptophan was for proline at position 480. This change converted the proline residue in the VLY undecapeptide to the consensus tryptophan residue, which has been shown to be crucial to the function of several other CDC that contain the consensus undecapeptide.

[0071] Notably, the P480W mutation produced a VLY toxoid that was substantially less effective at lysing human erythrocytes (FIG. 4C). In addition, this proline to tryptophan mutation abolished p38 activation and IL-8 transcription in HeLa cells (FIG. 4A-B) compared to wild-type VLY (FIG. 4A-B). This proline residue does not dictate species-specificity but appears to be crucial for toxin function. These findings underscore the importance of D4, and specifically the undecapeptide, to CDC function, even among the hCD59-dependent CDCs. These results confirm that proline at amino acid residue 480 of VLY is necessary for efficient pore-formation and cell activation by VLY. Because the P480W toxoid is significantly less toxic (lytic activity is reduced) than native VLY toxin, it can be used in vaccines that elicit a specific immune response to VLY toxin.

[0072] In order to investigate further the potential role of proline in pore forming activity, we made the converse mutation at the corresponding location (W435P) in pneumolysin, the species-non-specific CDC from *S. pneumoniae*. This pneumolysin mutant lysed erythrocytes, but only at concentrations much higher than wild-type toxin (FIG. 4D). This indicates that a substitution of tryptophan (W) for proline caused a loss of pore-forming activity. Construction of the corresponding mutation in PLY (i.e. substitution of proline for tryptophan at position 435) similarly led to a substantial decrease in its lytic activity.

[0073] Certain embodiments of the invention are also directed to toxoids of PLY and penumolysin, wherein there is an amino acid substitution of tryptophan for proline in the undecapeptide, and to the use of the PLY toxoids to elicit an immune response in a host.

[0074] These findings emphasize the importance of the structure of the undecapeptide region to the function of CDCs. Likewise, the substitution of a lysine residue for the conserved cysteine in the undecapeptide is a modification unique to VLY. Prior reports have demonstrated that the *G. vaginalis* hemolysin is not thiol-activated (35). The lack of enhancing effect of a reducing agent is consistent with this modification in the undecapeptide. Of note, in other CDC family members, the conserved cysteine residue confers thiol-activating properties but is not essential for pore-forming activity (37).

[0075] The following newly identified toxoids all have decreased pore-forming activity compared to the wild-type toxin, and are therefore useful in making a vaccine or antibodies against VLY or eliciting an immune response in a patient against *G. vaginalis*. Certain embodiments are directed to the toxoids listed below:

P480W, described above

N500I at position 500 change asparagines to isoleucine.

P480W, N500I double mutant

V471R, K473C double mutant

V471R, K473C, P480W triple mutant

[0076] Other embodiments are directed to toxoids in which:

1. Some or all of domain 4 is deleted. (a portion of the sequence can be removed, or substituted or a premature stop codon can be inserted); and

2. Some or all of the amino acids in the undecapeptide (aa472-483) are replaced with alanine or phenylalanine; substitutions for the proline at position 480 in native VLY as stated before are particularly useful.

[0077] Certain embodiments of the present invention are directed to isolated and purified VLY recombinant toxoid proteins or an immunologic fragment or variant thereof, including P480W, N500I, V471R, K473C, P480W- N500I double mutant, V471R-K473C double mutant, and V471R-K473C-P480W triple mutant, or immunologic fragments or variants thereof, and to other purified toxoids described herein (including purified recombinant forms). Other embodiments include recombinant purified VLY protein or toxoids that have a

6xHis tag on both the N-terminus (beginning) and C-terminus (end) of the protein to maximize purification. The tags can be removed after the VLY protein or toxoids are isolated and purified.

[0078] Other embodiments are directed to a composition comprising any of these toxoids or an immunologic fragment or variant thereof, for example to be used as immunogens in vaccines. Other embodiments are directed to a method for generating an immunologic response to VLY in an animal by administering to a patient a composition that includes one or more VLY toxoids such as the VLY (P480W) toxoid (or an immunologic fragment or variant thereof.

New rabbit polyclonal anti-VLY antibodies detect G. vaginalis by western blot and immunofluorescence

[0079] Recombinant purified VLY toxin generated and purified as described in the Examples was submitted to Cocalico Biologicals, Inc. (Reamstown, PA). According to their protocol, adult rabbits were injected with a minimum of 100 µg antigen mixed with Complete Freund's Adjuvant subcutaneous and/or intramuscularly at multiple sites. Booster doses containing a minimum of 50 µg antigen mixed with Incomplete Freund's Adjuvant were administered on days 14, 21 and 49. A test bleed was performed on day 56. Prior to the first immunization, serum was collected from each rabbit to serve as negative control. We named the new anti-VLY polyclonal antibody thus derived "polyAnti-VLY;" certain embodiments of the invention are directed to this antibody and its therapeutic use to treat *G. vaginalis* infections and BV, and to prevent transmission of HIV to another from a woman infected with *G. vaginalis* or having BV.

[0080] Western blot analysis of lysed *G. vaginalis* 14018 revealed a single band using polyclonal immune serum (polyAnti-VLY) as the primary antibody (FiG. 5A). This corresponds to the predicted 57 kDa molecular mass of VLY and to our prior findings using cross-reacting anti-pneumolysin antibody (50). There were no visible bands detected on membranes probed with pre-immune serum and processed identically (data not shown). Immunofluorescent detection of VLY associated with whole *G. vaginalis* was detected microscopically using immune serum and fluorescently labeled anti-rabbit secondary antibodies (FiG. 5B). Preimmune serum did not lead to detectable fluorescence of *G. vaginalis* (FiG. 5B).

[0081] Certain embodiments of the present invention are directed to the new rabbit polyAnti-VLY antibody, and fragments and variants thereof and to compositions that include the antibody that are intended for use in a mammal, particularly a human. Other embodiments are directed generally to polyclonal antibodies to VLY made by immunizing an animal with the isolated recombinant VLY toxins or a VLY toxoid as herein described, or an immunologically active fragment or variant thereof. Anti-VLY antibodies will bind to free VLY in the body. Other embodiments are directed to monoclonal anti-VLY antibodies made using well known hybridoma technology. In certain embodiments the anti-VLY antibodies can be humanized (as antibody or antibody fragments) and then administered therapeutically to a patient to confer passive immunity to *G. vaginalis* or BV or to neutralize VLY either systemically or locally in the vagina to prevent it from binding to CD 59. The antibodies can also be used in ELISA or RIA assays to detect the presence of VLY in a biological sample from a patient as is described below.

New ELISA that detects VLY production by *G. vaginalis*

[0082] We developed a sandwich ELISA assay capable of quantifying VLY at ng/ml concentrations in the supernatant of growing *G. vaginalis* using rabbit polyAnti-VLY (diluted 1:1000 in blocking solution) as the detection antibody and known concentrations of recombinant VLY toxin diluted in *G. vaginalis* culture medium as a standard. As a secondary antibody we used goat anti-rabbit HRP antibody 1:100 dilution). We found that VLY toxin production peaked at between 24 and 36 hours of *G. vaginalis* in culture (FIG. 6A) and directly correlated with bacterial concentration as determined by optical density (FiG. 6 B). The ELISA technique is robust, even in the setting of potential inhibitors (such as serum) and will be useful for quantifying VLY production both in vitro and in vivo. The ELISA based assay in particular, is sensitive, robust and directly correlates with the concentration of G. vaginalis, reported to be an independent predictor of BV and subsequent preterm delivery. (61-65)

[0083] As an alternative means to assess VLY regulation, we also developed a sensitive real-time PCR assay targeting the VLY gene. This assay, which can be used either to detect DNA from organisms with the toxin gene or to monitor VLY RNA levels during different *G. vaginalis* growth conditions, represents an important strategy for our continued investigations.

[0084] Certain embodiments of the invention are directed to a kit for detecting VLY toxin or fragment that includes an anti-VLY antibody (including rabbit polyAnti-VLY) as the detection antibody, or anti-pneumolysin antibody, anti-PLY antibody, anti-ILY antibody or any other antibody that cross reacts with VLY. Such a kit can be used as a diagnostic tool for *G. vaginalis* infections and bacterial vaginosis by testing the presence of VLY in a biological sample from a patient. The biological sample is preferably a vaginal swab that has been diluted in sterile saline or PBS before the assay is run. It is expected that the swab will have not only vaginal cells, but also free VLY toxin. Another sample can be obtained by instilling 1-5 ml sterile saline into the vaginal of patient, and then collecting a biological sample from the vagina for the assay.

[0085] An embodiment of a kit for a sandwich ELISA optionally includes a secondary antibody (specific for the detection antibody)conjugated to an enzyme or other compound known in the art (including fluorescent labels/biotin avidin, radiolabels) that permits detection of the binding of the secondary antibody to the detection antibody.

[0086] In one embodiment the rabbit polyAnti-VLY is conjugated to an enzyme for an indirect assay. A major disadvantage of the indirect ELISA is that the method of antigen immobilization is non-specific; any proteins in the sample will stick to the microtiter plate well, so small concentrations of analyte in serum must compete with other serum proteins when binding to the well surface. The sandwich ELISA provides a solution to this problem: (1) Plate is coated with a capture antibody; (2) sample is added, and any antigen present binds to capture antibody; (3) detecting antibody is added, and binds to antigen; (4) enzyme-linked secondary antibody is added, and binds to detecting antibody; (5) substrate is added, and is converted by enzyme to detectable form.

[0087] In yet another embodiment the kit includes a capture antibody that binds VLY toxin to the substrate, thereby preventing nonspecific binding of peptides or proteins in the sample. The capture antibody can be anti-penumolysin antibody that we used (such as clone 1F11 or other clones that cross-reacts with VLY), or soluble CD59, anti-VLY antibodies including polyAnti-VLY, anti-PLY antibody, anti-pneumolysin, or monoclonal anti-VLY.

[0088] An ELISA may be run in a qualitative or quantitative format. Qualitative results provide a simple positive or negative result for a sample. The cutoff between positive and negative is determined by the analyst and may be statistical. Two or three times the standard deviation is often used to distinguish positive and negative samples. In quantitative ELISA,

the optical density or fluorescent units of the sample is interpolated into a standard curve, which is typically a serial dilution of the target.

Antiserum against VLY inhibits toxin-mediated cytolysis

[0089] To test the biological activity of our antiserum to VLY, we studied toxin-mediated cytolysis of human erythrocytes, which are susceptible to hemolysis at various concentrations of purified recombinant VLY for 30 minutes (FiG. 7A). Pre-incubation of VLY with polyAnti-VLY (immune serum 1:50 dilution) prior to exposure to human erythrocytes resulted in significantly less hemolysis compared to untreated cells or cells exposed to pre-immune serum-treated VLY (FiG. 7A) Inhibition of VLY-mediated lysis by immune serum was dose-dependent (FiG. 7B).

[0090] Similarly, VLY-mediated cell lysis of human epithelial cell lines HeLa (FiG. 8A) and VK2 (FiG. 8B) was markedly reduced in the setting of immune serum. In order to generate a probe for toxin-hCD59 interactions, we created a GFP:VLYD4 fusion protein (not shown). This protein binds to hCD59-expressing human epithelial cells but does not form pores. It also can be used therapeutically to treat BV or *G. vaginalis* infections.

Treatment of G. vaginalis infections and BV

[0091] The results above show that anti-VLY antibodies in general and polyAnti-VLY in particular can be used therapeutically to treat *G. vaginalis* infections by neutralizing free VLY. Such antibodies can be administered locally in the vagina as a topical formulation, or systemically. When the anti-VLY antibodies are administered locally/topically to the vagina, It is expected that the disadvantages of using rabbit antibodies instead of human antibodies is minimized.

[0092] New compositions that come within the scope of the invention for treating BV or *G. vaginalis* infections, or for preventing transmission of HIV from a BV/HIV-infected woman include:

- polyclonal and monoclonal anti-VLY antibodies (hereafter "anti-VLY antibodies") including the new anti-VLY antibody we described and named "rabbit polyAnti-VLY"

Compositions comprising two or more of the following:

- anti-VLY antibodies, or fragments or variants thereof
- soluble CD59 or a fragment or variant thereof that binds to VLY

- anti-CD59 monoclonal or polyclonal antibodies that prevent VLY from binding to CD59 on the surface of epithelial cells or that prevent activation of surface CD59 by VLY, or fragments or variants thereof
- antibiotics known to be used in the treatment of BV or *G. vaginalis* infections, described herein
- anti-pneumolysin antibody, or fragments or variants thereof
- anti-VLY antibody, or fragments or variants thereof
- VLY toxoids which will compete with VLY toxin for binding to surface CD59 and that can be used for vaccines.

Reducing the transmission of HIV by an infected woman

[0093] BV has been repeatedly associated with both a significant risk of HIV acquisition and increased viral shedding among those already infected 43-45. *In vitro*, treatment of HIV-infected cells with *Gardnerella* leads to increased production of viral transcripts. *J Infect Dis* 179: 924, *Lancet* 353: 525, and *J Bacteriol190: 3896,* incorporated herein by reference. Treatment of HIV-infected U -1 cells with purified VLY toxin *in vitro* caused a significant increase in HIV transcripts and p24 release. (Unpublished observations). Therefore, treatment or prevention of BV in women will also greatly reduce their susceptibility to becoming infected with HIV. An embodiment of the invention is directed to methods for reducing or preventing the transmission of HIV by a woman infected with both HIV and BV to another through intercourse by administering to the woman before she engages in a sexual activity, a therapeutically effective amount of a composition comprising a protective agent as described herein. In one embodiment the agent is applied topically to the vagina of the infected woman before engaging in the sexual activity. Vagina and birth canal are used synonymously herein.

[0094] The woman can be treated either topically or systemically or both. The protective agent(s) bind to VLY, or bind to CD59 receptor on target cells blocking the binding of VLY. In one embodiment, VLY toxoids as described herein bind to CD59 thereby preventing the toxin VLY from binding and lysing cells. Without being bound by theory we speculate that the protective agent neutralizes VLY produced from *G. vaginalis* infected cells thereby preventing it from binding to CD59 receptor on HIV-infected cells; this prevents VLY from increasing viral shedding by HIV-infected cells, which in turn reduces the risk of HIV infection to the uninfected sexual partner. A therapeutically effective amount of the

protective agent is an amount that reduces or prevents transmission of HIV, preferably by reducing shedding by HIV-infected cells thereby reducing the viral load of HIV in a biological sample taken from the birth canal of the woman. A therapeutically effective amount that reduces viral load in a biological sample can be determined using routine experimentation.

[0095] Approximately 7,000 human immunodeficiency virus (HIV)-infected women give birth in the United States each year. This number is many times higher in Africa. Without treatment, about one-fourth of them transmit the virus to their children. The anti-HIV drug zidovudine (AZT), given to HIV-infected pregnant women before and during childbirth and to their infants after childbirth, reduces HIV transmission by as much as two-thirds. Treatment with AZT is now the standard of care in the U.S. for preventing HIV infection in infants. However, additional means are needed for the prevention of maternal to fetal transmission of HIV and other enveloped viruses both in the U.S. and worldwide. We have discovered compositions and methods that reduce the risk of a woman who is both HIV and BV positive transmitting HIV to an unborn fetus during the birth process.

[0096] One embodiment of the invention is directed to a method of reducing maternal to fetal transmission of HIV where the pregnant woman is infected with both HIV and BV, by administering a therapeutically effective amount of a protective agent either topically or systemically, or both, especially before and during a vaginal birth. Treatment of the pregnant HIV- and BV-infected individual should begin as soon as she is identified to reduce the viral load in the peripheral blood of the woman to both treat the BV and reduce the risk of the fetus becoming infected with HIV either before or during the birth.

[0100] When birth is approaching, treatment should be undertaken during labor until birth, with repeated topical application. Routine experimentation will determine the optimum schedule of administration. A therapeutically effective amount includes an amount that reduces or eliminates free HIV virus in a biological sample from the pregnant woman, such as a blood sample or a sample of vaginal secretion.

[0101] In another embodiment for preventing transmission of BV, *G. vaginalis* or HIV to a newborn, the preventative agent is administered topically to the newborn immediately after birth, for example, to an exposed tissue of the newborn such as the umbilical cord. The application of the preventive agent directly to the newborn can contribute to the reduction or elimination of a HIV viral particles that may be shed by any HIV-infected cells that remain from maternal-derived biological material on the newborn.

[0102] The methods described herein to reduce or prevent maternal to fetal transmission of an enveloped virus can be used in combination with one or more known treatments of HIV or BV, including using antiviral agents or antibacterial agents

Antibodies for use in the present invention

[0103] "Antibody" or "antibodies" as described herein include intact molecules as well as fragments thereof that are capable of binding to an epitope of a VLY polypeptide or VLY toxoid, as described herein, to a fragment or variant thereof. The term "epitope" refers to an antigenic determinant on an antigen to which an antibody binds. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains, and typically have specific three-dimensional structural characteristics, as well as specific charge characteristics. Epitopes generally have at least five contiguous amino acids. The terms "antibody" and "antibodies" include polyclonal antibodies, monoclonal antibodies, humanized or chimeric antibodies, single chain Fv antibody fragments, Fab fragments, and $F(ab)_2$ fragments. Polyclonal antibodies are heterogeneous populations of antibody molecules that are specific for a particular antigen, while monoclonal antibodies are homogeneous populations of antibodies to a particular epitope contained within an antigen. Monoclonal antibodies are particularly useful.

[0104] The term "specifically binds" as used herein refers to the situation in which one member of a specific binding pair does not significantly bind to molecules other than its specific binding partner(s) as measured by a technique available in the art, e.g., competition ELISA. The term is also applicable where e.g. an antigen-binding domain of an antibody of the invention is specific for a particular epitope that is carried by a number of antigens (for example by VLY or fragment thereof such as domain 4 and the undecapeptide, or a VLY toxoid), in which case the antibody carrying the antigen-binding domain will be able to specifically bind to the various antigens carrying the epitope. The term "epitope" refers to that portion of a molecule capable of being recognized by and bound by an antibody at one or more of the antibody's antigen-binding regions.

[0105] "Specific binding" of an antibody also means that the antibody exhibits appreciable affinity for antigen or a preferred epitope and, preferably, does not exhibit significant crossreactivity. "Appreciable" or preferred binding include binding with an affinity of at least 10^6, 10^7, 10^8, 10^9 M^{-1}, or 10^{10} M^{-1}. Affinities greater than 10^7 M^{-1}, preferably greater than 10^8 M^{-1} are more preferred. Values intermediate of those set forth

herein are also intended to be within the scope of the present invention and a preferred binding affinity can be indicated as a range of affinities.

[0106] Antibody fragments that have specific binding affinity (as defined below) for VLY polypeptides can be generated by known techniques. Such antibody fragments include, but are not limited to, $F(ab')_2$ fragments that can be produced by pepsin digestion of an antibody molecule, and Fab fragments that can be generated by deducing the disulfide bridges of $F(ab')_2$ fragments. Alternatively, Fab expression libraries can be constructed. See, for example, Huse et al. (1989) Science 246:1275-1281. Single chain Fv antibody fragments are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge (e.g., 15 to 18 amino acids), resulting in a single chain polypeptide. Single chain Fv antibody fragments can be produced through standard techniques, such as those disclosed in U.S. Pat. No. 4,946,778.

[0107] Once produced, antibodies or fragments thereof can be tested for recognition of a VLY polypeptide or toxoid by standard immunoassay methods including, for example, enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay assay (RIA). See, Short Protocols in Molecular Biology eds. Ausubel et al., Green Publishing Associates and John Wiley & Sons (1992). Suitable antibodies typically have equal binding affinities for recombinant and native proteins.

[0108] An "antibody" refers to an intact immunoglobulin or to an antigen-binding portion thereof that competes with the intact antibody for specific binding. Antigen-binding portions may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. Antigen-binding portions include, inter alia, Fab, Fab', F(ab').sub.2, Fv, dAb, and complementarity determining region (CDR) fragments, single-chain antibodies (scFv), chimeric antibodies, diabodies and polypeptides that contain at least a portion of an immunoglobulin that is sufficient to confer specific antigen binding to the polypeptide. An "immunoglobulin" is a tetrameric molecule. In a naturally-occurring immunoglobulin, each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy terminal portion of each chain defines a constant region primarily responsible for effector function.

[0109] The monoclonal antibodies for use in the invention may be obtained by any technique which provides for the production of antibody molecules by continuous cell lines

in culture. These include, but are not limited to, the hybridoma technique of Kohler and Milstein, (1975, Nature 256:495-497; and U.S. Pat. No. 4,376,110), the human B-cell hybridoma technique (Kosbor et al., 1983, Immunology Today 4:72; Cole et al., 1983, Proc. NatL Acad. Sci. USA 80:2026-2030), and the EBV-hybridoma technique (Cole et al., 1985, Monoclonal Antibodies And Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclasses thereof. The hybridoma producing the mAb may be cultivated *in vitro* or *in vivo*.

[0110] Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, DELTA, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See generally, Fundamental Immunology Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). The variable regions of each light/heavy chain pair form the antibody binding site such that an intact immunoglobulin has two binding sites. Immunoglobulin chains exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. The CDRs from the two chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N terminus to C terminus, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance 44 with the definitions of Kabat Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk J. Mol. Biol. 196:901 917 (1987); Chothia et al. Nature 342:878 883 (1989).

[0111] An Fab fragment is a monovalent fragment consisting of the VL, VH, CL and CH I domains; a F(ab').sub.2 fragment is a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment consists of the VH and CHI domains; an Fv fragment consists of the VL and VH domains of a single arm of an antibody; and a dAb fragment (Ward et al., Nature 341:544 546, 1989) consists of a VH domain. A single-chain antibody (scFv) is an antibody in which a VL and VH regions are paired to form a monovalent molecules via a synthetic linker that enables them to be made as a single protein chain (Bird et al., Science 242:423 426, 1988 and Huston et al., Proc. NatI. Acad. Sci. USA 85:58795883, 1988). Diabodies are bivalent, bispecific antibodies in which VH and VL

domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see e.g., Holliger, P., et al., Proc. Natl. Acad. Sci. USA 90:6444 6448, 1993, and Poljak, R. J., et al., Structure 2: 1121 1123, 1994). One or more CDRs may be incorporated into a molecule either covalently or noncovalently to make it an immunoadhesin. An immunoadhesin may incorporate the CDR(s) as part of a larger polypeptide chain, may covalently link the CDR(s) to another polypeptide chain, or may incorporate the CDR(s) noncovalently. The CDRs permit the immunoadhesin to specifically bind to a particular antigen of interest.

[0112] An antibody may have one or more binding sites. If there is more than one binding site, the binding sites may be identical to one another or may be different. For instance, a naturally occurring immunoglobulin has two identical binding sites, a single-chain antibody or Fab fragment has one binding site, while a "bispecific" or "bifunctional" antibody has two different binding sites. An "isolated antibody" is an antibody that (1) is not associated with naturally associated components, including other naturally-associated antibodies, that accompany it in its native state, (2) is free of other proteins from the same species, (3) is expressed by a cell from a different species, or (4) does not occur in nature.

[0113] The term "human antibody" includes all antibodies that have one or more variable and constant regions derived from human immunoglobulin sequences. In a preferred embodiment, all of the variable and constant domains are derived from human immunoglobulin sequences (a fully human antibody). These antibodies may be prepared in a variety of ways, as described below.

[0114] A humanized antibody is an antibody that is derived from a non-human species, in which certain amino acids in the framework and constant domains of the heavy and light chains have been mutated so as to avoid or abrogate an immune response in humans. Alternatively, a humanized antibody may be produced by fusing the constant domains from a human antibody to the variable domains of a non-human species. Examples of how to make humanized antibodies may be found in U.S. Pat. Nos. 6,054,297, 5,886,152 and 5,877,293, incorporated herein by reference.

[0115] The term "chimeric antibody" refers to an antibody that contains one or more regions from one antibody and one or more regions from one or more other antibodies. Fragments or analogs of antibodies can be readily prepared by those of ordinary skill in the

art following the teachings of this specification. Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. Preferably, computerized comparison methods are used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known. Bowie et al. Science 253:164 (1991).

Pharmaceutical Compositions

[0116] The present invention also includes pharmaceutical compositions and formulations of the protective agents described herein. Pharmaceutical compositions of the present invention contain the therapeutic agent in an amount sufficient to prevent or treat the diseases described herein in a subject. These pharmaceutical compositions are suitable for administration to a subject in need of prophylaxis or therapy for any of the described diseases or conditions. The subject is preferably a human but can be non-human as well. A suitable subject can be an individual who is suspected of having, has been diagnosed as having, or is at risk of developing one of the described diseases.

[0117] In some embodiments the protective agent is formulated in a lubricant as described in Porat, U.S. Patent No. 624,198, incorporated herein by reference, for intra-vaginal application or application to a newborn baby. In a preferred embodiment the lubricant-protective agent composition has a natural pH corresponding to that of the vagina. The lubricant may be any effective lubricant or combination of lubricants acceptable for cosmetic applications. Medical and pharmaceutical studies have shown that HIV develops mainly in the blood cells and is carried by various body fluids to other cells. The lubricant reduces the friction between the penis and the vaginal wall, thus reducing the rupture of blood cells which might otherwise occur and therefore reducing the amount of blood that is commingled. The protective agents of the present invention can also be formulated into gels and foams for application before or during sexual intercourse that are known in the art. In some embodiments the protective anti-shedding agents of the invention are included in disinfectant foam that coats the walls of the vagina and temporarily forms a closed layer of foam that traps and kills any HIV virus that may be present in addition to reducing or

preventing further shedding. Schmittmann, et al., United States Patent No. 6,022,545, incorporated herein by reference.

[0118] The preventive agent can be formulated into any topical composition known in the art that is suitable for its intended use as described herein, including creams, lotions, ointments, gels, lubricants, liquids, sprays, powders, or absorbent materials. The preventative agents can also be formulated for systemic administration for treating or preventing *G. vaginalis or BV,* or for use as a vaccine, or for reducing shedding in HIV -BV infected women as described herein. A composition of the preventive agents for either topical or systemic administration can also include a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" includes solvents, dispersion media, coatings, antiviral agents, antibacterial agents, antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Supplementary active compounds can also be incorporated into the compositions. Other topical formulations for preventing or reducing HIV transmission during birth are described in Sheele et al., 7,151,091, which is incorporated herein by reference.

[0119] Therapeutic compositions may contain, for example, such normally employed additives as binders, fillers, carriers, preservatives, stabilizing agents, emulsifiers, buffers and excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, cellulose, magnesium carbonate, and the like. These compositions typically contain 1%-95% of active ingredient, preferably 2%-70% active ingredient.

[0120] The protective agents can also be mixed with diluents or excipients which are compatible and physiologically tolerable. Suitable diluents and excipients are, for example, water, saline, dextrose, glycerol, or the like, and combinations thereof. In addition, if desired, the compositions may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, stabilizing or pH buffering agents.

[0121] In some embodiments, the therapeutic compositions of the present invention are prepared either as liquid solutions or suspensions, as sprays, or in solid forms. Oral formulations usually include such normally employed additives such as binders, fillers, carriers, preservatives, stabilizing agents, emulsifiers, buffers and excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations, or powders, and typically

contain 1 %-95% of active ingredient, preferably 2%-70%. One example of an oral composition useful for delivering the therapeutic compositions of the present invention is described in U.S. Pat. No. 5,643,602 (incorporated herein by reference).

[0122] Additional formulations which are suitable for other modes of administration, such as topical administration, include salves, tinctures, creams, lotions, pessary, transdermal patches, ointments, gels, lubricants, liquid, sprays, powders, absorbent materials, and suppositories. For salves and creams, traditional binders, carriers and excipients may include, for example, polyalkylene glycols or triglycerides. One example of a topical delivery method is described in U.S. Pat. No. 5,834,016 (incorporated herein by reference). Other liposomal delivery methods may also be employed (See, e.g., U.S. Pat. Nos. 5,851,548 and 5,711,964, both of which are herein incorporated by reference). The composition can include an inert carrier. The composition can be impregnated in a towlette, sponge or capsule.

[0123] The formulations may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[0124] Sustained-release preparations may also be prepared. Suitable examples of sustained release preparations include semipermeable matrices of solid hydrophobic polymers containing the protective agents, which matrices are in the form of shaped articles, e.g., films, or microcapsule. Examples of sustained release matrices include, but are not limited to, polyesters, hydro gels (for example, poly (2-hydroxyethyl-methacrylate), or poly (vinylalcohol)), polylactides, copolymers of L-glutamic acid and y ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT (injectable micro spheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods.

[0125] The protective agents of the present invention may be administered by any suitable means, preferably topically in the birth canal but also including systemic routes, such as parenteral, and subcutaneous. Parenteral infusions include intramuscular, intravenous, intra-arterial, intra-peritoneal, or subcutaneous administration.

[0126] For the prevention or treatment of disease, the appropriate dosage of antibody or other protective agent will depend on the type of disease to be treated, the severity and course

of the disease, whether the drug is administered for protective or therapeutic purposes, previous therapy, the patient's clinical history and response to the drugs and the discretion of the attending physician.

[0127] The protective agents and vaccines are suitably administered to the patient at one time or over a series of treatments.

[0128] The amount of antibody to be administered therapeutically ranges typically from about lug to 100 ug/ml. This amount typically varies and depends on the route of administration. The therapeutic agents of the invention can be administered by one or more separate administrations, topical or systemic administration, or by continuous infusion. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until the symptoms of *G. vaginalis* or BV are sufficiently reduced or eliminated, or until the HIV viral load is reduced in women with HIV and BV. The progress of this therapy is easily monitored by conventional techniques and assays, and may be used to adjust dosage to achieve a therapeutic effect.

Protein Modifications

[0129] VLY protein and toxoids, and their biologically active analogs, derivatives, fragments and variants for use in the present invention can be modified according to known methods in medicinal chemistry to increase its stability, half-life, uptake or efficacy. Certain known modifications are described below.

[0130] As is also well known, polypeptides are not always entirely linear. For instance, polypeptides may be branched as a result of ubiquitination, and they may be circular, with or without branching, generally as a result of post-translation events, including natural processing events and events brought about by human manipulation which do not occur naturally. Circular, branched and branched circular polypeptides may be synthesized by non-translational natural processes and by synthetic methods.

[0131] Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. Blockage of the amino or carboxyl group in a polypeptide, or both, by a covalent modification, is common in naturally-occurring and synthetic polypeptides. For instance, the amino terminal residue of polypeptides made in E. coli, prior to proteolytic processing, almost invariably will be N-formylmethionine.

[0132] The modifications can be a function of how the protein is made. For recombinant polypeptides, for example, the modifications will be determined by the host cell posttranslational modification capacity and the modification signals in the polypeptide amino acid sequence. Accordingly, when glycosylation is desired, a polypeptide should be expressed in a glycosylating host, generally a eukaryotic cell. Insect cells often carry out the same posttranslational glycosylations as mammalian cells, and, for this reason, insect cell expression systems have been developed to efficiently express mammalian proteins having native patterns of glycosylation. Similar considerations apply to other modifications. The same type of modification may be present in the same or varying degree at several sites in a given polypeptide. Also, a given polypeptide may contain more than one type of modification.

[0133] VLY protein and toxoids can be isolated and purified from cells that naturally express it, purified from cells that naturally express it but have been modified to overproduce it, e.g., purified from cells that have been altered to express it (recombinantly), synthesized using known protein synthesis methods, or by modifying cells that naturally encode VLY or VLY toxoid to express it.

Protein Modification	**Description**
Acetylation	Acetylation of N-terminus or e-lysines. Introducing an acetyl group into a protein, specifically, the substitution of an acetyl group for an active hydrogen atom. A reaction involving the replacement of the hydrogen atom of a hydroxyl group with an acetyl group (CH_3CO) yields a specific ester, the acetate. Acetic anhydride is commonly used as an acetylating agent, which reacts with free hydroxyl groups. Acylation may facilitate addition of other functional groups. A common reaction is acylation of e.g., conserved lysine residues with a biotin appendage.
ADP-ribosylation	Covalently linking proteins or other compounds via an arginine-specific reaction.
Alkylation	Alkylation is the transfer of an alkyl group from one molecule to another. The alkyl group may be transferred as an alkyl carbocation, a free radical or a carbanion (or their equivalents). Alkylation is accomplished by using certain

Protein Modification	Description
	functional groups such as alkyl electrophiles, alkyl nucleophiles or sometimes alkyl radicals or carbene acceptors. A common example is methylation (usually at a lysine or arginine residue).
Amidation	Reductive animation of the N-terminus. Methods for amidation of insulin are described in U.S. 4,489,159.
Carbamylation	Nigen et al. describes a method of carbamylating hemoglobin.
Carboxylation	Carboxylation typically occurs at the glutamate residues of a protein, which may be catalyzed by a carboxylase enzyme (in the presence of Vitamin K – a cofactor).
Citrullination	Citrullination involves the addition of citrulline amino acids to the arginine residues of a protein, which is catalyzed by peptidylarginine deaminase enzymes (PADs). This generally converts a positively charged arginine into a neutral citrulline residue, which may affect the hydrophobicity of the protein (and can lead to unfolding).
Condensation of amines with aspartate or glutamate	Such reactions, may be used, e.g., to attach a peptide to other proteins labels.
Covalent attachment of flavin	Flavin mononucleotide (FAD) may be covalently attached to serine and/or threonine residues. May be used, e.g., as a light-activated tag.
Covalent attachment of heme moiety	A heme moiety is generally a prosthetic group that consists of an iron atom contained in the center of a large heterocyclic organic ring, which is referred to as a porphyrin. The heme moiety may be used, e.g., as a tag for the peptide.
Attachment of a nucleotide or nucleotide derivative	May be used as a tag or as a basis for further derivatising a peptide.
Cross-linking	Cross-linking is a method of covalently joining two proteins. Cross-linkers contain reactive ends to specific functional groups (primary amines, sulfhydryls, etc.) on proteins or other molecules. Several chemical groups may be targets for reactions in proteins and peptides. For example, Ethylene

Protein Modification	Description
	glycol bis[succinimidylsuccinate, Bis[2-(succinimidooxycarbonyloxy)ethyl]sulfone, and Bis[sulfosuccinimidyl] suberate link amines to amines.
Cyclization	For example, cyclization of amino acids to create optimized delivery forms that are resistant to, e.g., aminopeptidases (e.g., formation of pyroglutamate, a cyclized form of glutamic acid).
Disulfide bond formation	Disulfide bonds in proteins are formed by thiol-disulfide exchange reactions, particularly between cysteine residues (e.g., formation of cystine).
Demethylation	See, e.g., U.S. 4,250,088 (Process for demethylating lignin).
Formylation	The addition of a formyl group to, e.g., the N-terminus of a protein. See, e.g., U.S. Patent Nos. 4,059,589, 4,801,742, and 6,350,902.
Glycylation	The covalent linkage of one to more than 40 glycine residues to the tubulin C-terminal tail.
Glycosylation	Glycosylation may be used to add saccharides (or polysaccharides) to the hydroxy oxygen atoms of serine and threonine side chains (which is also known as O-linked Glycosylation). Glycosylation may also be used to add saccharides (or polysaccharides) to the amide nitrogen of asparagine side chains (which is also known as N-linked Glycosylation), e.g., via oligosaccharyl transferase.
GPI anchor formation	The addition of glycosylphosphatidylinositol to the C-terminus of a protein. GPI anchor formation involves the addition of a hydrophobic phosphatidylinositol group - linked through a carbohydrate containing linker (e.g., glucosamine and mannose linked to phosphoryl ethanolamine residue) - to the C-terminal amino acid of a protein.
Hydroxylation	Chemical process that introduces one or more hydroxyl groups (-OH) into a protein (or radical). Hydroxylation reactions are typically catalyzed by hydroxylases. Proline is the principal residue to be hydroxylated in proteins, which occurs at the C^{γ} atom, forming hydroxyproline (Hyp). In

Protein Modification	Description
	some cases, proline may be hydroxylated at its C^{β} atom. Lysine may also be hydroxylated on its C^{δ} atom, forming hydroxylysine (Hyl). These three reactions are catalyzed by large, multi-subunit enzymes known as prolyl 4-hydroxylase, prolyl 3-hydroxylase and lysyl 5-hydroxylase, respectively. These reactions require iron (as well as molecular oxygen and α-ketoglutarate) to carry out the oxidation, and use ascorbic acid to return the iron to its reduced state.
Iodination	See, e.g., U.S. 6,303,326 for a disclosure of an enzyme that is capable of iodinating proteins. U.S. 4,448,764 discloses, e.g., a reagent that may be used to iodinate proteins.
ISGylation	Covalently linking a peptide to the ISG15 (Interferon-Stimulated Gene 15) protein, for, e.g., modulating immune response.
Methylation	Reductive methylation of protein amino acids with formaldehyde and sodium cyanoborohydride has been shown to provide up to 25% yield of N-cyanomethyl ($-CH_2CN$) product. The addition of metal ions, such as Ni^{2+}, which complex with free cyanide ions, improves reductive methylation yields by suppressing by-product formation. The N-cyanomethyl group itself, produced in good yield when cyanide ion replaces cyanoborohydride, may have some value as a reversible modifier of amino groups in proteins. (Gidley et al.) Methylation may occur at the arginine and lysine residues of a protein, as well as the N- and C-terminus thereof.
Myristoylation	Myristoylation involves the covalent attachment of a myristoyl group (a derivative of myristic acid), via an amide bond, to the alpha-amino group of an N-terminal glycine residue. This addition is catalyzed by the N-myristoyltransferase enzyme.
Oxidation	-Oxidation of cysteines. -Oxidation of N-terminal Serine or Threonine residues (followed by hydrazine or aminooxy condensations). -Oxidation of glycosylations (followed by hydrazine or aminooxy condensations).
Palmitoylation	Palmitoylation is the attachment of fatty acids, such as

Protein Modification	Description
	palmitic acid, to cysteine residues of proteins. Palmitoylation increases the hydrophobicity of a protein.
(Poly)glutamylation	Polyglutamylation occurs at the glutamate residues of a protein. Specifically, the gamma-carboxy group of a glutamate will form a peptide-like bond with the amino group of a free glutamate whose alpha-carboxy group may be extended into a polyglutamate chain. The glutamylation reaction is catalyzed by a glutamylase enzyme (or removed by a deglutamylase enzyme). Polyglutamylation has been carried out at the C-terminus of proteins to add up to about six glutamate residues. Using such a reaction, Tubulin and other proteins can be covalently linked to glutamic acid residues.
Phosphopantetheinylation	The addition of a 4'-phosphopantetheinyl group.
Phosphorylation	A process for phosphorylation of a protein or peptide by contacting a protein or peptide with phosphoric acid in the presence of a non-aqueous apolar organic solvent and contacting the resultant solution with a dehydrating agent is disclosed e.g., in U.S. 4,534,894. Insulin products are described to be amenable to this process. See, e.g., U.S. 4,534,894. Typically, phosphorylation occurs at the serine, threonine, and tyrosine residues of a protein.
Prenylation	Prenylation (or isoprenylation or lipidation) is the addition of hydrophobic molecules to a protein. Protein prenylation involves the transfer of either a farnesyl (linear grouping of three isoprene units) or a geranyl-geranyl moiety to C-terminal cysteine(s) of the target protein.
Proteolytic Processing	Processing, e.g., cleavage of a protein at a peptide bond.
Selenoylation	The exchange of, e.g., a sulfur atom in the peptide for selenium, using a selenium donor, such as selenophosphate.
Sulfation	Processes for sulfating hydroxyl moieties, particularly tertiary amines, are described in, e.g., U.S. 6,452,035. A process for sulphation of a protein or peptide by contacting the protein or peptide with sulphuric acid in the presence of a non-aqueous apolar organic solvent and contacting the resultant solution with a dehydrating agent is disclosed. Insulin products are described to be amenable to this process.

Protein Modification	Description
	See, e.g., U.S. 4,534,894.
SUMOylation	Covalently linking a peptide a SUMO (small ubiquitin-related Modifier) protein, for, e.g., stabilizing the peptide.
Transglutamination	Covalently linking other protein(s) or chemical groups (e.g., PEG) via a bridge at glutamine residues
tRNA-mediated addition of amino acids (e.g., arginylation)	For example, the site-specific modification (insertion) of an amino acid analog into a peptide.

[0134] In the foregoing specification, the invention has been described with reference to specific embodiments thereof. It will, however, be evident that various modifications and changes may be made thereto without departing from the broader spirit and scope of the invention. The specification and drawings are, accordingly, to be regarded in an illustrative rather than a restrictive sense.

[0135] The invention is illustrated herein by the experiments described above and by the following examples, which should not be construed as limiting. The contents of all references, pending patent applications and published patents, cited throughout this application are hereby expressly incorporated by reference. Those skilled in the art will understand that this invention may be embodied in many different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will fully convey the invention to those skilled in the art. Many modifications and other embodiments of the invention will come to mind in one skilled in the art to which this invention pertains having the benefit of the teachings presented in the foregoing description. Although specific terms are employed, they are used as in the art unless otherwise indicated.

SEQUENCES:

VLY protein from [organism=Strain 14018 and 14019 *Gardnerella vaginalis]* DNA Sequence ID NO: 1

ATGAAGAGTACAAAGTTCTACCGTAATGCAGCAATGTTGCTCCTCGCGGGCGC
AACTATTGTTCCACAATGCTTAG
CAGCACCAGCAATGGCCGCTCCTTCCGCTAAGGATTCTGAACCAGCTACATCTT
GCGCAGCTAAGAAAGACTCGTT
GAATAATTATTTGTGGGATTTGCAATACGATAAAACAAACATTCTCGCCCGTCA
TGGCGAAACCATTGAGAACAAA
TTCTCCAGCGACAGCTTCAACAAGAACGGTGAATTCGTTGTTGTTGAGCATCAG
AAGAAGAACATCACCAATACAA
CTTCAAATTTGTCGGTTACTTCCGCCAACGATGATCGCGTATACCCAGGTGCTC
TTTTCCGTGCTGATAAGAATTT
GATGGACAATATGCCAAGCCTGATTTCTGCAAACCGCGCTCCAATAACGTTGA
GCGTTGATTTGCCGGGATTCCAC
GGCGGCGAAAGTGCTGTAACTGTTCAGCGCCCAACCAAGAGCTCTGTAACTTC
CGCAGTGAACGGCTTAGTTTCTA
AGTGGAATGCACAATATGGAGCAAGTCATCATGTTGCAGCTCGCATGCAGTAC
GATTCTGCAAGCGCACAAAGCAT
GAACCAGCTCAAGGCTAAGTTTGGTGCTGATTTTGCCAAGATTGGTGTTCCGCT
GAAGATTGATTTCGATGCAGTA
CACAAGGGTGAGAAGCAGACTCAAATTGTGAACTTCAAGCAAACTTACTACAC
CGTAAGCGTTGATGCACCAGATA
GCCCAGCAGATTTCTTTGCTCCTTGCACTACGCCAGACAGCTTGAAGAACCGTG
GCGTTGACAACAAGCGCCCACC
AGTTTACGTGTCAAACGTAGCTTATGGTCGCTCAATGTACGTAAAGTTCGATAC
CACCAGCAAGAGCACTGATTTC
CAGGCTGCGGTAGAAGCAGCAATTAAGGGCGTAGAAATCAAGCCAAACACCGA
ATTCCATCGCATTCTCCAGAATA
CTTCTGTTACTGCAGTGATTCTTGGTGGCAGCGCTAATGGTGCAGCTAAAGTTA
TTACAGGCAATATCGATACGCT

TAAGGCTTTGATTCAGGAAGGTGCAAATTTGAGCACCTCTAGCCCAGCGGTTC
CAATTGCATACACCACTTCCTTC
GTCAAGGATAACGAAGTAGCAACTTTGCAATCCAACAGCGATTATATTGAAACG
AAGGTTTCTTCTTATCGCAATG
GCTACTTGACTTTGGACCACCGTGGAGCTTATGTAGCTCGCTACTACATCTACT
GGGATGAGTACGGCACCGAAAT
TGACGGCACTCCTTACGTGCGTTCTCGCGCTTGGGAAGGCAATGGTAAGTATC
GTACAGCTCACTTCAACACCACT
ATTCAGTTCAAAGGAAATGTACGCAATCTACGAATCAAGTTGGTTGAAAAGACT
GGTTTGGTTTGGGAACCATGGC
GCACAGTATATGACCGTTCTGATTTGCCACTAGTTCGTCAGCGTACTATTAGCA
ACTGGGGCACAACCTTGTGGCCTCGCGTTGCTGAAACTGTAAAGAACGACTGA

VL Y protein from [organism=Strain 14018 and 14019 *Gardnerella vaginalis*] Amino Acid
Sequence ID NO: 2

MKSTKFYRNAAMLLLAGATIVPQCLAAPAMAAPSAKDSEPATSCAAKKDSLNNYL
WDLQYDKTNILARHGETIENKFSSDSFNKNGEFVVVEHQKKNITNTTSNLSVTSAN
DDRVYPGALFRADKNLMDNMPSLISANRAPITLSVDLPGFHGGESA VTVQRPTKSS
VTSA VNGL VSKWNAQYGASHHV AARMQYDSASAQSMNQLKAKFGADF
AKIGVPL
KIDFDAVHKGEKQTQIVNFKQTYYTVSVDAPDSPADFFAPCTTPDSLKNRGVDNKR
PPVYVSNV A YGRSMYVKFDTTSKSTDFQAA
VEAAIKGVEIKPNTEFHRILQNTSVTA
VILGGSANGAAKVITGNIDTLKALIQEGANLSTSSPA VPIA YTTSFVKDNEV
ATLQSN
SDYIETKVSSYRNGYLTLDHRGA YV ARYYIYWDEYGTEIDGTPYVRSRA WEGNGK
YRTAHFNTTIQFKGNVRNLRIKL VEKTGL VWEPWRTVYDRSDLPLVRQRTISNWG
TTL WPRV AETVKND

VLY protein from [*organism=Gardnerella Vaginalis* 49145] DNA SEQ ID NO: 3

ATGAAGAGTACAAAGTTCTACCGTAATGCAGCAATGTTGCTCCTCGCGGGCGC
AACTATTGTTCCACAATGCTTAG
CAGCACCAGCAATGGCCGCTCCTTCCGCTAAGGATTCTGAACCAGCTACATCTT
GCGCAGCTAAGAAAGACTCGTT
GAATAATTATTTGTGGGATTTGCAATACGATAAAACAAACATTCTCGCCCGTCA
TGGCGAAACCATTGAGAACAAA
TTCTCCAGCGACAGCTTCAACAAGAACGGTGAATTCGTTGTTGTTGAGCATCAG
AAGAAGAACATCACCAATACAA
CTTCAAATTTGTCGGTTACTTCCGCCAACGATGATCGCGTATACCCAGGTGCTC
TTTTCCGTGCTGATAAGAATTT
GATGGACAATATGCCAAGCCTGATTTCTGCAAACCGCGCTCCAATAACGTTGA
GCGTTGATTTGCCGGGATTCCAC
GGCGGCGAAAGTGCTGTAACTGTTCAGCGCCCAACCAAGAGCTCTGTAACTTC
CGCAGTGAACGGCTTAGTTTCTA
AGTGGAATGCACAATATGGAGCAAGTCATCATGTTGCAGCTCGCATGCAGTAC
GATTCTGCAAGCGCACAAAGCAT
GAACCAGCTCAAGGCTAAGTTTGGTGCTGATTTTGCCAAGATTGGTGTTCCGCT
GAAGATTGATTTCGATGCAGTA
CACAAGGGTGAGAAGCAGACTCAAATTGTGAACTTCAAGCAAACTTACTACAC
CGTAAGCGTTGATGCACCAGATA
GCCCAGCAGATTTCTTTGCTCCTTGCACTACGCCAGACAGCTTGAAGAACCGTG
GCGTTGACAACAAGCGCCCACC
AGTTTACGTGTCAAACGTAGCTTATGGTCGCTCAATGTACGTAAAGTTCGATAC
CACCAGCAAGAGCACTGATTTC
CAGGCTGCAGTAGAAGCAGCAATTAAGGGCGTAGAAATCAAGCCAAACACCGA
ATTCCATCGCATTCTCCAAAATA
CTTCTGTTACTGCAGTGATTCTTGGTGGCAGCGCTAATGGTGCAGCTAAAGTTA
TTACAGGCAACATCGATACGTT
GAAGGCTTTGATTCAGGAAGGTGCAAATTTGAGCACCTCTAGCCCAGCAGTTC
CAATTGCATACACCACTTCCTTC
GTCAAGGATAACGAAGTAGCAACTTTGCAATCCAACAGCGATTATATTGAAACG
AAGGTTTCCTCTTACCGCAATG
GCTACTTGACTTTGGACCACCGTGGAGCTTACGTAGCTCGCTACTACATCTACT

GGGATGAGTACGGCACCGAAAT
TGACGGCACTCCTTACGTGCGTTCTCGCGCTTGGGAAGGCAATGGTAAGTATC
GTACAGCTCACTTCAATACCACT
ATTCAGTTCAAAGGAAATGTACGCAATCTACGAATCAAGTTGGTTGAAAAGACT
GGTTTAGTTTGGGAACCATGGC
GCACAGTATATGACCGTTCTGATTTGCCACTAGTTCATCAGCGTACTATTAGCA
ACTGGGGCACAACCTTGTGGCCTCGCGTTGCTGAAACTGTAAAGAACGACTGA

VLY DOMAIN 4 DNA SEQ ID NO: 4 FROM *Gardnerella vaginalis* 14018 and 14019 nucleic acid 1126 to the end 1551 V

TACACCACTTCCTTC
GTCAAGGATAACGAAGTAGCAACTTTGCAATCCAACAGCGATTATATTGAAACG
AAGGTTTCTTCTTATCGCAATG
GCTACTTGACTTTGGACCACCGTGGAGCTTATGTAGCTCGCTACTACATCTACT
GGGATGAGTACGGCACCGAAAT
TGACGGCACTCCTTACGTGCGTTCTCGCGCTTGGGAAGGCAATGGTAAGTATC
GTACAGCTCACTTCAACACCACT
ATTCAGTTCAAAGGAAATGTACGCAATCTACGAATCAAGTTGGTTGAAAAGACT
GGTTTGGTTTGGGAACCATGGC
GCACAGTATATGACCGTTCTGATTTGCCACTAGTTCGTCAGCGTACTATTAGCA
ACTGGGGCACAACCTTGTGGCCTCGCGTTGCTGAAACTGTAAAGAACGACTGA

VLY DOMAIN 4 DNA SEQ ID NO: 12
FROM *Gardnerella vaginalis* 49145 nucleic acid 1126 to the end 1551 V

TACACCACTTCCTTCGTCAAGGATAACGAAGTAGCAACTTTGCAATCCAACAGC
GATTATATTGAAACGAAGGTTTCCTCTTACCGCAATGGCTACTTGACTTTGGAC
CACCGTGGAGCTTACGTAGCTCGCTACTACATCTACTGGGATGAGTACGGCAC
CGAAATTGACGGCACTCCTTACGTGCGTTCTCGCGCTTGGGAAGGCAATGGTA
AGTATCGTACAGCTCACTTCAATACCACTATTCAGTTCAAAGGAAATGTACGCA
ATCTACGAATCAAGTTGGTTGAAAAGACTGGTTTAGTTTGGGAACCATGGCGC
ACAGTATATGACCGTTCTGATTTGCCACTAGTTCATCAGCGTACTATTAGCAAC

TGGGGCACAACCTTGTGGCCTCGCGTTGCTGAAACTGTAAAGAACGACTGA

VLYDOMAIN 4
Amino Acid SEQ ID NO: 5
FROM *Gardnerella vaginalis* 14018 and 14019

YTTSFVKDNEV ATLQSNSDYIETKVSSYRNGYLTLDHRGA YV
ARYYIYWDEYGTEI
DGTPYVRSRA WEGNGKYRTAHFNTTIQFKGNVRNLRIKL VEKTGL VWEPWRTVY
DRSDLPL VRQRTISNWGTTLWPRV AETVKND

VLYDOMAIN 4
Amino Acid SEQ ID NO: 13
FROM *Gardnerella vaginalis 49145:*

YTTSFVKDNEV ATLQSNSDYIETKVSSYRNGYLTLDHRGA YV
ARYYIYWDEYGTEI
DGTPYVRSRA WEGNGKYRTAHFNTTIQFKGNVRNLRIKL VEKTGL VWEPWRTVY
DRSDLPL VHQRTISNWGTTL WPRV AETVKND

VLY Undecapeptide
FROM *Gardnerella vaginalis* 14018 and 14019DNA SEQ ID NO: 6
nucleic acid residues 1414-1446

GAAAAGACTGGTTTGGTTTGGGAACCATGGCGC

VLY Undecapeptide
FROM *Gardnerella vaginalis* 49145 DNA SEQ ID NO: 14
nucleic acid residues 1414-1446

GAAAAGACTGGTTTAGTTTGGGAACCATGGCGC

VLY UNDECAPEPTIDE

FROM *Gardnerella vaginalis* (CONSERVED)
Amino acids 472 to 482 AMINO ACID SEQUENCE SEQ ID NO: 7

EKTGLVWEPWR

VLY UNDECAPEPTIDE P480W TOXOID FROM *Gardnerella vaginalis* (CONSERVED)
Undecapeptide amino acids 472 to 482 Amino Acid SEQ ID NO: 8
EKTGLVWEWWR

Codon optimized VLY gene sequence (same protein sequence as VLY from strain 14018) DNA SEQ ID NO: 9
ATGAAAAGCACCAAATTTTATCGTAACGCGGCGATGCTGCTGCTGGCAGGTGCA
ACCATTGTGCCGCAGTGCCTGGCAGCACCGGCAATGGCAGCACCGAGCGCAAAA
GATAGC
GAACCGGCGACCAGCTGCGCGGCGAAAAAAGATAGCCTGAACAACTATCTGTGG
GATCTG
CAGTATGATAAAACCAACATTCTGGCGCGTCATGGCGAAACCATTGAAAACAAA
TTTAGC
AGCGATAGCTTTAACAAAAACGGCGAATTTGTGGTGGTGGAACATCAGAAGAAA
AACATT
ACCAACACCACCAGCAACCTGAGCGTGACCAGCGCGAACGATGATCGTGTGTAT
CCGGGC
GCGCTGTTTCGTGCGGATAAAAACCTGATGGATAACATGCCGAGCCTGATTAGCG
CGAAC
CGTGCGCCGATTACCCTGAGCGTGGATCTGCCGGGCTTTCATGGCGGCGAAAGCG
CGGTG
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AGCAAA
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AGCGCG
AGCGCGCAGAGCATGAACCAGCTGAAAGCGAAATTTGGCGCGGATTTTGCGAAA
ATTGGC

GTGCCGCTGAAAATTGATTTTGATGCGGTGCATAAAGGCGAAAAACAGACCCAG
ATTGTG
AACTTTAAACAGACCTATTATACCGTGAGCGTGGATGCGCCGGATAGCCCGGCG
GATTTC
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CCACC
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ATTAAA
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TGGGC
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GCGCTG
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ACCACC
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GAAACC
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TGCGT
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ATTCAG
TTTAAAGGCAACGTGCGTAACCTGCGTATTAAACTGGTGGAAAAAACCGGCCTG
GTGTGG
GAACCGTGGCGTACCGTGTATGATCGTAGCGATCTGCCGCTGGTGCGTCAGCGTA
CCATT
AGCAACTGGGGCACCACCCTGTGGCCGCGTGTGGCGGAAACCGTGAAAAACGAT
TAA

Gardnerella vaginalis strain ARG3 VLY DNA SEQ ID NO: 10

ATGAAGAGTACAAAGTTCTACCGTAATGCAGCAATGTTGCTCCTCGCGGGCGCA
ACTATTGTTCCACAATGCTTAGCAGCACCAGCAATGGCCGCTCCTTCCGCTAAGG

ATTCTGAACCAGCTACATCTTGCGCAGCTAAGAAAGACTCGTTGAATAATTATTT
GTGGGATTTGCAATACGATAAAACAAACATTCTCGCCCGTCATGGCGAAACCATT
GAGAACAAATTCTCCAGCGACAGCTTCAACAAGAACGGTGAATTCGTTGTTGTTG
AGCATCAGAAGAAGAACATCACCAATACAACTTCAAATTTGTCGGTTACTTCCGC
CAACGATGATCGCGTATACCCAGGTGCTCTTTTCCGTGCTGATAAGAATTTGATG
GACAATATGCCAAGCCTGATTTCTGCAAACCGCGCTCCAATAACGTTGAGCGTTG
ATTTGCCGGGATTCCACGGCGGCGAAAGTGCTGTAACTGTTCAGCGCCCAACCAA
GAGCTCTGTAACTTCCGCAGTGAACGGCTTAGTTTCTAAGTGGAATGCACAATAT
GGAGCAAGTCATCATGTTGCAGCTCGCATGCAGTACGATTCTGCAAGCGCACAA
AGCATGAACCAGCTCAAGGCTAAGTTTGGTGCTGATTTTGCCAAGATTGGTGTTC
CGCTGAAGATTGATTTCGATGCAGTACACAAGGGTGAGAAGCAGACTCAAATTG
TGAACTTCAAGCAAACTTACTACACCGTAAGCGTTGATGCACCAGATAGCCCAGC
AGATTTCTTTGCTCCTTGCACTACGCCAGACAGCTTGAAGAACCGTGGCGTTGAC
AACAAGCGCCCACCAGTTTACGTGTCAAACGTAGCTTATGGTCGCTCAATGTACG
TAAAGTTCGATACCACCAGCAAGAGCACTGATTTCCAGGCTGCGGTAGAAGCAG
CAATTAAGGGCGTAGAAATCAAGCCAAACACCGAATTCCATCGCATTCTCCAGA
ATACTTCTGTTACTGCAGTGATTCTTGGTGGCAGCGCTAATGGTGCAGCTAAAGT
TATTACAGGCAATATCGATACGCTTAAGGCTTTGATTCAGGAAGGTGCAAATTTG
AGCACCTCTAGCCCAGCGGTTCCAATTGCATACACCACTTCCTTCGTCAAGGATA
ACGAAGTAGCAACTTTGCAATCCAACAGCGATTATATTGAAACGAAGGTTTCTTC
TTATCGCAATGGCTACTTGACTTTGGACCACCGTGGAGCTTATGTAGCTCGCTAC
TACATCTACTGGGATGAGTACGGCACCGAAATTGACGGCACTCCTTACGTGCGTT
CTCGCGCTTGGGAAGGCAATGGTAAGTATCGTACAGCTCACTTCAACACCACTAT
TCAGTTCAAAGGAAATGTACGCAATCTACGAATCAAGTTGGTTGAAAAGACTGG
TTTGGTTTGGGAACCATGGCGCACAGTATATGACCGTTCTGATTTGCCACTAGTT
CGTCAGCGTACTATTAGCAATTGGGGCACAACCTTGTGGCCTCGCGTTGCTGAAA
CTGTAAAGAACGACTGA

ARG3 VLY protein sequence SEQ ID NO. 11

MKSTKFYRNAAMLLLAGATIVPQCLAAPAMAAPSAKDSEPATSCAAKKDSLNNYL
WDLQYDKTNILARHGETIENKFSSDSFNKNGEFVVVEHQKKNITNTTSNLSVTSAND
DRVYPGALFRADKNLMDNMPSLISANRAPITLSVDLPGFHGGESAVTVQRPTKSSVT
SAVNGLVSKWNAQYGASHHVAARMQYDSASAQSMNQLKAKFGADFAKIGVPLKID
FDAVHKGEKQTQIVNFKQTYYTVSVDAPDSPADFFAPCTTPDSLKNRGVDNKRPPV
YVSNVAYGRSMYVKFDTTSKSTDFQAAVEAAIKGVEIKPNTEFHRILQNTSVTAVIL
GGSANGAAKVITGNIDTLKALIQEGANLSTSSPAVPIAYTTSFVKDNEVATLQSNSDY
IETKVSSYRNGYLTLDHRGAYVARYYIYWDEYGTEIDGTPYVRSRAWEGNGKYRTA
HFNTTIQFKGNVRNLRIKLVEKTGLVWEPWRTVYDRSDLPLVRQRTISNWGTTLWP
RVAETVKND*

EXAMPLES

EXAMPLE 1 MATERIALS AND METHODS

Bacterial strains and cell lines

[0136] *Gardnerella vaginalis* strains 14018, 14019, and 49145 were obtained from ATCC. ARG3 is a clinical isolate of *G. vaginalis* kindly provided by Susan Whittier. Cells were grown in brain-heart infusion supplemented with 5% fetal bovine serum ,0.3% Tween 80, and 0.1% soluble starch or in 10% fetal bovine serum (HyClone), 5% Fildes enrichment (Remel) and 4 ng/ml of amphotericin. There are many efficient ways to culture *Gardnerella vaginalis* known in the art. Cultures were typically incubated at 37°C and 5% CO_2. *E. coli* strains TOP10 and BL21AI (Invitrogen) were grown in LB, with kanamycin (30 μg/ml) selection as appropriate. HeLa cells were grown at 37°C / 5% CO_2 in MEM supplemented with 10% fetal bovine serum and 10 μg/ml ciprofloxacin. CHO-K1 cells (CCL-61) were grown at 37°C / 5% CO_2 in F12 Kaighn's Modification (Invitrogen) with 10% FBS and 10 μg/ml ciprofloxacin.

Human cervical endothelial cells (HeLa, ATCC CCL-2) were grown at 37°C and 5% CO_2 in minimal essential medium (Invitrogen) supplemented with 10% fetal bovine serum and 10 μg/ml ciprofloxacin. Human vaginal endothelial cells (VK2, ATCC CRL-2616) were grown in serum free keratinocyte growth media (Invitrogen) with 0.1 ng/ml EGF, 0.05 mg/ml bovine pituitary extract and 0.4 mM calcium chloride (Biol Reprod 1997, Oct 57(4):847-55, Generation of papillomavirus-immortalized cell lines from normal human ectocervical, endocervical, and vaginal epithelium that maintain expression of tissue-specific differentiation proteins; Fichorova RN, Rheinwald JG, Anderson DJ).

Cloning, sequencing, and analysis of the VLY gene

[0137] The *G. vaginalis* genomic region containing VLY was amplified from *G. vaginalis* 14018 by PCR using *Pfx* proofreading polymerase (Invitrogen) and primers V1 (ATGCAGCGAAGCATGCCATGC) and V2 (TCAGTCGTTCTTTACAGTTTC). This PCR product was cloned into vector pCR2.1/TOPO (Invitrogen) and transformed into *E. coli* TOP10 according to the manufacturer's instructions. (50) The insert was bidirectionally sequenced using vector-specific primers. The predicted VLY open reading frame was amplified by PCR using the cloned genomic region as template, *Pfx* polymerase, and primers V3 (GCCGCCGCCCATATGAAGAGTACAAAG) and V6

(GCCGGATCCTCAGTCGTTCTTTACAGT), adding unique restriction sites indicated by underlining. The resulting product was cut with restriction enzymes NdeI and BamHI, cloned into the vector pET28a (Novagen) to generate a construct with an N-terminal hexahistidine transcriptional fusion, and confirmed by sequencing. Site-directed mutagenesis to construct (pET28a/VLY(P480W)) was performed with the QuikChange II XL kit (Stratagene) according to the manufacturer's instructions. Mutagenic primers used were P480Wsense (TGGTTGAAAAGACTGGTTTGGTTTGGGAATGGTGGCGCACAGTATAT) and P480Wanti (ATATACTGTGCGCCACCATTCCCAAACCAAACCAGTCTTTTCAACCA).

[0138] Improved purity and greater yield were achieved by generating a truncated construct (excluding the first 50 amino acids from the N-terminal region) using the primer VLY50up (5'- GCCGCC**CATATG**TCGTTGAATAATTATTTGTGG-3') along with the previously described V6 primer. The PCR product was cloned into the pET28a vector (Novagen), confirmed by sequencing, and transformed into *E. coli* BL21-AI competent cells (Invitrogen) for expression and purification as previously described. [11] The lytic activity of this truncated recombinant toxoid was unaltered (data not shown). The DNA sequence encoding VLY protein is set forth in SEQ ID NO: 1 for strains 14018 an 14019; the amino acid sequence encoding VLY protein is set forth in SEQ ID NO:2.

[0139] Protein sequence prediction, alignment, and phylogenetic analyses were performed using MacVector software (Version 9.5, MacVector Inc.). Protein sequences for other CDC family members (Appendix) were obtained from the Comprehensive Microbial Resource (J. Craig Venter Institute, http://cmr.tigr.org) or from the GenBank/Entrez Protein database (National Center for Biotechnology Information).

Expression and purification of recombinant toxins

[0140] *E. coli* BL21AI carrying the pET28a/VLY or pET28a/VLY(P480W) plasmid were grown in one liter cultures at 37°C on a rotary shaker for 3 hr, and protein expression was induced with 1 mM IPTG and 0.02% L-arabinose (Sigma). After 6 hr, bacteria were pelleted, lysed with BugBuster solution (Novagen) in the presence of protease inhibitor cocktail, lysozyme (100 μg/ml), and benzonase nuclease, all from Sigma. Lysates were cleared by centrifugation and tagged recombinant toxin purified using Ni-NTA agarose (Qiagen) according to the manufacturer's instructions. Purified toxin was extensively dialysed against LPS-free PBS (Gibco) to remove imidazole and concentrated (Amicon Ultra, 10 kD MW cutoff). Protein concentrations were determined using a modified Bradford assay (Bio-Rad).

[0141] The coding sequence of the pneumolysin gene was amplified by PCR using primers NdeI-Ply-up (GGAATTCCATATGGCAAATAAAGCAG) and Ply-down-XhoI (CCGCTCGAGGTCATTTTCTACCTTATC) using genomic DNA of *S. pneumoniae* strain TIGR4 as a template. These primers added unique restriction sites as indicated by underlining and led to amplification of the entire pneumolysin sequence, omitting the stop codon to allow addition of a C-terminal hexahistidine taG. The product was confirmed by sequencing, digested with NdeI and XhoI (New England Biolabs), and cloned into pET29a (Novagen) cut with NdeI and XhoI. The plasmid was transformed into *E. coli* BL21-AI, and induction and purification were performed as for VLY. Site-directed mutagenesis used primers W435Psense (ACCGGGCTTGCCTGGGAACCGTGGCGTACG) and W435Panti (CGTACGCCACGGTTCCCAGGCAAGCCCGGT).

Anti-pneumolysin Western blot

[0142] *G. vaginalis* 14018 was grown on chocolate agar and fresh colonies scraped from the plate and resuspended in lysis buffer (BugBuster, EMD Chemicals, Gibbstown, NJ) with benzonase nuclease. The lysate was boiled for 5 minutes, and 30 µl of lysate separated on a 4-12% polyacrylamide gel (Invitrogen). Purified VLY (500 ng total) was run as a positive control. The proteins were transferred to PVDF membranes, blocked with 5% milk, and probed with murine monoclonal anti-pneumolysin (clone 9.1/2/3/6; Novocastra, Newcastle Upon Tyne, UK; 1:100 dilution). Detection was with HRP-conjugated anti-mouse IgG (Santa Cruz Biotechnology, Santa Cruz, CA) and ECL.

Anti-VLY Western Blot

[0143] *G. vaginalis* 14018 was grown on an HBT plate and fresh colonies were resuspended in lysis buffer (BugBuster, EMD Chemicals, Gibbstown NJ) with benzonase nuclease. The lysate was boiled and separated on a 10% polyacrylamide gel. Proteins were transferred to polyvinylidene difluoride membranes, blocked with 5% milk and probed using rabbit polyclonal anti-VLY antiserum (1:500,000 dilution). Detection was with HRP-conjugated anti-rabbit IgG (Santa Cruz Biotechnology) and ECL. Membranes probed with pre-immune serum served as a negative control.

Erythrocyte lysis assay

[0144] The use of human erythrocytes was approved by the Columbia University Institutional Review Board. Human blood was obtained by venipuncture and erythrocytes immediately isolated by centrifugation and repeated washing in sterile PBS. For human samples, a 1% solution of packed erythrocytes in sterile PBS was prepared and added to a 96-well polystyrene V-bottomed plate (100 µl/well). Blood from other species tested was obtained commercially (Fisher Scientific) and erythrocytes washed in sterile PBS prior to use. A 1% solution of packed erythrocytes in sterile PBS was combined with an equal volume of toxin diluted in PBS. The total volume for the assay was 200 µl per well of a 96-well polystyrene V-bottom plate. The negative control for lysis consisted of PBS without toxin added to erythrocytes, and the positive control for 100% lysis was 0.05% Triton X-100. Incubation was for 30 min at 37°C / 5% CO_2. At the conclusion of the assay, the plates were spun at 2000 rpm to pellet erythrocytes and supernatants removed for measurement of optical density at 415 nm. Where noted, toxins were preincubated with cholesterol (stock solution 100 mg/ml in chloroform; working concentration 1-10 µg/ml) or control (chloroform alone at the corresponding dilution) for 10 min at room temperature prior to use in the assay. Antibody inhibition experiments were performed using anti-CD55 (clone IA10, BD Pharmingen), anti-CD59 (clone YTH53.1, GeneTex), or irrelevant antibody control. Preincubation of erythrocytes with antibody (9 ng/ml final concentration) was for 1 hr at 4°C with constant rotation, followed by two PBS washes to remove unbound antibody prior to use in the assay.

CHO cell transfection and LDH release assay

[0145] The coding sequence for human CD59 was amplified from cDNA from A549 (CCL-185) respiratory epithelial cells using primers CD59-1 (GCCGCCCTCGAGCCACCAATGGGAATCCAAGGAG) and CD59-2 (GCCGCCGAATTCTTAGGGATGAAGGCTCCAGGC) and cloned into the XhoI and EcoRI sites of pIRES2-EGFP (Clontech). Sequence was confirmed using vector specific primers. CHO-K1 cells were transfected with purified plasmid DNA (either pIRES2-EGFP/CD59 or the corresponding empty vector control) using a Nucleofector (Amaxa) according to the manufacturer's instructions. Transfected cells were plated into 6-well dishes and used 48 hr after transfection. Greater than 90% transfection efficiency was assessed by fluorescence microscopy (data not shown). Cells were weaned from serum overnight and stimulated with VLY or pneumolysin diluted in serum-free F12 media for 30 min at 37°C /

5% CO_2. Cell viability was confirmed at the end of the experiment by visual inspection of the monolayer and trypan blue exclusion and exceeded 90%. The positive control for complete lysis was 1% Triton X-100 in serum-free F12. The concentration of lactate dehydrogenase in supernatants was assessed with a commercial kit (Roche) according to the manufacturer's instructions.

Epithelial p38 MAPK phosphorylation

[0146] Western blot analysis of epithelial p38 MAPK phosphorylation was performed as previously described (32).

Real-time PCR

[0147] HeLa cells were weaned from serum overnight and treated for 2 hr with medium alone, VLY (10 μg/ml), or VLY(P480W) (10 μg/ml). Cells were lysed in RLT+ buffer (Qiagen) and RNA purified using a commercially available kit (RNeasy Plus; Qiagen). Reverse transcription of 1.5 μg of RNA per sample to generate cDNA was with the high-capacity cDNA kit (Applied Biosystems). Real-time PCR (Applied Biosystems StepOne) with SYBR green detection (PowerSYBR, Applied Biosystems) was performed using primers for interleukin-8 (TACTCCAAACCTTTCCAACCC and AACTTCTCCACAACCCTCTG) and GAPDH (GGGCGCCTGGTCACCAGGGCTG and GGGGCCATCCACAGTCTTCTG). Relative quantitation used the $\Delta\Delta C_T$ method with normalization to GAPDH.

Generation of antibodies

[0148] Purified VLY toxin was generated and submitted to Cocalico Biologicals, Inc. (Reamstown, PA). According to their protocol, adult rabbits were injected with a minimum of 100 μg antigen mixed with Complete Freund's Adjuvant subcutaneous and/or intramuscularly at multiple sites. Booster doses containing a minimum of 50 μg antigen mixed with Incomplete Freund's Adjuvant were administered on days 14, 21 and 49. A test bleed was performed on day 56. Prior to the first immunization, serum was collected from each rabbit to serve as negative control.

Immunofluorescence

[0149] *G. vaginalis* 14018 was grown to in culture media and bacterial cells were fixed on a glass chamber slide using 4% paraformaldehyde. Non-specific binding sites were blocked using 5% normal donkey serum and 0.2% triton X-100. Pre-immune or immune serum was added to each slide (1:500 dilution) for 1 h at room temperature. Following serial washes with PBS and 0.2% triton X-100, donkey anti-rabbit conjugated to Alexa Fluor (AF)-488 (Invitrogen; 1:1000 dilution) was added for 30 min in the dark with gentle shaking. After washing, chambers were removed from the slide and cover slips were mounted with ProLong Gold antifade with DAPI (Invitrogen). Slides to which no primary antibody was added served as negative controls.

ELISA based assay for VLY production

[0150] Four strains of *G. vaginalis* (14018, 14019, 49145, and ARG3) were grown on HBT plates, colonies were scraped and inoculated into 30 ml of liquid media. A 500 µl aliquot of each culture was obtained every 6 hours for determination of OD_{600}. An additional 1 ml sample from each was pelleted by centrifugation and supernatant stored at -20 °C prior to ELISA.

[0151] Immuno-96 MicroWell plates (Nunc) were coated with anti-pneumolysin antibody (clone 1F11, previously shown to cross-react with VLY) diluted 1:500 in coating buffer (0.1 M sodium carbonate, pH: 9.5) and incubated at 4°C overnight. Wells were washed with PBS and 0.05% Tween 20. Non-specific binding sites were blocked using PBS with 10% fetal bovine serum for 1 h. Supernatants (100 µl) were added to each well and plates were incubated at room temperature for 2 h. Known concentrations of recombinant VLY toxin diluted in *G. vaginalis* culture media were used as standards. Rabbit polyclonal anti-VLY antiserum (diluted 1:1000 in blocking solution) was added to each well for 30 min at room temperature. After washing, goat anti-rabbit HRP antibody (Santa Cruz biotechnology Inc., 1:1000 dilution) was added for 30 min. Wells were thoroughly washed and 100 µl of TMB substrate (Thermo Scientific) was added to each well and plate was incubated in the dark for 15 min. 50 µl of stop solution (2N sulfuric acid) was added to each well and OD_{450} determined.

Cytotoxicity assay

[0152] 24-well plates were seeded with VK2 or HeLa human epithelial cells in appropriate media and grown to >90% confluence. 12 hours prior to use, HeLa cells were

weaned from serum. Recombinant VLY toxin diluted in media (10 μg/ml) or vehicle control was added to each well. Where indicated, toxin was preincubated with pre-immune or immune sera for 30 min at 4°C prior to use in the assay. The plates were incubated for 45 min at 37°C and 5% CO_2. Supernatant was removed and the concentration of lactate dehydrogenase was determined using a commercial kit (Roche) per the manufacturer's instructions.

Statistical analysis

[0153] Statistical comparisons were performed using two-tailed unpaired t-tests or one-way analysis of variance (ANOVA) with Tukey post-test as appropriate (Prism, GraphPad Software).

Sequence data availability

[0154] The sequence data for the *G. vaginalis* genome for strains 14018, 14019, and 49145 are available in GenBank under the accession numbers EU522486-EU522488.

EXAMPLE 2

Domain 4 of VLY plays a role in species specificity

[0155] Consistent with these findings, the hCD59 binding site has been localized to this domain for ILY (22) and for VLY (FiG. 8 renumber). Using overlap-extension PCR, we generated a toxin chimera, containing domains 1-3 of VLY and domain 4 of PLY, a species non-selective CDC (FiG. 8A). Unlike the parent VLY, the chimera lysed human and non-human erythrocytes with equal efficacy (FiG. 8B), and does not require hCD59 for CHO cell lysis (FiG. 8C). This indicates that D4 plays a major role in species-selectivity among the CDCs. In order to generate a probe for toxin-hCD59 interactions, we created a GFP:VLYD4 fusion protein (FiG. 8D). This protein binds to hCD59-expressing human epithelial cells but does not form pores (FiG. 8E). Thus, this protein will be an invaluable tool for studies delineating requirements for binding between D4 and hCD59.

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CLAIMS

What is claimed is:

1. An isolated nucleic acid encoding VLY protein from *G. vaginalis*, comprising a member selected from the group comprising nucleotide sequences SEQ ID No: 1 from strains 14018 or 14019, SEQ ID NO: 3 from strain 49145, SEQ ID NO: 10 from strain ARG3, and degenerate variants or fragments thereof.

2. An isolated nucleic acid encoding domain 4 of VLY from *G. vaginalis*, comprising a member selected from the group comprising nucleotide sequences SEQ ID No: 4 from strains 14018 or 14019, SEQ ID NO: 12 from strain 49145, and degenerate variants or fragments thereof.

3. An isolated nucleic acid encoding the undecapeptide of VLY from *G. vaginalis*, comprising a member selected from the group comprising nucleotide sequences SEQ ID No: 6, from strains 14018 or 14019, SEQ ID NO: 14 from strain 49145, and degenerate variants or fragments thereof.

4. Isolated and purified VLY protein from *G. vaginalis*, the amino acid sequence of which comprises a member selected from the group comprising amino acid sequences SEQ ID NO: 2, SEQ ID NO: 11, and biologically active fragments or variants thereof.

5. Isolated and purified undecapeptide region of VLY of *G. vaginalis*, the amino acid sequence of which comprises SEQ ID NO: 7, or a biologically active fragment or variant thereof.

6. Isolated and purified domain 4 of VLY of *G. vaginalis*, the amino acid sequence of which comprises a member selected from the group comprising amino acid sequences SEQ ID NO: 5 from strains 14018 and 14019, SEQ ID NO: 13 from strain 49145, and biologically active fragments or variants thereof.

7. Isolated and purified immunogenic *G. vaginalis* VLY polypeptide fragment, comprising a member selected from the group comprising at least ten consecutive amino acid

residues of SEQ ID NO: 2 from strains 14018 or 14019, SEQ ID NO: 11 from strain ARG3 VLY, and biologically active variants thereof.

8. The isolated and purified polypeptide of claim 7, wherein the ten consecutive residues are selected from the group comprising the nucleic acids that encode domain 4 of VLY as set forth in SEQ ID NO: 5 from strains 14018 or 14019, SEQ ID NO: 13 from strain 49145, and variants thereof.

9. Isolated and purified pore-forming toxoid of VLY from *G. vaginalis*, which toxoid differs from VLY identified by SEQ ID NO: 2, by a single amino acid substitution, and immunologically active fragments or variants thereof.

10. Isolated and purified pore-forming toxoid of claim 9, wherein the amino acid substitution is a tryptophan residue at position 480 of SEQ ID NO. 2.

11. Isolated and purified pore-forming toxoid of claim 9, wherein the amino acid substitution is an isoleucine residue at position 500 of SEQ ID NO: 2.

12. Isolated and purified pore-forming toxoid of claim 9, wherein the amino acid substitution is an R at position 471 of SEQ ID NO. 2.

13. Isolated and purified pore-forming toxoid of claim 9, wherein the amino acid substitution is a C at position 473 of SEQ ID NO. 2.

14. Isolated and purified pore-forming toxoid of claim 9, wherein the amino acid substitution is an R at position 471 and a C at position 473 of SEQ ID NO. 2.

15. Isolated and purified pore-forming toxoid of claim 9, wherein the amino acid substitution is a W at position 480, and further comprises a second amino acid substitution of an isoleucine at position 500 of SEQ ID NO. 2.

16. Isolated and purified pore-forming toxoid of claim 15, further comprising a third amino acid substitution of a C at position 473 of SEQ ID NO. 2.

17. A method of eliciting an immune response in an animal to *G. vaginalis* VLY, comprising introducing into the animal a composition comprising a isolated and purified *G. vaginalis* VLY toxoid protein as claimed in one of claims 9-17, or immunologically active fragment or variant thereof.

18. A vaccine comprising isolated and purified *G. vaginalis* VLY toxoid protein as claimed in one of claims 9-17, or an immunologically active fragment or variant thereof.

19. A method for permeabilizing a cell membrane, comprising contacting the cell with a composition comprising isolated and purified *G. vaginalis* VLY protein, or a biologically active fragment or variant thereof.

20. A method for diagnosing and treating a G. *vaginalis* infection or *bacterial vaginosis* in a patient, comprising:

a. obtaining a biological sample from the patient,

b. detecting the presence of VLY protein having the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 11, or a biologically active fragment or variant thereof in the biological sample taken from the patient,

c. and if the VLY protein is detected then administering to the patient a therapeutically effective amount of an antibiotic known to treat *G. vaginalis* or a protective agent that is a member selected from the group comprising anti-VLY antibody, anti-PLY antibody, anti-CD59 antibody, anti-pneumolysin antibody, soluble CD59, *G. vaginalis* VLY toxoid, or a biologically active fragment or variant thereof

21. A method of treating or preventing a G. *vaginalis infection* or bacterial vaginosis in a patient, by administering to the patient a therapeutically effective amount of a protective agent that is a member selected from the group comprising anti-VLY antibody, anti-PLY antibody, anti-CD59 antibody, anti-pneumolysin antibody, anti-ILY antibody, soluble CD59, *G. vaginalis* VLY toxoid, and biologically active fragments or variants thereof.

22. A composition comprising two compounds selected from the group comprising soluble CD59, anti CD59, VLY antibody, anti-PLY antibody, anti-pneumolysin antibody,

anti-ILY antibody, VLY toxoids or immunologically active fragments or variants thereof, and antibiotics known to treat bacterial vaginosis or *G. vaginalis*.

23. An ELISA kit for diagnosing *G. vaginalis* infection in a patient, comprising a detection antibody that specifically binds to VLY protein identified by SEQ ID NO: 2 or SEQ ID NO: 11, or to a biologically active fragment thereof.

24. The ELISA kit of claim 23, wherein the detection antibody is a member selected from the group comprising rabbit polyAnti-VLY antibody, anti-pneumolysin antibody, anti-PLY antibody and anti-ILY antibody.

25. The ELISA kit of claim 23, wherein the detection antibody is covalently bound to an enzyme, and further comprising a substrate for the enzyme.

26. The ELISA kit of claim 23, further comprising a secondary antibody covalently bound to an enzyme, which secondary antibody specifically binds to the detection antibody, and further comprising a substrate for the enzyme.

27. The ELISA kit of claim 23, further comprising a capture molecule specifically binds to VLY.

28. The ELISA kit of claim 23, wherein the capture molecule is a member selected from the group comprising soluble CD59, anti-VLY antibody, anti-PLY antibody, anti-ILY antibody and anti-pneumolysin.

29. A kit for detecting VLY, comprising a substrate to which VLY binds, soluble CD59 covalently bound to an enzyme, and a substrate for the enzyme.

30. The kit of claim 29, further comprising a capture molecule that is a member selected from the group comprising soluble CD59, anti-VLY antibody, anti-PLY antibody, and anti-pneumolysin.

31. A method for reducing or preventing the transmission of *HIV* to another human by a woman infected with HIV and *bacterial vaginosis*, comprising administering to the woman before the woman engages in a sexual activity, a therapeutically effective amount of a composition comprising a protective agent selected from the group comprising soluble CD59, anti CD59 antibody, anti-VLY antibody, anti-PLY antibody, polyAntiPLY antibody, or anti-pneumolysin antibody, VLY toxoids or biologically active fragments or variants thereof.

32. The method of claim 31, wherein the composition is applied topically to the vagina of the woman before engaging in the sexual activity.

33. The method of claim 31, wherein the composition is administered systemically to the woman before engaging in the sexual activity.

34. A method of reducing or preventing maternal to fetal transmission of HIV from a pregnant woman diagnosed as being infected with both HIV and *bacterial vaginosis*, comprising administering to the woman prior to a vaginal birth of a fetus, a therapeutically effective amount of a protective agent selected from the group comprising soluble CD59, anti CD59 antibody, anti-VLY antibody, anti-PLY antibody, polyAntiPLY antibody, or anti-pneumolysin, VLY toxoids and fragments or variants thereof.

35. The method of claim 34, wherein the composition is administered as soon as the woman is identified and continues until the fetus is born.

36. The method of claim 34, wherein the composition is applied topically to the vagina of the woman.

37. The method of claim 34, wherein the composition is administered systemically to the woman.

38. The polyclonal rabbit anti-VLY antibody poly-Anti-VLY antibody, or an immunologically active fragment or variant thereof.

FIG. 1A

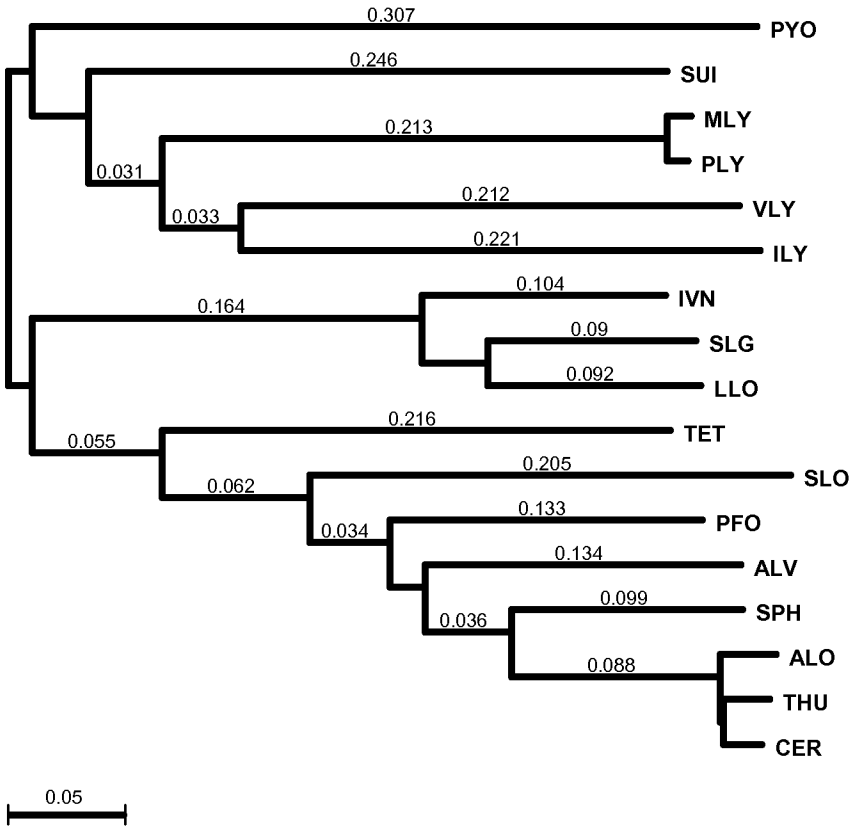


FIG. 1B

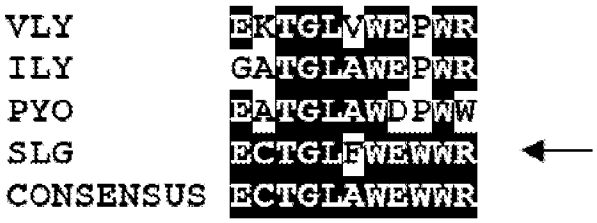


FIG. 1C

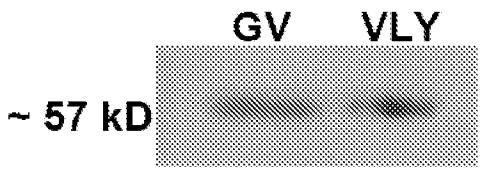


FIG. 2A

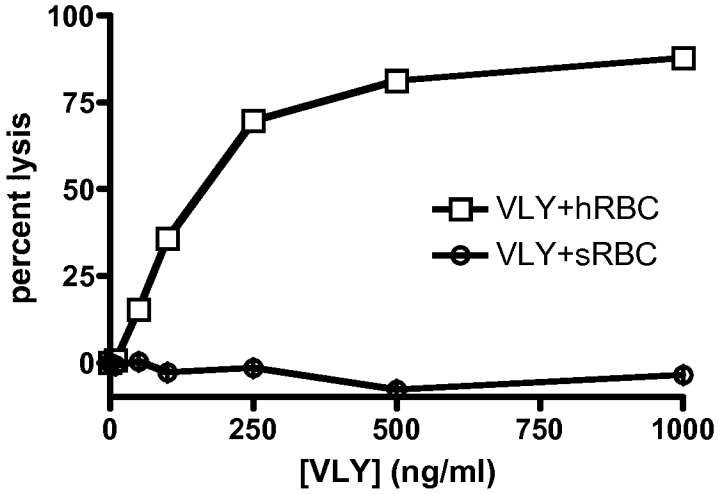


FIG. 2B

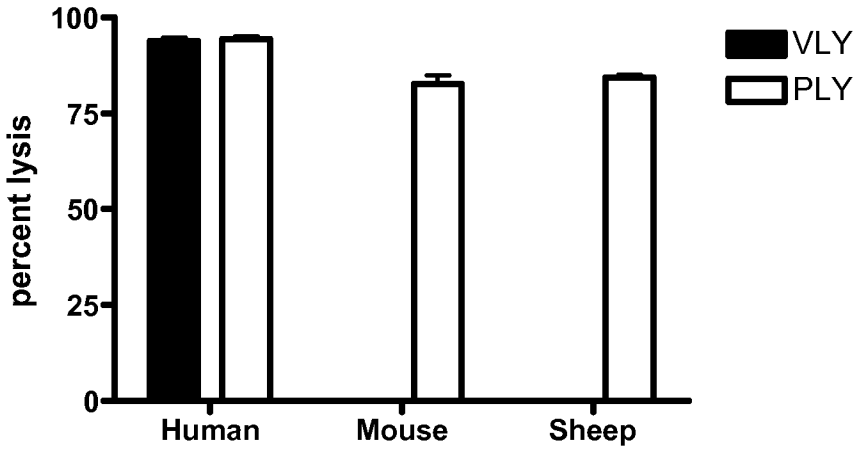


FIG. 2C

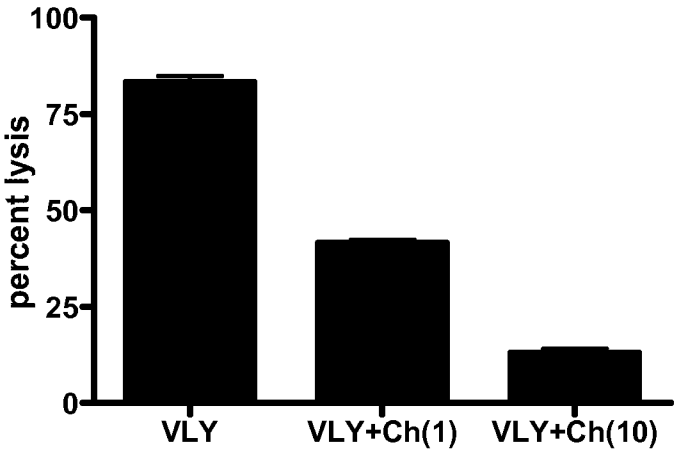


FIG. 3A

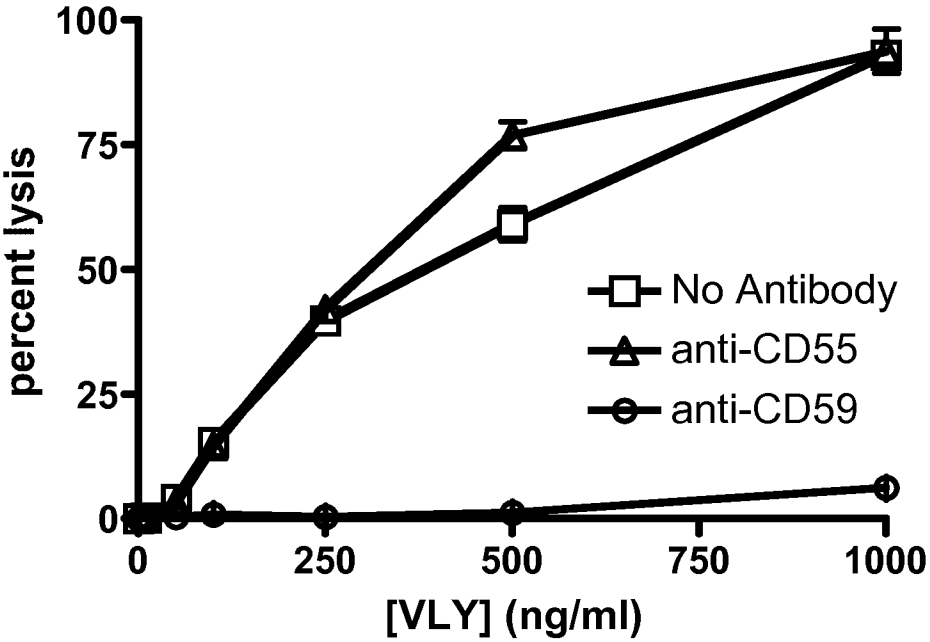

100
75
50
25
0
percent lysis
0
250
500
750
1000
[VLY] (ng/ml)
No Antibody
anti-CD55
anti-CD59

FIG. 3B

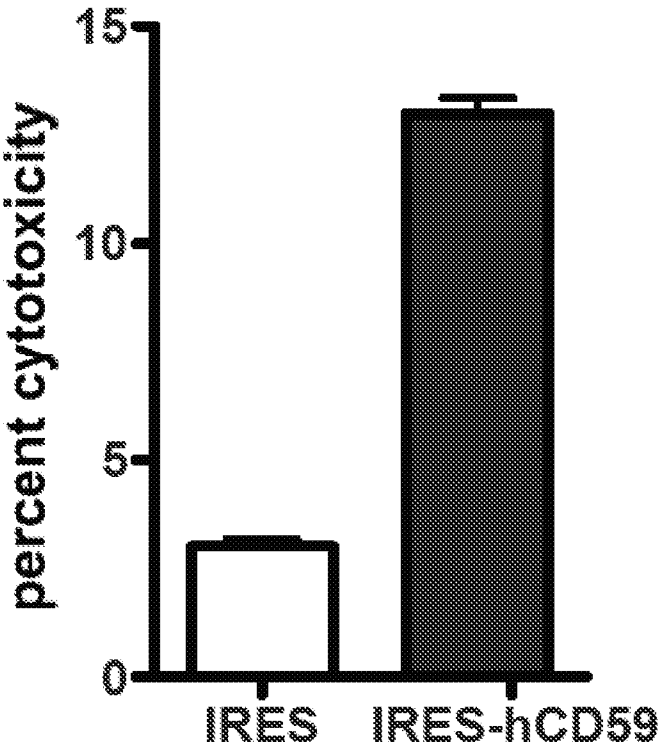

15
10
5
0
percent cytotoxicity
IRES
IRES-hCD59

FIG. 3C

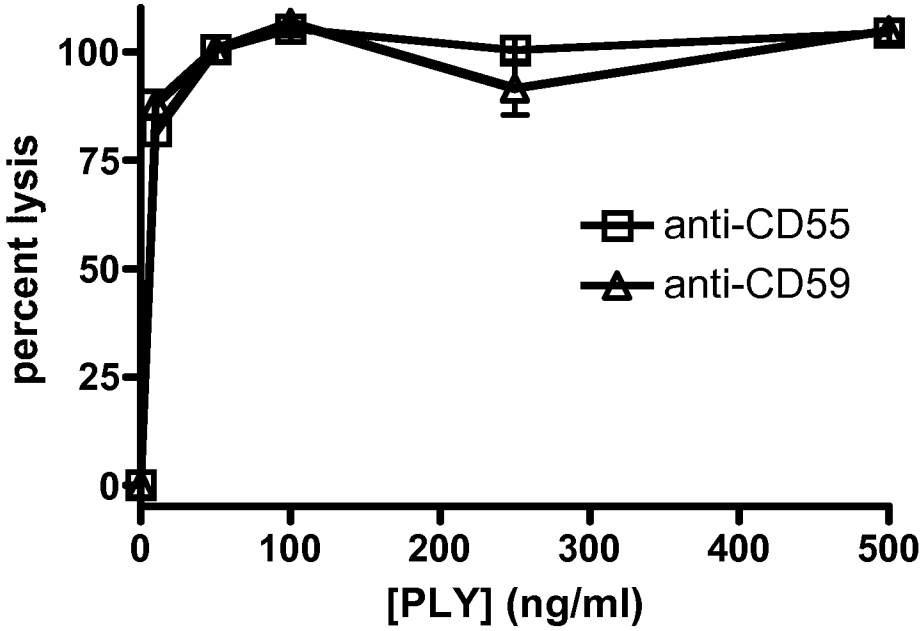

percent lysis
100
75
50
25
0
0
100
200
300
400
500
[PLY] (ng/ml)
anti-CD55
anti-CD59

FIG. 3D

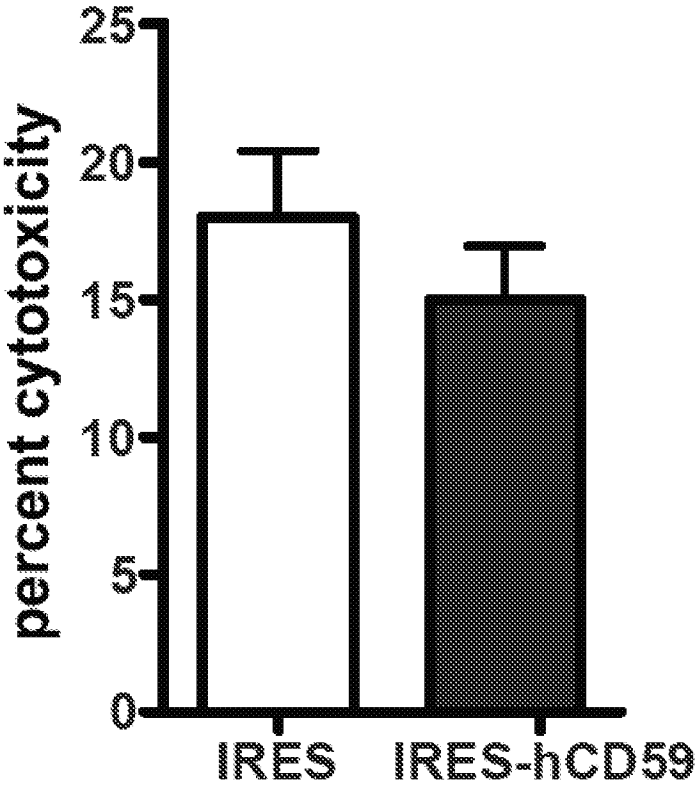

percent cytotoxicity
25
20
15
10
5
0
IRES
IRES-hCD59

FIG. 4A

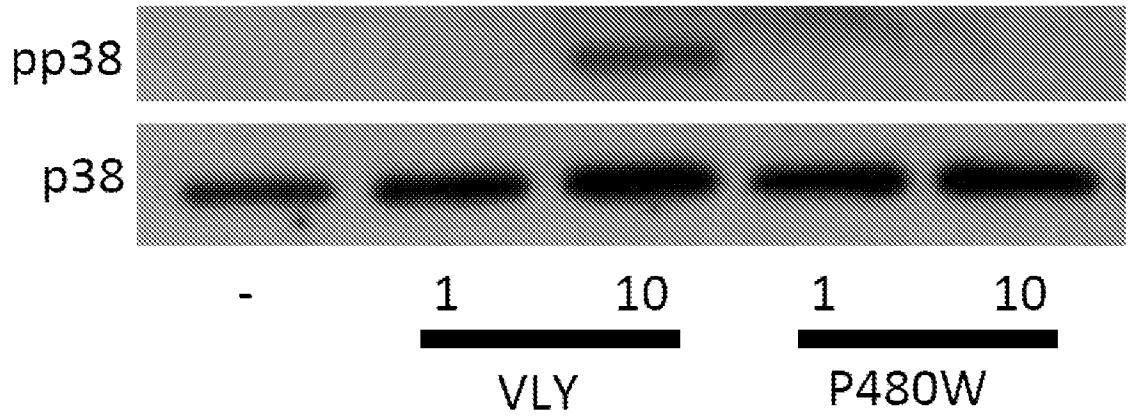

pp38
p38
-
1
10
1
10
VLY
P480W

FIG. 4B

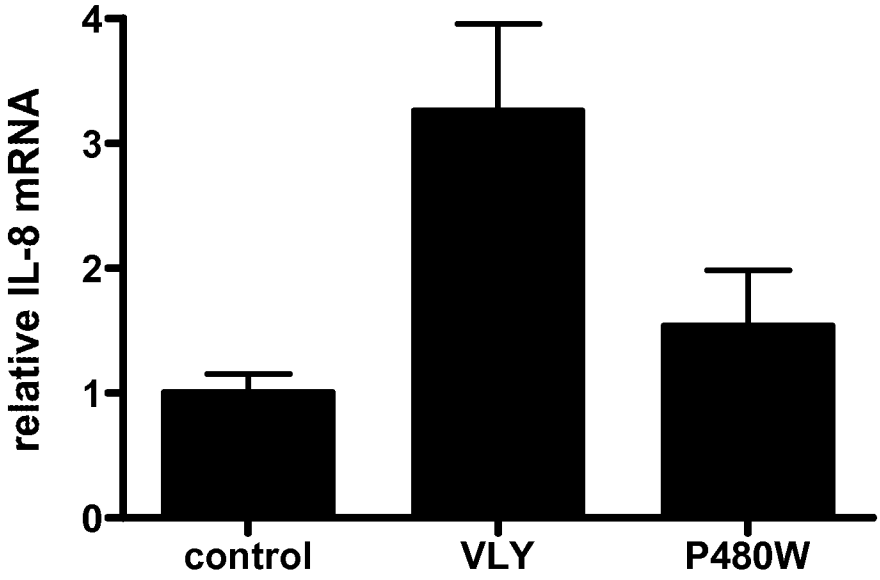

relative IL-8 mRNA
0
1
2
3
4
control
VLY
P480W

FIG. 4C

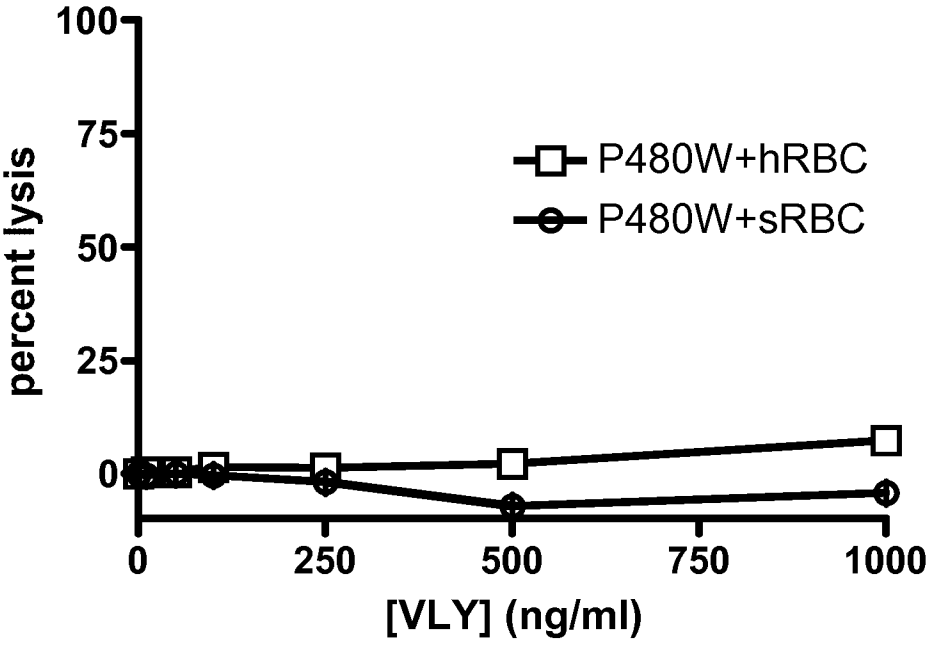

100
75
50
25
0
percent lysis
P480W+hRBC
P480W+sRBC
0
250
500
750
1000
[VLY] (ng/ml)

FIG. 4D

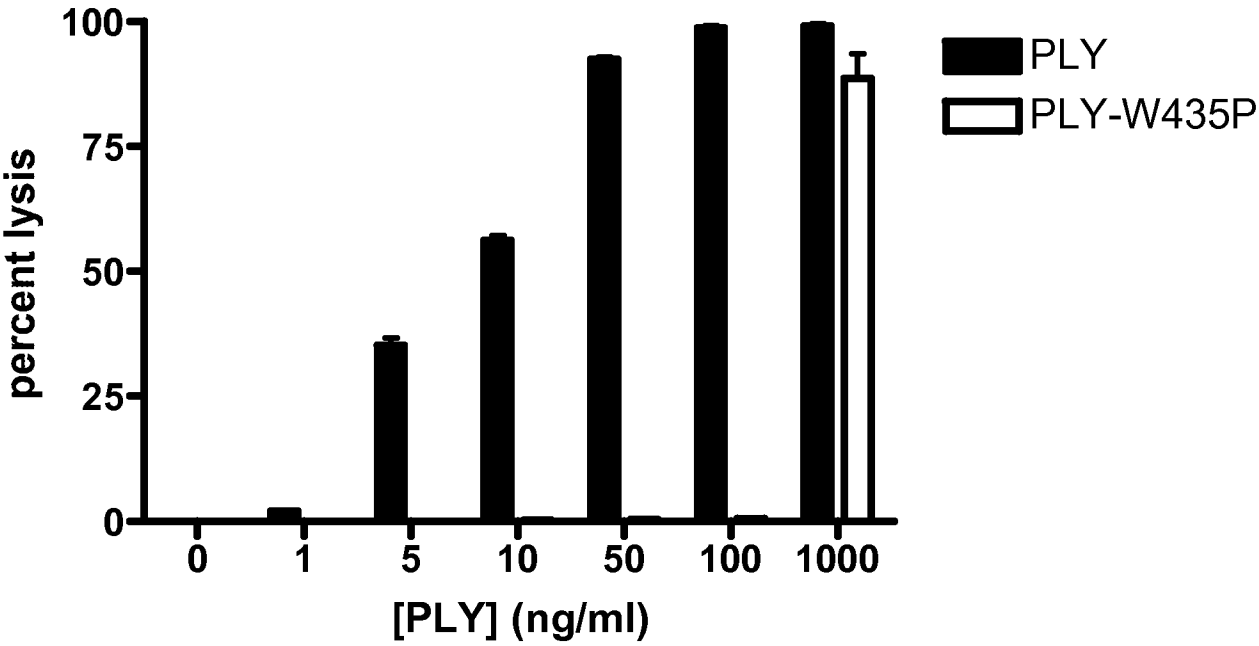

100
75
50
25
0
percent lysis
PLY
PLY-W435P
0
1
5
10
50
100
1000
[PLY] (ng/ml)

FIG. 5A

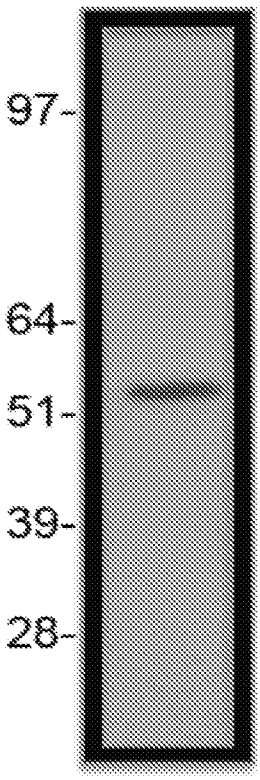
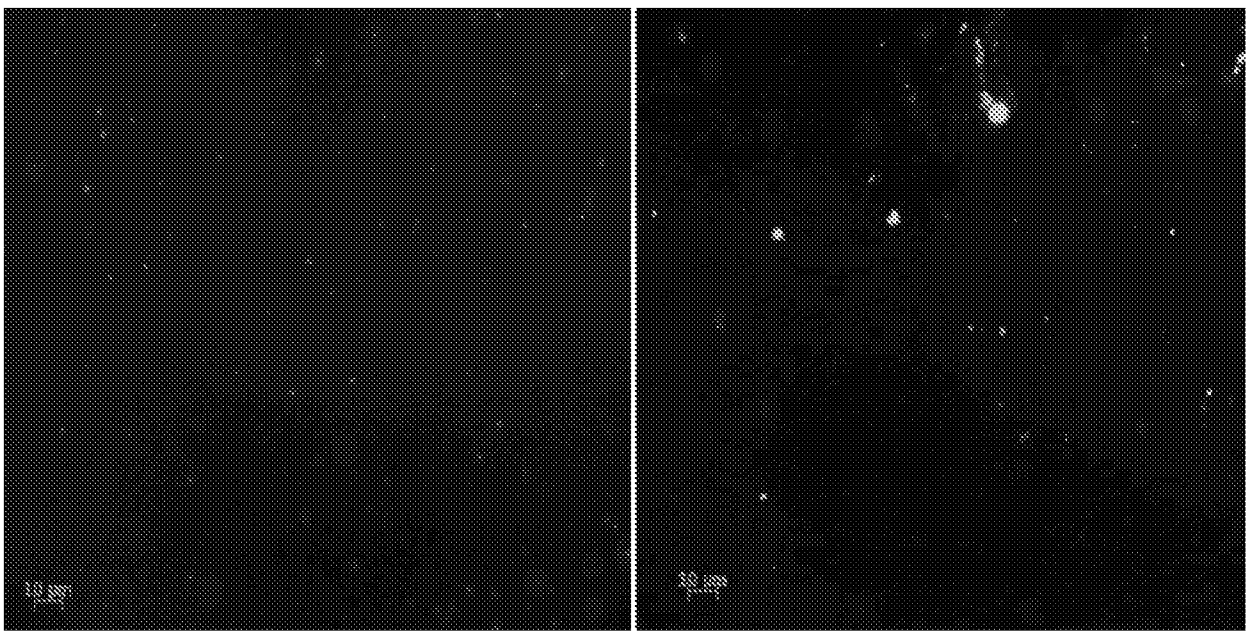


FIG. 5B



Pre-immune Anti-VLY

FIG. 6A

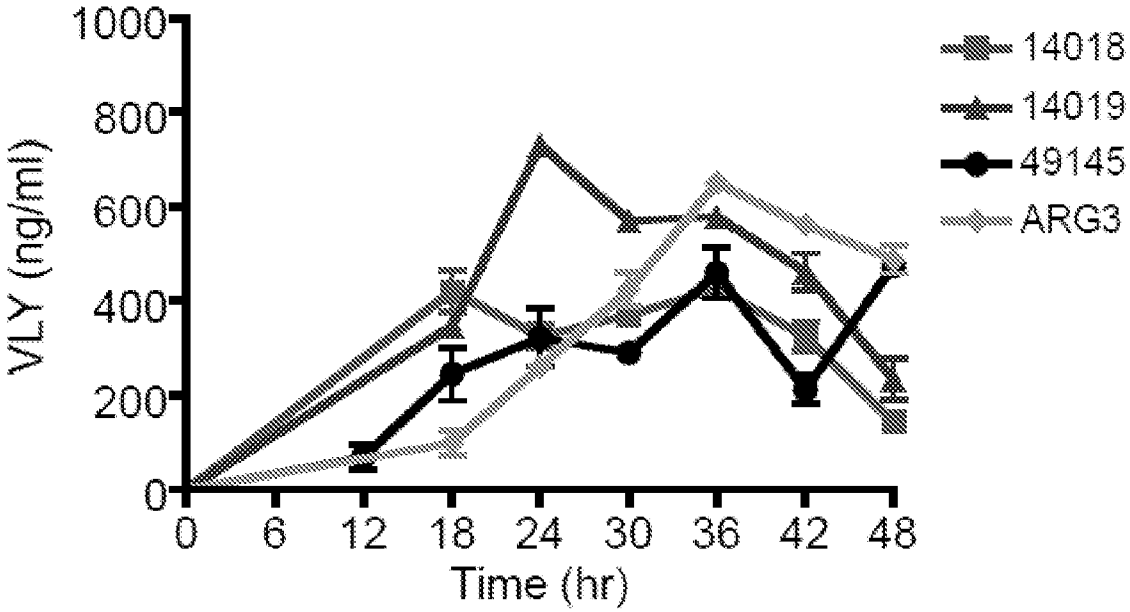

1000
800
600
400
200
0
VLY (ng/ml)
0 6 12 18 24 30 36 42 48
Time (hr)
14018
14019
49145
ARG3

FIG. 6B

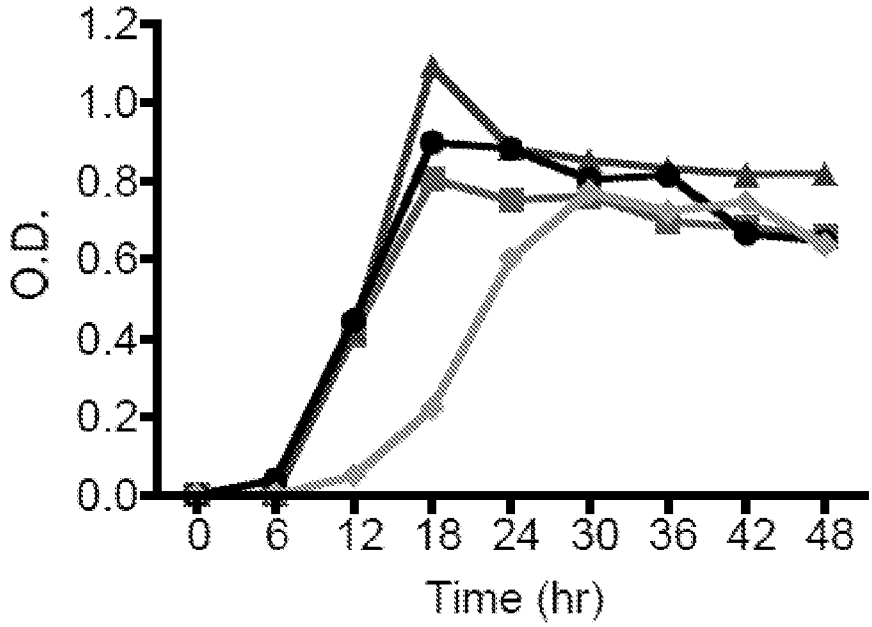

1.2
1.0
0.8
0.6
0.4
0.2
0.0
O.D.
0 6 12 18 24 30 36 42 48
Time (hr)

FIG. 7A

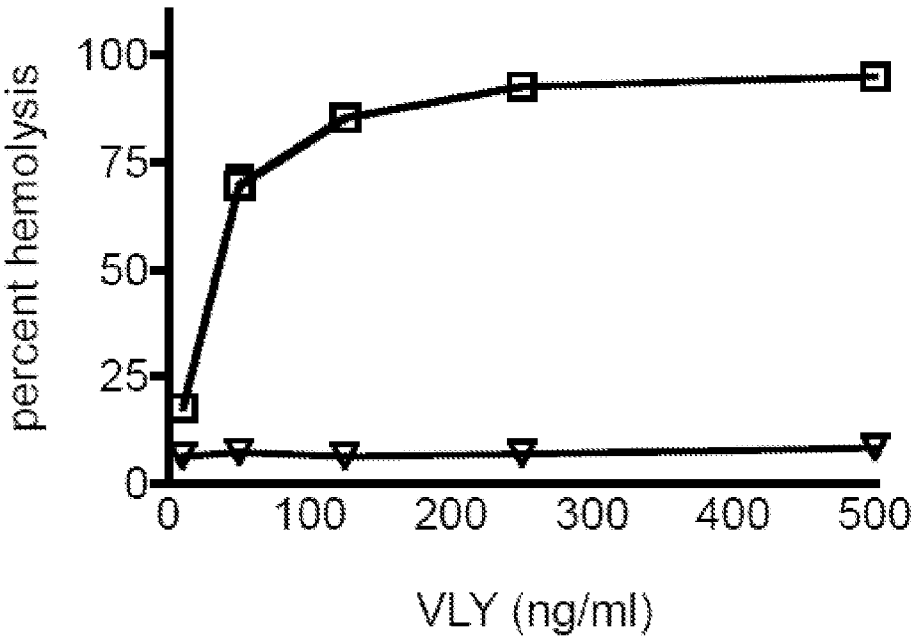

percent hemolysis
100
75
50
25
0
0
100
200
300
400
500
VLY (ng/ml)

FIG. 7B

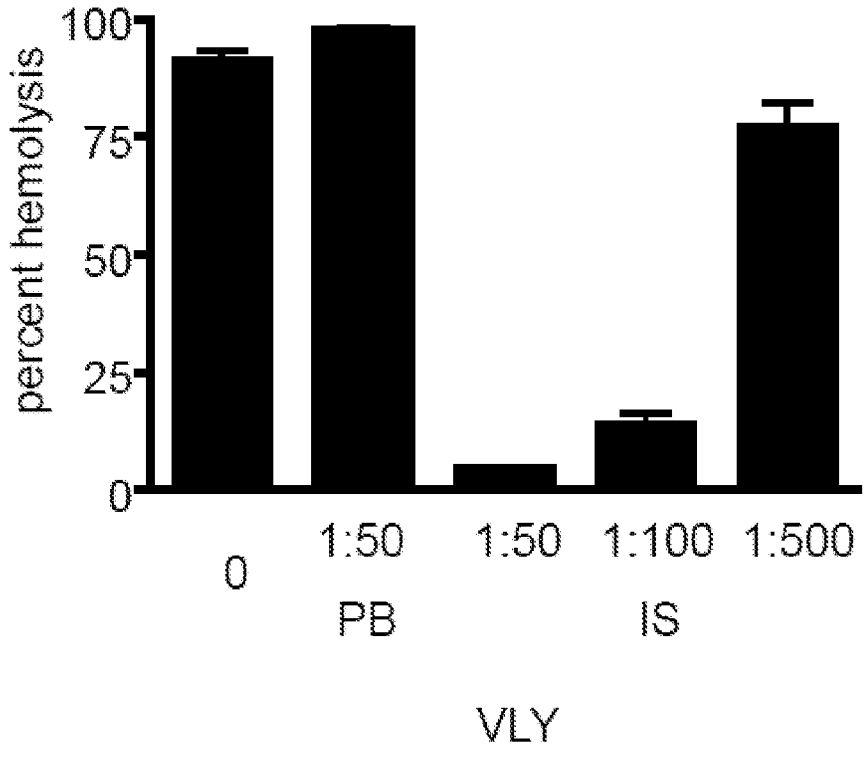

percent hemolysis
100
75
50
25
0
0
1:50
PB
1:50
1:100
1:500
IS
VLY

FIG. 8A

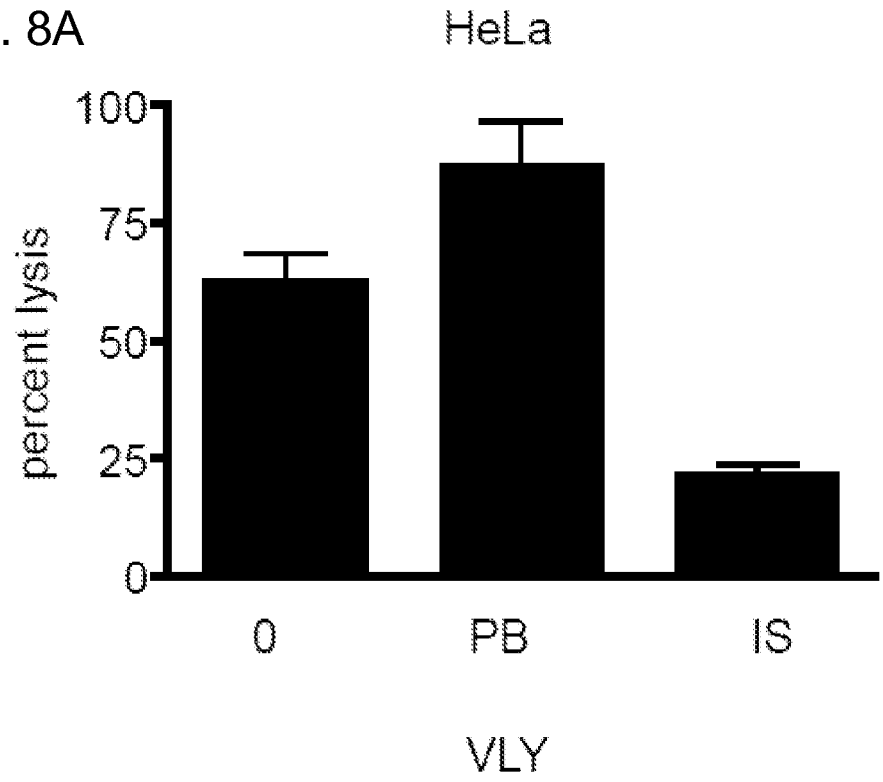
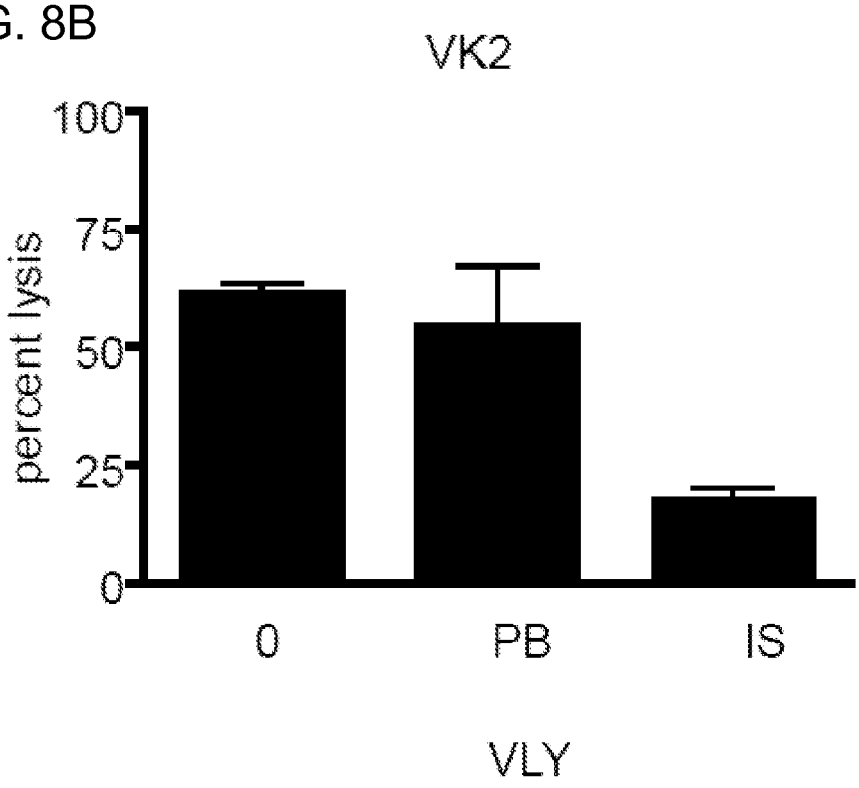

HeLa
100
75
50
25
0
percent lysis
0
PB
IS
VLY

FIG. 8B


VK2
100
75
50
25
0
percent lysis
0
PB
IS
VLY