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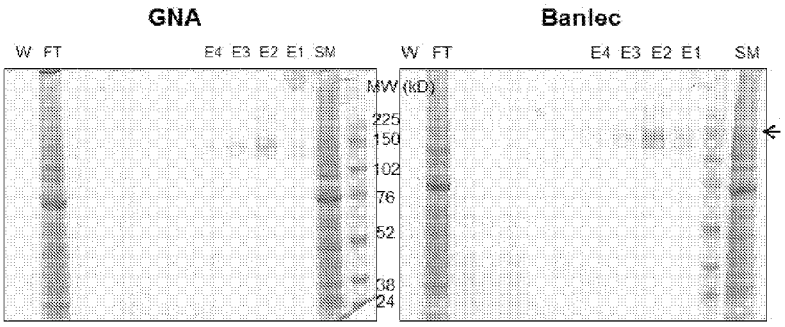

FIGURE 1

(57) Abstract: The present invention relates to non-naturally occurring or engineered banana lectins, for the purification of glycoproteins which may be advantageous for therapeutic, prophylactic, veterinary or vaccine production for humans or animals.

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USE OF MODIFIED BANANA LECTIN IN PURIFICATION OF GLYCOPROTEINS

FIELD OF THE INVENTION

[0001] This application claims the benefit of U.S. Provisional Application Number 61/990,886 filed on May 9, 2014.

[0002] The foregoing applications, and all documents cited therein or during their prosecution ("appln cited documents") and all documents cited or referenced in the appln cited documents, and all documents cited or referenced herein ("herein cited documents"), and all documents cited or referenced in herein cited documents, together with any manufacturer's instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated by reference herein, are hereby incorporated herein by reference, and may be employed in the practice of the invention. More specifically, all referenced documents are incorporated by reference to the same extent as if each individual document was specifically and individually indicated to be incorporated by reference.

[0003] The present invention relates to banana lectins, advantageously non-naturally occurring engineered banana lectins, for the purification of glycoproteins that may be advantageous for vaccines or other therapeutic or prophylactic purposes.

FEDERAL FUNDING LEGEND

[0004] This invention was supported, in part, by the National Cancer Institute (NIH) grant number: Grant No. RO1 CA 144043. The federal government may have certain rights to this invention.

BACKGROUND OF THE INVENTION

[0005] A lectin is a sugar-binding protein and specifically binds to carbohydrate moieties of a glycoprotein or glycolipid. Lectin affinity chromatography has been widely used for purification of glycoproteins, especially tag-free recombinant glycoproteins. However, most lectins are toxic at high doses and can induce allergies or other immune reactions based on their mitogenic properties. The possibility of leaching lectin during glycoprotein purification by lectin affinity chromatography limits the usage of lectin in the process of glycoprotein production for human use.

[0006] BanLec (also BanLec-I or Banana lectin) is a lectin from the jacalin-related lectin family isolated from bananas, advantageous those from *Musa acuminate* and *Musa*

balbisiana. BanLec is one of the predominant proteins in the pulp of ripe bananas and has binding specificity for mannose and mannose-containing oligosaccharide.

[0007] A banana is an edible fruit produced by plants of the genus *Musa*. BanLec has a number of similarities to Concanavalin A and binds to mannose-related carbohydrate structures. It was discovered due to its highly immunogenic properties—BanLec induces a strong IgG4 antibody response—and appears to be an important antigen involved in banana allergies. BanLec protein expression can be induced by the plant hormone methyl jasmonate. BanLec binds to high mannose carbohydrate structures, including those found on viruses containing glycosylated envelope proteins such as human immunodeficiency virus type-1 (HIV-1).

[0008] Despite the possibilities, BanLec in its natural form is highly mitogenic, meaning that it activates T cells. For use in viral antigen purification, mitogencity is a potential problem both in terms of toxicity and as a mitogenic lectin may be easily displaced from a purification column.

[0009] A lectin from bananas (termed BanLec) binds to high mannose structures and thus useful for purifying viral protein antigens that are rich in mannose. Such antigens include, but are not limited to, the envelope proteins from hepatitis C virus, the F and G glycoproteins of respiratory syncytial virus, multiple glycoproteins from herpes viruses, hemagglutinin (HA) and neuraminidase of influenza viruses, and the like. However, BanLec in its natural form is highly mitogenic.

[0010] Citation or identification of any document in this application is not an admission that such document is available as prior art to the present invention.

SUMMARY OF THE INVENTION

[0011] Applicants have developed several new versions of BanLec that are not mitogenic but surprisingly readily interact with viral envelope proteins. These mutant forms of BanLec may be made by altering histidine 84 of the molecule, especially by changing H84 to a threonine. H84T is quite effective at purifying the gp120 envelope protein of HIV as well as the envelope proteins of other viruses, such as the HA of influenza and HCMV gB, which have mannose on their surface. Therefore, H84T and other mutant forms of BanLec have the potential to be used in high volume purification of viral antigens for use in vaccines or other therapeutic or prophylactic purposes.

[0012] Accordingly, the present invention relates to a method of isolating a mannose-containing glycoprotein from a biological sample which may comprise immobilizing banana

lectin (BanLec) on a carrier, contacting the biological sample with the BanLec and eluting the mannose-containing glycoprotein with mannose, a mannose analogue or a mannose derivative, thereby isolating the mannose-containing glycoprotein from the biological sample.

[0013] In one embodiment, the mannose-containing glycoprotein may be a mannose-containing viral glycoprotein. In another embodiment, the mannose-containing viral glycoprotein may be an envelope protein. The viral protein may be isolated from hepatitis C virus, herpes virus, human cytometalovirus (HCMV), human immunodeficiency virus (HIV), influenza virus, respiratory syncytial virus or another virus. In an advantageous embodiment, the glycoprotein may be HCMV gB, HCMV gH/gL or influenza hemagglutinin (HA).

[0014] Preferably, the BanLec may be a recombinant BanLec (rBanLec), advantageously a mutant rBanLec. The mutant rBanLec may comprise a mutation at histidine 84 (H84), such as a mutation to threonine (H84T). In another embodiment, the mutant rBanLec lacks mitogenic activity.

[0015] In another advantageous embodiment, the mannose-containing glycoprotein may be eluted with a mannose derivative, such as methyl α-D-mannopyrannoside.

[0016] These and other embodiments are disclosed or are obvious from and encompassed by, the following Detailed Description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] The following detailed description, given by way of example, but not intended to limit the invention solely to the specific embodiments described, may best be understood in conjunction with the accompanying drawings.

[0018] FIG. 1 depicts purification of HCMV gB ectodomain with BanLec and GNA lectin chromatography. SM is start material, FT is flow through, W is wash and E is elution. The arrow indicates gB.

[0019] FIG. 2 depicts purification of HCMV gH/gL with BanLec and GNA lectin chromatography. Lanes 1 and 12 are markers, lane 2 is starting material, lane 3 is flow through (GNA), lane 4 is wash 1 (GNA), lane 5 is elution 1 (GNA), lane 6 is elution 2 (GNA), lane 7 is elution 2 (Banlec), lane 8 is elution 1 (Banlect), lane 9 is wash 1 (Banlec), lane 10 is flow through (Banlec) and lane 12 is starting material.

[0020] FIG. 3 depicts purification of Flu HA with BanLec and GNA lectin chromatography.

[0021] FIG. 4 depicts a HIV gp120 protein purified from a CHO cell line using H84T BanLec. The HIV envelope protein gp120 was purified from the supernatants of a CHO cell

line expressing the protein using either snowdrop lectin – agarose (Sigma L8775) or BanLec H84T-agarose. HIV-1 -Bal gp120 (from the NIH AIDS reagent program) was used as a positive control. The proteins were analyzed via western blot probed with human anti-gp120 antibody obtained from the NIH AIDS reagent program.

DETAILED DESCRIPTION OF THE INVENTION

[0022] BanLec in its natural form is highly mitogenic, meaning that it activates T cells. For use in viral antigen purification, mitogencity is a potential problem both in terms of toxicity and as a mitogenic lectin may be easily displaced from a purification column.

[0023] Applicants have recently developed several new versions of BanLec that are not mitogenic but surprisingly readily interact with viral envelope proteins. The result is unexpected as it was expected that the reduced mitogenic activity of the H84T variant was thought to play a role in carbohydrate binding and that mutations in this region may lead to structural alterations that affect carbohydrate recognition (see, e.g., lines 18-26 of page 22 of WO 2011/130145). These mutant forms of BanLec are made by altering histidine 84 of the molecule, especially by changing H84 to a threonine. H84T is quite effective at purifying the gp120 envelope protein of HIV as well as the envelope proteins of other viruses, such as the hemagglutinin of influenza and HCMV gB, which have mannose on their surface. Therefore, H84T and other mutant forms of BanLec have the potential to be used in high volume purification of viral antigens for use in vaccines or other therapeutic or prophylactic purposes.

[0024] In other embodiments, other mutant forms of BanLec may include mutations at sugar binding sites in addition to H84, such as but not limited to D35, G60 and F131. It is within the purview of the skilled artisan to determine whether mutants at these specific positions, as well as other positions in the BanLec proteins are mitogenic.

[0025] The present invention relates to method of isolating a mannose-containing glycoprotein from a biological sample which may comprise: immobilizing a recombinant mutant banana lectin (rBanLec) on a carrier, contacting the biological sample with the BanLec and eluting the mannose-containing glycoprotein with mannose, a mannose analogue or a mannose derivative, thereby isolating the mannose-containing glycoprotein from the biological sample, wherein the recombinant mutant banana lectin has at least 100-fold lower mitogenic activity than wild-type banana lectin.

[0026] In another embodiment, the recombinant mutant banana lectin has at least 500-fold lower mitogenic activity than wild-type banana lectin. In another embodiment, the recombinant mutant banana lectin has at least 750-fold lower mitogenic activity than wild-

type banana lectin. In another embodiment, the recombinant mutant banana lectin has at least 1000-fold lower mitogenic activity than wild-type banana lectin. In another embodiment, the recombinant mutant banana lectin has at least 1250-fold lower mitogenic activity than wild-type banana lectin. In another embodiment, the recombinant mutant banana lectin has at least 1500-fold lower mitogenic activity than wild-type banana lectin. In another embodiment, the recombinant mutant banana lectin has at least 1750-fold lower mitogenic activity than wild-type banana lectin. In another embodiment, the recombinant mutant banana lectin has at least 2000-fold lower mitogenic activity than wild-type banana lectin. In yet another embodiment, the recombinant mutant banana lectin has at least 500-fold to about 2000-fold lower mitogenic activity than wild-type banana lectin.

[0027] As used herein, a biological sample may refer to bodily fluid (such as, but not limited to, blood, plasma or spinal fluid), a cell, a cell line, a tissue or an organ or parts thereof or fragments thereof. The biological sample may be partially purified, such as, but not limited to, a cell lysate.

[0028] As used herein, a wild type banana lectin may refer to purified lectin from bananas (termed BanLec) that binds to high mannose structures which is highly mitogenic. A wild type banana lectin may also refer to a recombinant BanLec having the sequence of purified BanLec.

[0029] The recombinant BanLec (rBanLec) of the present invention may have at least 100-fold lower mitogenic activity than wild-type banana lectin, about 1000-fold lower mitogenic activity than wild-type banana lectin, about -fold lower mitogenic activity than wild-type banana lectin, about 1500-fold lower mitogenic activity than wild-type banana lectin, or about 2000-fold lower mitogenic activity than wild-type banana lectin. Alternatively, the rBanLec of the present invention may have mitogenic activity that may be reduced at least 10%, at least 20%, at least 50%, at least 7 5%, at least 85%, at least 90% or at least 95% relative to wild type BanLec.

[0030] Mitogenic activity may be measured by ELISA as follows (see, e.g., Swanson et al., J. Biol. Chem., 2010, 285(12): 8646-55, the disclosure of which is incorporated by reference). Peripheral blood lymphocytes (PBLs) are isolated and resuspended in media at a concentration of about 2 x 10^6 cells/ml. 50 µl of cells may be added per well of a 96 well plate followed by 50 µl of media containing lectin at various concentrations or saline. The cells may be incubated at 37°C for about three days prior to an eighteen hour addition of bromodeoxyuridine (5-bromo-2'-deoxyuridine) (BrdU), a synthetic analogue of thymidine.

Proliferation may be measured by BrdU incorporation which may be detected by chemiluminescent ELISA. Mitogenic activity may be quantified as a stimulation index, which is the signal of the stimulated cells divided by the signal of the non-treated cells (relative light units (RLU) of treated PBL / RLU of untreated PBL).

[0031] A loop found on the third Greek key of members of the JRL family (known as the carbohydrate recognition loop) may be important for ligand specificity [Jeyaprakash et al., 2004, J Mol Biol, 338(4): p. 757-70]. The length of the loop appears to play a role in determining the size of the binding pocket. Longer loops can restrict the size of the binding site to short oligosaccharides, while shorter loops have a more open binding pocket that can accommodate larger structures such as those of the high-mannose variety. While the loop lengths do not appear to correlate with specificity or affinity for different monosaccharides, the specificity for larger oligosaccharides with different components, e.g. mannose versus galactose, are likely determined by this loop [Jeyaprakash et al., 2004, J Mol Biol, 338(4): p. 757-70; Nakamura-Tsuruta et al., 2008, Febs J, 275(6): p. 1227-39; Houles Astoul et al., 2002, Biochem J, **367**(Pt 3): p. 817-24]. Applicants hypothesized that the ligand specificity of BanLec could be influenced by mutating amino acids found in the loop [M. Swanson, "Molecular Engineering of a Banana Lectin that Inhibits HIV-1 Replication", PhD dissertation, Department of Immunology, The University of Michigan, 2010].

[0032] Applicants targeted the histidine found at amino acid position 84 of BanLec (H84) since it is found in the carbohydrate recognition loop and has been predicted to play a role in the recognition of oligosaccharides [Meagher et al., 2005, Glycobiology 15(10): p. 1033-42; Singh et al., 2005, Glycobiology, 15(10): p. 1025-32]. Applicants made several different amino acid substitutions at random and tested for a decrease in mitogenic activity. The decrease in mitogenic activity was determined as above, comparing the highest mutant concentrations that still yielded a stimulation index value less than ten to rBanLec [M. Swanson, "Molecular Engineering of a Banana Lectin that Inhibits HIV-1 Replication", PhD dissertation, Department of Immunology, The University of Michigan, 2010]. The H84A mutant appeared to have no change in mitogenic activity and H84Y may have increased activity. [M. Swanson, "Molecular Engineering of a Banana Lectin that Inhibits HIV-1 Replication", PhD dissertation, Department of Immunology, The University of Michigan, 2010]. At higher concentrations, Applicants observed a drop in the stimulation index for this mutant, which is likely from activation-induced cellular death [M. Swanson, "Molecular Engineering of a Banana Lectin that Inhibits HIV-1 Replication", PhD dissertation,

Department of Immunology, The University of Michigan, 2010]. Other mutants such as H84S, H84M and H84T were found to have markedly decreased mitogenic activity. One of the most promising mutants was H84T, which did not induce a stimulation index greater than ten in the three donors tested [M. Swanson, "Molecular Engineering of a Banana Lectin that Inhibits HIV-1 Replication", PhD dissertation, Department of Immunology, The University of Michigan, 2010].

[0033] A rabbit RBC agglutination assay was used to determine if H84T had impaired cross-linking ability, binding specificity, or binding affinity. Interestingly, Applicants found H84T's agglutination activity was reduced when compared to rBanLec [M. Swanson, "Molecular Engineering of a Banana Lectin that Inhibits HIV-1 Replication", PhD dissertation, Department of Immunology, The University of Michigan, 2010]. Applicants also tested the agglutination activity of the H84S mutant and found that it to be lower than rBanLec, but greater than H84T [M. Swanson, "Molecular Engineering of a Banana Lectin that Inhibits HIV-1 Replication", PhD dissertation, Department of Immunology, The University of Michigan, 2010]. These results suggest that the H84T or other mutants may have altered cross-linking ability or carbohydrate affinity, which may contribute to the decrease in mitogenic activity. In addition, this agglutination assay may be useful for predicting the mitogenic activity of additional H84 mutants.

[0034] The present invention also encompasses the use of additional BanLec mutants, advantageously H84 mutants, that preferably have decreased or lack mitogenic activity. Such mutants may be tested by methods known to one of the art, such as the described rabbit RBC agglutination assay. In an advantageous embodiment, the mutant is H84T, however, H84S and H84M are also contemplated for the present invention.

[0035] The affinity of H84T and H84S for the mannose like ligand D-methy-α-mannopyranoside was tested by isothermal titration calorimetry (ITC) [Mo et al., 2001, Eur J Biochem, 268(9): p. 2609-15.] . Interestingly, no striking differences were observed [M. Swanson, "Molecular Engineering of a Banana Lectin that Inhibits HIV-1 Replication", PhD dissertation, Department of Immunology, The University of Michigan, 2010]. These results suggest that the H84T mutant's decrease in mitogenic activity is not due to a change in its affinity for D-methy-α-mannopyranoside, and that the protein is properly folded, and that the decrease in agglutination activity is not due to a proportion of the protein being non-functional.

[0036] The BanLec for use of the methods of the present invention may be advantageously produced by recombinant methods by utilizing a BanLec cDNA sequence that is codon-optimized for expression in *E. coli* that may be sub-cloned into an expression vector that introduced a C-terminal His-tag [M. Swanson, "Molecular Engineering of a Banana Lectin that Inhibits HIV-1 Replication", PhD dissertation, Department of Immunology, The University of Michigan, 2010; Gavrovic-Jankulovic et al., 2008, Int J Biochem Cell Biol, 40(5): p. 929-41].

[0037] BanLec binds to high mannose structures and thus useful for purifying viral protein antigens that are rich in mannose. Such antigens include, but are not limited to, the envelope proteins from HCMV, hepatitis C virus, HIV, multiple herpes viruses, influenza viruses, respiratory syncytial virus, and the like.

[0038] The present invention encompasses methods for binding HCMV glycoproteins containing mannose, such as, but not limited to, HCMV gB and HCMV gH/gL, and HCMV complex consisting of gH/gL/pUL128/pUL130/pUL131.

[0039] The present invention encompasses methods for binding influenza glycoproteins containing mannose, such as, but not limited to, influenza hemagglutinin (HA) and neuraminidase.

[0040] The present invention encompasses methods for binding HCV glycoproteins containing mannose, such as, but not limited to, E1 and E2.

[0041] The present invention encompasses methods for binding RSV glycoproteins containing mannose, such as, but not limited to, F and/or G glycoproteins..

[0042] The present invention encompasses methods for binding HSV glycoproteins containing mannose, such as, but not limited to, gA, gB, gC, gD,gE, gH and gL glycoproteins.

[0043] The present invention encompasses methods for binding rotavirus glycoproteins containing mannose, such as, but not limited to, VP7 glycoprotein. For example, to purify a rotavirus VP7 glycoprotein, the lectin of the present invention may be substitute for the monoclonal antibody of Dormitzer et al., Virology, 277, 420-428 (2000).

[0044] The present invention also encompasses isolation of any mannose-containing glycoprotein that may be formulated into vaccines or other therapeutic, prophylactic or veterinary purposes. For example, fungi and tuberculosis have mannose on their surfaces, so these mannose-containing antigens might be purified with the lectin of the present invention.

[0045] The glycoproteins may be isolated by binding to BanLec, and mutants thereof, such as the H84 mutants. Lectin affinity chromatography is known to one of skill in the art

where the lectins may be immobilized in a column or in gel beads. Glycoproteins are then adsorbed by lectins and eluted with a specific carbohydrate. So, it can be employed to fractionate and purify glycoproteins based on their specific features.

[0046] In a non-limiting example, glycoproteins may be purified as follows: A twist-off bottom of a 2 ml Pierce centrifuge tube may be removed and placed into a 15ml centrifuge tube. About 0.25ml of a resin (which may comprise BanLec and mutants thereof) may be applied to the column (for 0.25 ml of resin, add 0.5 ml as resin is stored in the ratio of 1:2 in storage buffer). The column may be centrifuged for 30sec at about 1000g, allowing the resin to settle. The column may be washed twice with 1ml of 1X PBS. The bottom portion of the column may be capped and about 1 ml of starting materials (such as a mixture which may comprise the glycoprotein to be purified) may be added. The top and bottom portions of the column are capped, making sure there are no leaks and incubate for about 3 hours at 4 °C on a rocker. The flow through may be collected and saved for gel electrophoresis. The column may be washed twice with 1 ml of 1X PBS, and the washes may be saved for gel electrophoresis. About 6 ml of 0.5 M methyl α-D-mannopyrannoside (0.583 g/6 ml) in cold 1X PBS may be prepared and used for elution in 0.5 ml fractions. Eluates with highest protein amounts (usually eluates 1 to 4 up to 1 to 6) may be pooled.

[0047] The glycoproteins may be eluted by mannose, a mannose analog or a mannose derivative. In an advantageous embodiment, the mannose analog or mannose derivative may be an inhibitor for mannose binding, such as, but not limited to, methyl α-D-mannopyrannoside. In other embodiments, a mannose analog may be 1-deoxymannojirimycin, 2-deoxy-D-glucose, 2- 2-deoxy-2-fluoro-mannose, 2-deoxy-2-chloro-mannose or 5-thio-D-Mannose. In yet another embodiment, a mannose derivative may be alpha-D-mannosamine, alpha-D-mannose-1-phosphate, beta-D-mannose-6-phosphate, guanosine diphosphate mannose (GDP)-4-keto-6-deoxy-D-mannose, GDP-D-mannose, methyl-beta-D-glycopyranoside, methyl-beta-D-mannopyranoside, manose-6-phosphate, N-acetyl-D-mannosamin-6-phosphate, N-acetyl-D-mannosamine or uridine diphosphate galactose (UDP)-N-acetyl-D-mannosamine. Mannose analogs and mannose derivatives are within the purview of one of skill in the art for practicing the present invention.

[0048] The present invention relates to therapeutic, prophylactic, veterinary or vaccine compositions which may comprise the purified glycoprotein. In an advantageous embodiment, the purified glycoprotein may be purified to about 90% or greater. Also especially preferred is a therapeutic, prophylactic, veterinary or vaccine composition which

comprises a substantially pure, recombinant, mature, glycosylated protein, wherein the protein is purified to about 90% or greater and said protein is immunogenic and induces a protective immune response when used as a vaccine and a substantially pure, recombinant glycoprotein, wherein said protein is purified to about 90% or greater.

[0049] Examples of compositions of the invention include liquid preparations for orifice, e.g., oral, nasal, anal, vaginal, peroral, intragastric, mucosal (e.g., perlingual, alveolar, gingival, olfactory or respiratory mucosa) etc., administration such as suspensions, syrups or elixirs; and preparations for parenteral, subcutaneous, intradermal, intramuscular or intravenous administration (e.g., injectable administration), such as sterile suspensions or emulsions. The choice of a composition can be determined by whether a local or systemic response is desired, as preparations for parenteral, subcutaneous, intradermal, intramuscular or intravenous administration may induce a systemic response, whereas compositions for orifice or mucosal administration may induce a local response. Such compositions may be in admixture with a suitable carrier, diluent, or excipient such as sterile water, physiological saline, glucose or the like. The compositions can also be lyophilized. The compositions can contain auxiliary substances such as wetting or emulsifying agents, pH buffering agents, gelling or viscosity enhancing additives, preservatives, flavoring agents, colors, and the like, depending upon the route administration and the preparation desired. Standard texts, such as "REMINGTON'S PHARMACEUTICAL SCIENCE", 22nd edition, 2012, incorporated herein by reference, may be consulted to prepare suitable preparations, without undue experimentation.

[0050] Compositions of the invention, are conveniently provided as liquid preparations, e.g., isotonic aqueous solutions, suspensions, emulsions or viscous compositions which may be buffered to a selected pH. If digestive tract absorption is preferred, compositions of the invention can be in the "solid" form of pills, tablets, capsules, caplets and the like, including "solid" preparations which are time-released or which have a liquid filling, e.g., gelatin covered liquid, whereby the gelatin is dissolved in the stomach for delivery to the gut. If nasal or respiratory (mucosal) administration is desired, compositions may be in a form and dispensed by a squeeze spray dispenser, pump dispenser or aerosol dispenser. Aerosols are usually under pressure by means of a hydrocarbon. Pump dispensers can preferably dispense a metered dose or, a dose having a particular particle size.

[0051] The choice of suitable carriers and other additives will depend on the exact route of administration and the nature of the particular dosage form, e.g., liquid dosage form (e.g.,

whether the composition is to be formulated into a solution, a suspension, gel or another liquid form), or solid dosage form (e.g., whether the composition is to be formulated into a pill, tablet, capsule, caplet, time release form, liquid-filled form and the like).

[0052] Solutions, suspensions and gels, normally contain a major amount of water (preferably purified water) in addition to the antigens, and optional adjuvant. Minor amounts of other ingredients such as pH adjusters (e.g., a base such as NaOH), emulsifiers or dispersing agents, buffering agents, preservative, wetting agents, jelling agents, (e.g., methylcellulose), colors and/or flavors may also be present. The inventive compositions can be isotonic, i.e., it can have the same osmotic pressure as blood and lacrimal fluid.

[0053] The desired isotonicity of the compositions of this invention may be accomplished using sodium chloride, or other pharmaceutically acceptable agents such as dextrose, boric acid, sodium tartrate, propylene glycol or other inorganic or organic solutes. Sodium chloride is preferred particularly for buffers containing sodium ions.

[0054] Viscosity of the compositions may be maintained at the selected level using a pharmaceutically acceptable thickening agent. Methylcellulose is preferred because it is readily and economically available and is easy to work with. Other suitable thickening agents include, for example, xanthan gum, carboxymethyl cellulose, hydroxpropyl cellulose, carbomer, and the like. The preferred concentration of the thickener will depend upon the agent selected. The important point is to use an amount which will achieve the selected viscosity. Viscous compositions are normally prepared from solutions by the addition of such thickening agents.

[0055] A pharmaceutically acceptable preservative can be employed to increase the shelf-life of the compositions. Benzyl alcohol may be suitable, although a variety of preservatives including, for example, parabens, thimerosal, chlorobutanol, or benzalkonium chloride may also be employed. A suitable concentration of the preservative will be from 0.02% to 2% based on the total weight although there may be appreciable variation depending upon the agent selected.

[0056] Formulations can be inhalables, e.g., sprays and the like. Aerosol spray preparations can be in a pressurized container with a suitable propellant such as a hydrocarbon propellant. Pump spray dispensers are commercially available, e.g., from Valois of America, Inc., Connecticut. Nasal spray dispensers are commonly fabricated from a flexible material such as plastic and cause a spray to dispense in response to being squeezed. Anti-inflammatories, such as "Vanceril" are commercially available in oral and nasal aerosol

form for mucosae administration; the anti-inflammatory "Vancerase" is commercially available in a pump-spray dispenser for nasal administrations; cold remedies such as "Dristan" are commercially available in nasal spray (squeeze) dispensers (so that the reader is aware that aerosol, pump and squeeze dispensers are known and available).

[0057] The therapeutic, prophylactic, veterinary or vaccine composition may be formulated in any convenient manner and in a dosage formulation consistent with the mode of administration and the elicitation of a protective response. The quantity of antigen or epitope of interest to be administered depends on the subject to be immunized. Suitable dosage ranges, however, are readily determinable by those skilled in the art and may be of the order of micrograms to milligrams, e.g., 5 to 500 μg of glycoprotein and 5 to 500 μg of glycoprotein in inventive compositions. For example, a standard dosage for influenza HA ranges from about 5 to 500 μg of HA, with a standard dose of about 15 μg and a high dose of about 60 μg of HA. Suitable regimes for initial administration and booster doses also are variable, but may include an initial administration followed by subsequent administration(s).

[0058] The therapeutically effective compositions of glycoproteins purified with this invention are prepared by mixing the ingredients following generally accepted procedures. For example the selected components may be simply mixed in a blender, or other standard device to produce a concentrated mixture which may then be adjusted to the final concentration. Compositions can be administered in dosages and by techniques well known to those skilled in the medical arts taking into consideration such factors as the age, sex, weight, and condition of the particular patient, and the particular route of administration, intraperineal, intravenous, intravascular, intramuscular, subcutaneous, intradermal, intranasal, oral, peroral, mucosal, intragastic, etc.

[0059] The compositions of glycoproteins purified with the invention may be injectable suspensions, solutions, sprays, lyophilized powders, syrups, elixirs and the like. Any suitable form of composition may be used. To prepare such a composition, having the desired degree of purity, is mixed with one or more pharmaceutically acceptable carriers and/or excipients. The carriers and excipients must be “acceptable” in the sense of being compatible with the other ingredients of the composition. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to, water, saline, phosphate buffered saline, dextrose, glycerol, ethanol, or combinations thereof, buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl

ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

[0060] An immunogenic or immunological composition can also be formulated in the form of an oil-in-water emulsion. The oil-in-water emulsion can be based, for example, on light liquid paraffin oil (European Pharmacopea type); isoprenoid oil such as squalane, squalene, EICOSANE™ or tetratetracontane; oil resulting from the oligomerization of alkene(s), *e.g.*, isobutene or decene; esters of acids or of alcohols containing a linear alkyl group, such as plant oils, ethyl oleate, propylene glycol di(caprylate/caprate), glyceryl tri(caprylate/caprate) or propylene glycol dioleate; esters of branched fatty acids or alcohols, *e.g.*, isostearic acid esters. The oil advantageously is used in combination with emulsifiers to form the emulsion. The emulsifiers can be nonionic surfactants, such as esters of sorbitan, mannide (*e.g.*, anhydromannitol oleate), glycerol, polyglycerol, propylene glycol, and oleic, isostearic, ricinoleic, or hydroxystearic acid, which are optionally ethoxylated, and polyoxypropylene-polyoxyethylene copolymer blocks, such as the Pluronic® products, *e.g.*, L121. The adjuvant can be a mixture of emulsifier(s), micelle-forming agent, and oil such as that which is commercially available under the name Provax® (IDEC Pharmaceuticals, San Diego, CA).

[0061] The immunogenic compositions of glycoproteins purified with the invention can contain additional substances, such as wetting or emulsifying agents, buffering agents, or adjuvants to enhance the effectiveness of the vaccines (Remington's Pharmaceutical Sciences, 18th edition, Mack Publishing Company, (ed.) 1980).

[0062] Adjuvants may also be included. Adjuvants include, but are not limited to, mineral salts (e.g., $AlK(SO_4)_2$, $AlNa(SO_4)_2$, $AlNH(SO_4)_2$, silica, alum, $Al(OH)_3$, $Ca_3(PO_4)_2$, kaolin, or carbon), polynucleotides with or without immune stimulating complexes (ISCOMs) (e.g., CpG oligonucleotides, such as those described in Chuang, T.H. et al, (2002) J. Leuk. Biol.

71(3): 538- 44; Ahmad-Nejad, P. et al (2002) Eur. J. Immunol. 32(7): 1958-68; poly IC or poly AU acids, polyarginine with or without CpG (also known in the art as IC31; see Schellack, C. et al (2003) Proceedings of the 34th Annual Meeting of the German Society of Immunology; Lingnau, K. et al (2002) Vaccine 20(29-30): 3498-508), JuvaVax™ (U.S. Patent No. 6,693,086), certain natural substances (e.g., wax D from *Mycobacterium tuberculosis*, substances found in *Cornyebacterium parvum, Bordetella pertussis*, or members of the genus *Brucella*), flagellin (Toll-like receptor 5 ligand; see McSorley, S.J. et al (2002) J. Immunol. 169(7): 3914-9), saponins such as QS21, QS17, and QS7 (U.S. Patent Nos. 5,057,540; 5,650,398; 6,524,584; 6,645,495), monophosphoryl lipid A, in particular, 3-de-O-acylated monophosphoryl lipid A (3D-MPL), imiquimod (also known in the art as IQM and commercially available as Aldara®; U.S. Patent Nos. 4,689,338; 5,238,944; Zuber, A.K. et al (2004) 22(13-14): 1791-8), and the CCR5 inhibitor CMPD167 (see Veazey, R.S. et al (2003) J. Exp. Med. 198: 1551-1562).

[0063] Aluminum hydroxide or phosphate (alum) are commonly used at 0.05 to 0.1% solution in phosphate buffered saline. Other adjuvants that can be used, especially with DNA vaccines, are cholera toxin, especially CTA1-DD/ISCOMs (see Mowat, A.M. et al (2001) J. Immunol. 167(6): 3398-405), polyphosphazenes (Allcock, H.R. (1998) App. Organometallic Chem. 12(10-11): 659-666; Payne, L.G. et al (1995) Pharm. Biotechnol. 6: 473-93), cytokines such as, but not limited to, IL-2, IL-4, GM-CSF, IL-12, IL-15 IGF-1, IFN-α, IFN-β, and IFN-γ (Boyer et al., (2002) J. Liposome Res. 121:137-142; WO01/095919), immunoregulatory proteins such as CD40L (ADX40; see, for example, WO03/063899), and the CD1a ligand of natural killer cells (also known as CRONY or α-galactosyl ceramide; see Green, T.D. et al, (2003) J. Virol. 77(3): 2046-2055), immunostimulatory fusion proteins such as IL-2 fused to the Fc fragment of immunoglobulins (Barouch et al., Science 290:486-492, 2000) and co-stimulatory molecules B7.1 and B7.2 (Boyer), all of which can be administered either as proteins or in the form of DNA, on the same expression vectors as those encoding the antigens of the invention or on separate expression vectors.

[0064] Immunization schedules (or regimens) are well known for animals (including humans) and can be readily determined for the particular subject and immunogenic composition. Hence, the immunogens can be administered one or more times to the subject. Preferably, there is a set time interval between separate administrations of the immunogenic composition. While this interval varies for every subject, typically it ranges from 10 days to several weeks, and is often 2, 4, 6 or 8 weeks. For humans, the interval is typically from 2 to

6 weeks. The immunization regimes typically have from 1 to 6 administrations of the immunogenic composition, but may have as few as one or two or four. The methods of inducing an immune response can also include administration of an adjuvant with the immunogens. In some instances, annual, biannual or other long interval (5-10 years) booster immunization can supplement the initial immunization protocol.

[0065] The terms "protein", "peptide", "polypeptide", and "amino acid sequence" are used interchangeably herein to refer to polymers of amino acid residues of any length. The polymer may be linear or branched, it may comprise modified amino acids or amino acid analogs, and it may be interrupted by chemical moieties other than amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling or bioactive component.

[0066] As used herein, the terms "antigen" or "immunogen" are used interchangeably to refer to a substance, typically a protein, which is capable of inducing an immune response in a subject. The term also refers to proteins that are immunologically active in the sense that once administered to a subject (either directly or by administering to the subject a nucleotide sequence or vector that encodes the protein) are able to evoke an immune response of the humoral and/or cellular type directed against that protein. As used herein, the term also refers to glycoproteins isolated by the present invention that may be administered for therapeutic purposes.

[0067] It should be understood that the proteins, including the antigens of the invention may differ from the exact sequences illustrated and described herein. Thus, the invention contemplates deletions, additions and substitutions to the sequences shown, so long as the sequences function in accordance with the methods of the invention. In this regard, particularly preferred substitutions will generally be conservative in nature, i.e., those substitutions that take place within a family of amino acids. For example, amino acids are generally divided into four families: (1) acidic--aspartate and glutamate; (2) basic--lysine, arginine, histidine; (3) non-polar--alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan; and (4) uncharged polar--glycine, asparagine, glutamine, cysteine, serine threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified as aromatic amino acids. It is reasonably predictable that an isolated replacement of leucine with isoleucine or valine, or vice versa; an aspartate with a glutamate or vice versa; a

threonine with a serine or vice versa; or a similar conservative replacement of an amino acid with a structurally related amino acid, will not have a major effect on the biological activity. Proteins having substantially the same amino acid sequence as the sequences illustrated and described but possessing minor amino acid substitutions that do not substantially affect the immunogenicity of the protein are, therefore, within the scope of the invention.

[0068] As used herein the terms "nucleotide sequences" and "nucleic acid sequences" refer to deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) sequences, including, without limitation, messenger RNA (mRNA), DNA/RNA hybrids, or synthetic nucleic acids. The nucleic acid may be single-stranded, or partially or completely double-stranded (duplex). Duplex nucleic acids may be homoduplex or heteroduplex.

[0069] As used herein the term "transgene" may be used to refer to "recombinant" nucleotide sequences that may be derived from any of the nucleotide sequences encoding the proteins of the present invention. The term "recombinant" means a nucleotide sequence that has been manipulated "by man" and which does not occur in nature, or is linked to another nucleotide sequence or found in a different arrangement in nature. It is understood that manipulated "by man" means manipulated by some artificial means, including by use of machines, codon optimization, restriction enzymes, etc.

[0070] For example, in one embodiment the nucleotide sequences may be mutated such that the activity of the encoded proteins in vivo is abrogated. In another embodiment the nucleotide sequences may be codon optimized, for example the codons may be optimized for human use. In preferred embodiments the nucleotide sequences of the invention are both mutated to abrogate the normal in vivo function of the encoded proteins, and codon optimized for human use.

[0071] As regards codon optimization, the nucleic acid molecules of the invention have a nucleotide sequence that encodes the antigens of the invention and may be designed to employ codons that are used in the genes of the subject in which the antigen is to be produced. Many viruses, including HIV and other lentiviruses, use a large number of rare codons and, by altering these codons to correspond to codons commonly used in the desired subject, enhanced expression of the antigens may be achieved. In a preferred embodiment, the codons used are "humanized" codons, i.e., the codons are those that appear frequently in highly expressed human genes (Andre et al., J. Virol. 72:1497-1503, 1998) instead of those codons that are frequently used by HIV. Such codon usage provides for efficient expression of the transgenic proteins in human cells. Any suitable method of codon optimization may be

used. Such methods, and the selection of such methods, are well known to those of skill in the art. In addition, there are several companies that will optimize codons of sequences, such as Geneart (geneart.com). Thus, the nucleotide sequences of the invention may readily be codon optimized.

[0072] The invention further encompasses nucleotide sequences encoding functionally and/or antigenically equivalent variants and derivatives of the antigens of the invention and functionally equivalent fragments thereof. These functionally equivalent variants, derivatives, and fragments display the ability to retain antigenic activity. For instance, changes in a DNA sequence that do not change the encoded amino acid sequence, as well as those that result in conservative substitutions of amino acid residues, one or a few amino acid deletions or additions, and substitution of amino acid residues by amino acid analogs are those which will not significantly affect properties of the encoded polypeptide. Conservative amino acid substitutions are glycine/alanine; valine/isoleucine/leucine; asparagine/glutamine; aspartic acid/glutamic acid; serine/threonine/methionine; lysine/arginine; and phenylalanine/tyrosine/tryptophan. In one embodiment, the variants have at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% homology or identity to the antigen, epitope, immunogen, peptide or polypeptide of interest.

[0073] For the purposes of the present invention, sequence identity or homology is determined by comparing the sequences when aligned so as to maximize overlap and identity while minimizing sequence gaps. In particular, sequence identity may be determined using any of a number of mathematical algorithms. A nonlimiting example of a mathematical algorithm used for comparison of two sequences is the algorithm of Karlin & Altschul, Proc. Natl. Acad. Sci. USA 1990; 87: 2264-2268, modified as in Karlin & Altschul, Proc. Natl. Acad. Sci. USA 1993;90: 5873-5877.

[0074] Another example of a mathematical algorithm used for comparison of sequences is the algorithm of Myers & Miller, CABIOS 1988;4: 11-17. Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 may be used. Yet another useful algorithm for identifying regions of local sequence similarity

and alignment is the FASTA algorithm as described in Pearson & Lipman, Proc. Natl. Acad. Sci. USA 1988; 85: 2444-2448.

[0075] Advantageous for use according to the present invention is the WU-BLAST (Washington University BLAST) version 2.0 software. WU-BLAST version 2.0 executable programs for several UNIX platforms may be downloaded from ftp ://blast.wustl.edu/blast/executables. This program is based on WU-BLAST version 1.4, which in turn is based on the public domain NCBI-BLAST version 1.4 (Altschul & Gish, 1996, Local alignment statistics, Doolittle ed., Methods in Enzymology 266: 460-480; Altschul et al., Journal of Molecular Biology 1990; 215: 403-410; Gish & States, 1993;Nature Genetics 3: 266-272; Karlin & Altschul, 1993; Proc. Natl. Acad. Sci. USA 90: 5873-5877; all of which are incorporated by reference herein).

[0076] The various recombinant nucleotide sequences and antigens of the invention are made using standard recombinant DNA and cloning techniques. Such techniques are well known to those of skill in the art. See for example, "Molecular Cloning: A Laboratory Manual", second edition (Sambrook et al. 1989).

[0077] The nucleotide sequences of the present invention may be inserted into "vectors." The term "vector" is widely used and understood by those of skill in the art, and as used herein the term "vector" is used consistent with its meaning to those of skill in the art. For example, the term "vector" is commonly used by those skilled in the art to refer to a vehicle that allows or facilitates the transfer of nucleic acid molecules from one environment to another or that allows or facilitates the manipulation of a nucleic acid molecule.

[0078] Any vector that allows expression of the antigens of the present invention may be used in accordance with the present invention. In certain embodiments, the antigens and/or antibodies of the present invention may be used in vitro (such as using cell-free expression systems) and/or in cultured cells grown in vitro in order to produce the encoded antigens and/or which may then be used for various applications such as in the production of proteinaceous vaccines or other therapeutic or prophylactic purposes. For such applications, any vector that allows expression of the antigens and/or antibodies in vitro and/or in cultured cells may be used.

[0079] For applications where it is desired that the antigens be expressed in vivo, for example when the transgenes of the invention are used in DNA or DNA-containing vaccines, any vector that allows for the expression of the antigens of the present invention and is safe

for use in vivo may be used. In preferred embodiments the vectors used are safe for use in humans, mammals and/or laboratory animals.

[0080] For the antigens of the present invention to be expressed, the protein coding sequence should be "operably linked" to regulatory or nucleic acid control sequences that direct transcription and translation of the protein. As used herein, a coding sequence and a nucleic acid control sequence or promoter are said to be "operably linked" when they are covalently linked in such a way as to place the expression or transcription and/or translation of the coding sequence under the influence or control of the nucleic acid control sequence. The "nucleic acid control sequence" may be any nucleic acid element, such as, but not limited to promoters, enhancers, IRES, introns, and other elements described herein that direct the expression of a nucleic acid sequence or coding sequence that is operably linked thereto. The term "promoter" will be used herein to refer to a group of transcriptional control modules that are clustered around the initiation site for RNA polymerase II and that when operationally linked to the protein coding sequences of the invention lead to the expression of the encoded protein. The expression of the transgenes of the present invention may be under the control of a constitutive promoter or of an inducible promoter, which initiates transcription only when exposed to some particular external stimulus, such as, without limitation, antibiotics such as tetracycline, hormones such as ecdysone, or heavy metals. The promoter may also be specific to a particular cell-type, tissue or organ. Many suitable promoters and enhancers are known in the art, and any such suitable promoter or enhancer may be used for expression of the transgenes of the invention. For example, suitable promoters and/or enhancers may be selected from the Eukaryotic Promoter Database (EPDB).

[0081] Although the present invention and its advantages have been described in detail, it should be understood that various changes, substitutions and alterations can be made herein without departing from the spirit and scope of the invention as defined in the appended claims.

[0082] The present invention will be further illustrated in the following Examples which are given for illustration purposes only and are not intended to limit the invention in any way.

Example: Purification of viral glycoprotein antigen by banana lectin (BanLec)

[0083] Lectin is a sugar-binding protein and specifically binds to carbohydrate moieties of glycoprotein. Lectin affinity chromatography has been widely used for purification of glycoproteins, especially tag-free recombinant glycoproteins, in research. However, most lectins are toxic at high doses and can induce allergies and reactogenicity. The possibility of

leaching lectin during glycoprotein purification by lectin affinity chromatography excludes the usage of lectin in the process of glycoprotein production for human use.

[0084] Banana lectin (BanLec) is the lectin isolated from the fruit of banana or expressed recombinantly from other cells or organisms and potentially is safer for producing glycoproteins for human use compared with most lectins currently used for glycoprotein purification.

[0085] The goal of the invention is to apply the capability of BanLec affinity chromatography to the purification of viral glycoprotein antigens. By using GNA (*Galanthus nivalis* (snowdrop) agglutinin) lectin chromatography as the benchmark, Applicants explored the purity and binding capacity achieved by Baclec on both highly glycosylated and lightly glycosylated proteins.

[0086] HCMV gB is the fusion machinery for HCMV cell entry and an attractive target for HCMV vaccine development. gB is hyperglycosylated carrying 18 N-linked glycosylation sites on each subunit. Applicants performed the purification with H84T BanLec and GNA lectin side-by-side on HCMV gB transiently expressed in HEK293 cell media. GNA conjugated agarose was used as a control. H84T BanLec was conjugated with cyanogen bromide activated agarose (Sigma C9210) in coupling buffer 0.1M $NaHCO_3$, 0.5m NaCl in the pH range of 8.3-8.5. Glycoproteins were incubated with H84T BanLec conjugated agarose in PBS and eluted by PBS containing 0.5 M methyl α-D-mannopyrannoside. Both H84T BanLec and GNA purified gB from the harvest media with comparable purity (FIG. 1). The yield of gB protein by H84T BanLec was 35% higher than the yield produced by GNA.

[0087] HCMV gH/gL is another surface glycoprotein complex critical for HCMV cell entry. Compared with gB, gH/gL is lightly glycosylated with ~6 N-linked glycosylation sites on each gH/gL complex. The purification of HEK 293 transient-expressed gH/gL showed that both H84T BanLec and GNA isolated gH/gL protein from harvest media with comparable yield and purity (FIG. 2)

[0088] Influenza hemagglutinin (HA) is the influenza virus surface glycoprotein essential for cell targeting and entry. It is therefore the main antigen in influenza subunit vaccines. HA is also lightly glycosylated with ~5-6 N-linked glycosylation sites on each subunit. Applicants performed HA purification with H84T BanLec and GNA lectin side-by-side from HA monobulk. The purity and yield by both BanLec and GNA lectin are comparable (FIG. 3). Applicants have further sucessfully used the modified BacLec to purify HIV gp120, for example (FIG. 4).

[0089] By testing the purification of three viral glycoproteins Applicants have demonstrated that BanLec is able to purify both heavily and lightly glycosylated viral glycoproteins with comparable purity and yield as GNA lectin which is widely used for glycoprotein purification. With its safer characteristics, H84T BanLec can potentially be used for glycoprotein purification for human use.

* * *

[0090] Accordingly, it is an object of the invention to not encompass within the invention any previously known product, process of making the product, or method of using the product such that Applicants reserve the right and hereby disclose a disclaimer of any previously known product, process, or method. It is further noted that the invention does not intend to encompass within the scope of the invention any product, process, or making of the product or method of using the product, which does not meet the written description and enablement requirements of the USPTO (35 U.S.C. §112, first paragraph) or the EPO (Article 83 of the EPC), such that Applicants reserve the right and hereby disclose a disclaimer of any previously described product, process of making the product, or method of using the product.

[0091] It is noted that in this disclosure and particularly in the claims and/or paragraphs, terms such as "comprises", "comprised", "comprising" and the like can have the meaning attributed to it in U.S. Patent law; e.g., they can mean "includes", "included", "including", and the like; and that terms such as "consisting essentially of" and "consists essentially of" have the meaning ascribed to them in U.S. Patent law, e.g., they allow for elements not explicitly recited, but exclude elements that are found in the prior art or that affect a basic or novel characteristic of the invention.

[0092] Having thus described in detail preferred embodiments of the present invention, it is to be understood that the invention defined by the above paragraphs is not to be limited to particular details set forth in the above description as many apparent variations thereof are possible without departing from the spirit or scope of the present invention.

WHAT IS CLAIMED IS:

1. A method of isolating a mannose-containing glycoprotein from a biological sample comprising: immobilizing a recombinant mutant banana lectin (rBanLec) on a carrier, contacting the biological sample with the BanLec and eluting the mannose-containing glycoprotein with mannose, a mannose analogue or a mannose derivative, thereby isolating the mannose-containing glycoprotein from the biological sample, wherein the recombinant mutant banana lectin has at least 100-fold lower mitogenic activity than wild-type banana lectin.

2. The method of claim 1, wherein the mannose-containing glycoprotein is a mannose-containing viral glycoprotein.

3. The method of claim 1 or 2, wherein the mannose-containing viral glycoprotein is an envelope glycoprotein.

4. The method of claim 2 or 3, viral glycoprotein is isolated from human cytomegalovirus (HCMV), hepatitis C virus, herpes virus, human immunodeficiency virus (HIV), influenza virus or respiratory syncytial virus.

5. The method of any one of claims 1-4, wherein the glycoprotein is HCMV gB, HCMV gH/gL, HIV gp120 or influenza hemagglutinin (HA).

6. The method of any one of claims 1-5, wherein the recombinant mutant rBanLec comprises a mutation at histidine 84 (H84).

7. The method of claim 6, wherein the H84 mutation comprises a mutation to threonine (H84T), methionine (H84M) or tryptophan (H84W), preferably H84T.

8. The method of any one of claims 1-5, wherein the mutant rBanLec substantially lacks mitogenic activity.

9. The method of any one of claims 1-8, wherein the mannose derivative is methyl α-D-mannopyrannoside.

10. A mannose-containing glycoprotein produced by the method of any one of claims 1-9.

11. A vaccine composition comprising a mannose-containing glycoprotein produced by the method of any one of claims 1-9.

12. A method for producing a vaccine composition which method comprises purifying a mannose-containing glycoprotein by the method of any one of claims 1-9 and combining the purified mannose-containing glycoprotein with a pharmaceutically acceptable carrier or diluent.

13. Use of a recombinant mutant banana lectin, which has at least 100-fold lower mitogenic activity than wild-type banana lectin, in the purification of a mannose-containing glycoprotein.

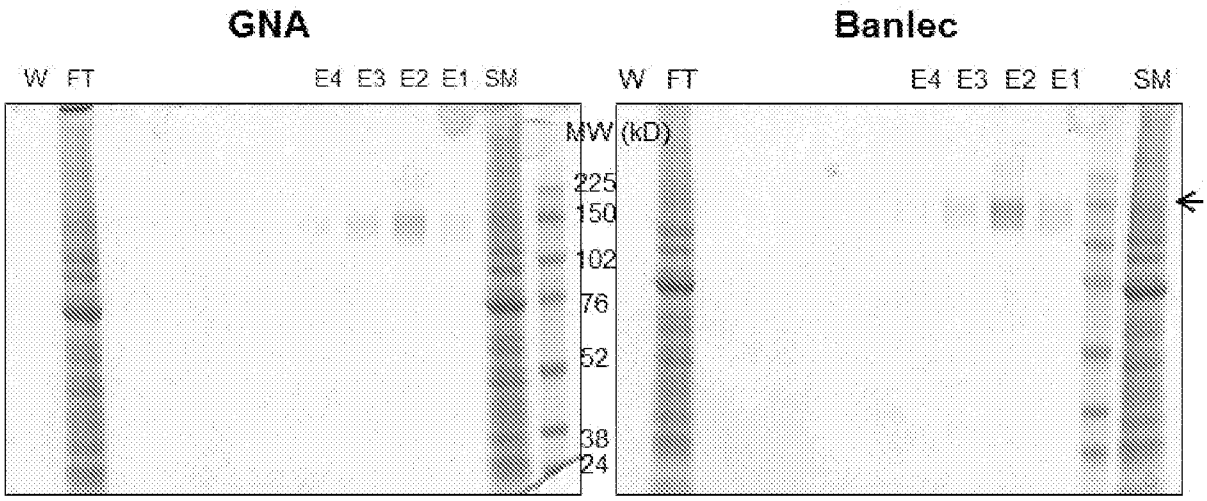
GNA
Banlec
W FT E4 E3 E2 E1 SM
W FT E4 E3 E2 E1 SM
MW (kD)
225
150
102
76
52
38
24

FIGURE 1

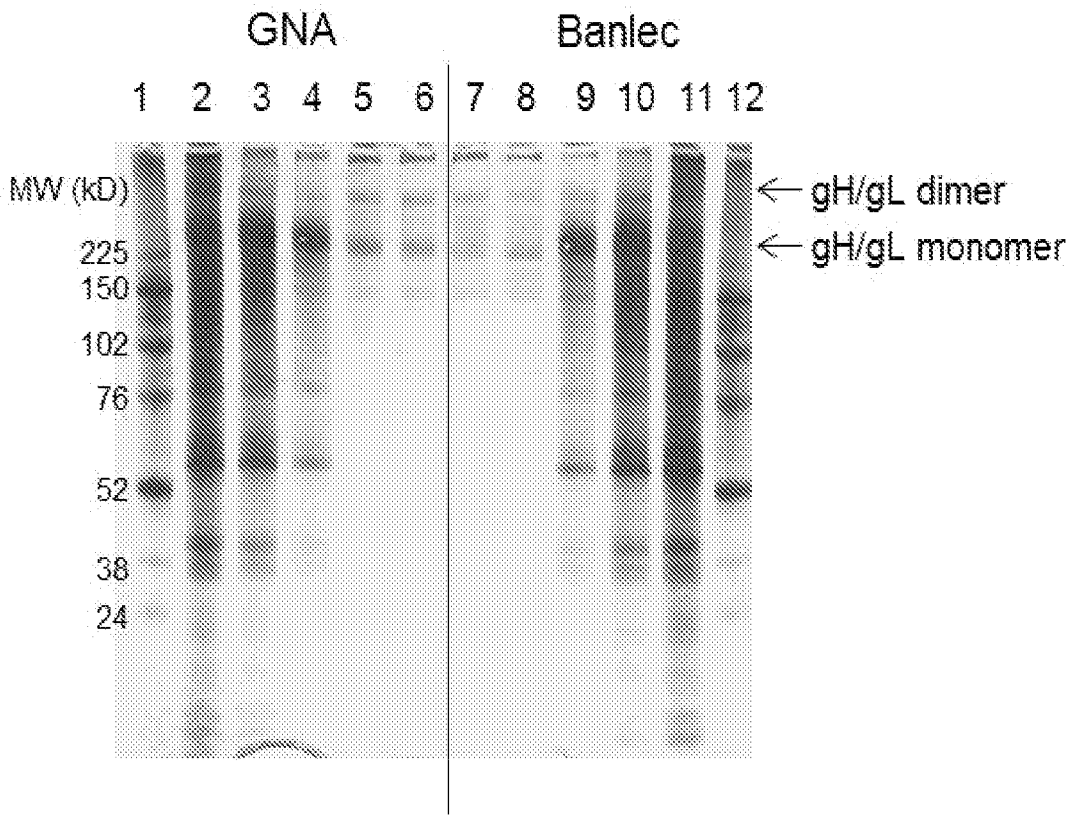
GNA
Banlec
1 2 3 4 5 6 7 8 9 10 11 12
MW (kD)
225
150
102
76
52
38
24
gH/gL dimer
gH/gL monomer

FIGURE 2

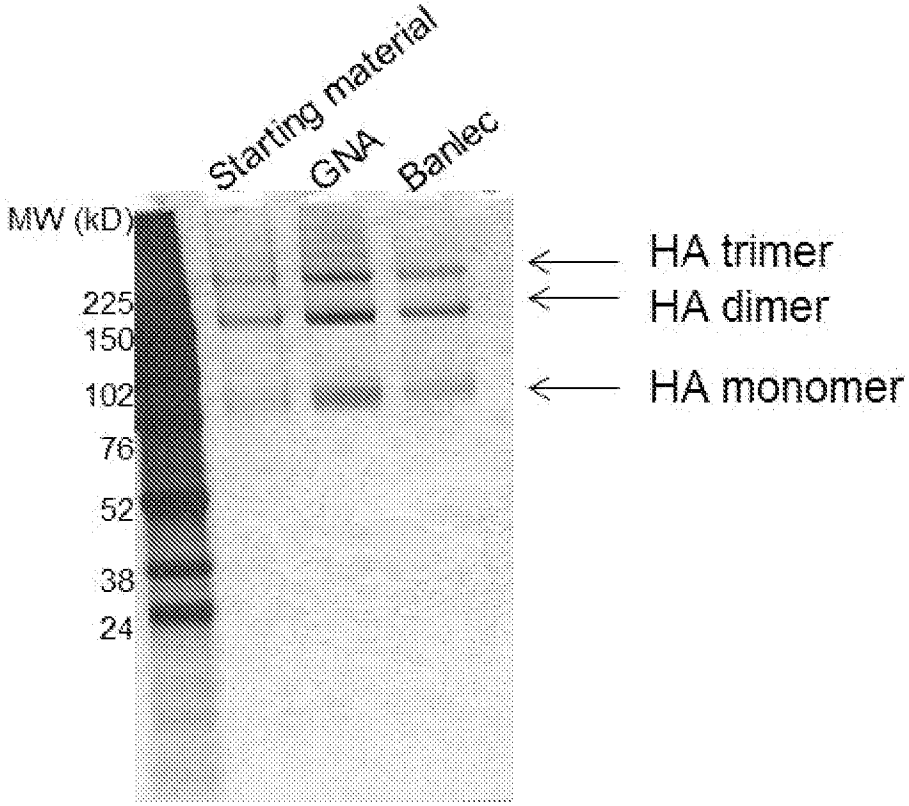
Starting material
GNA
Banlec
MW (kD)
225
150
102
76
52
38
24
HA trimer
HA dimer
HA monomer

FIGURE 3

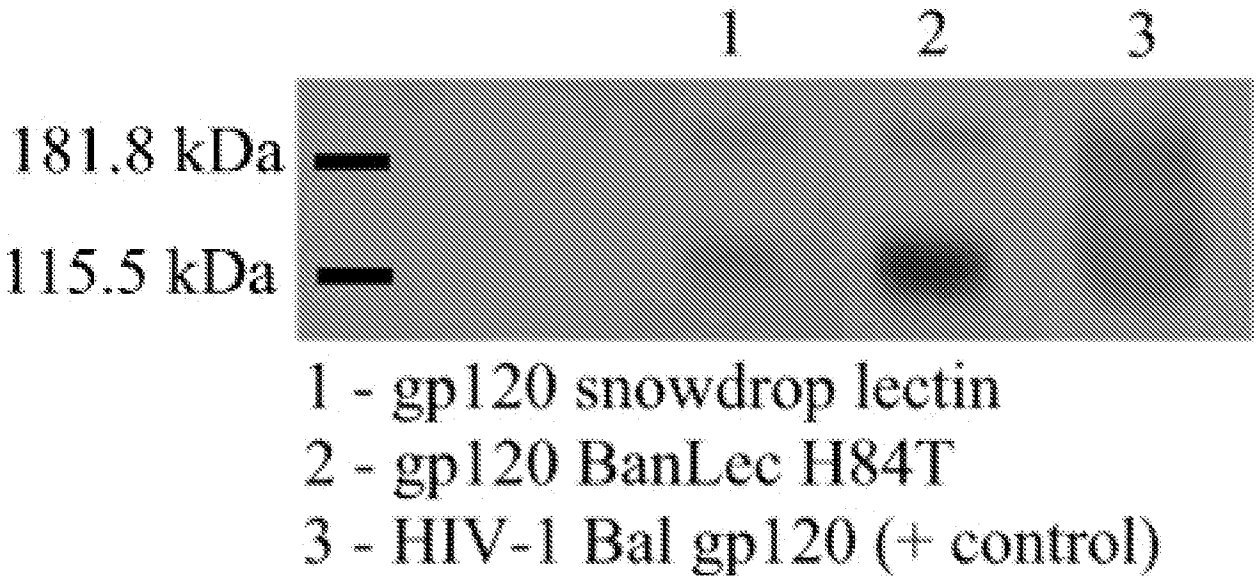
1
2
3
181.8 kDa
115.5 kDa
1 - gp120 snowdrop lectin
2 - gp120 BanLec H84T
3 - HIV-1 Bal gp120 (+ control)

FIGURE 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/029815

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/42 A61K38/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2011/130145 A1 (UNIV MICHIGAN [US]; MARKOVITZ DAVID [US]; SWANSON MICHAEL [US]; GOLDST) 20 October 2011 (2011-10-20) cited in the application page 2 - page 4; figures 1,2; sequences 1-3	1-13
A	CHEUNG A H K ET AL: "Musa acuminata (Del Monte banana) lectin is a fructose-binding lectin with cytokine-inducing activity", PHYTOMEDICINE, GUSTAV FISCHER VERLAG, STUTTGART, DE, vol. 16, no. 6-7, 1 June 2009 (2009-06-01) , pages 594-600, XP026102725, ISSN: 0944-7113, DOI: 10.1016/J.PHYMED.2008.12.016 [retrieved on 2009-02-04] abstract; table 2	1-13

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
17 June 2015	02/07/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Krüger, Julia

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/029815

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GAVROVIC-JANKULOVIC ET AL: "A novel recombinantly produced banana lectin isoform is a valuable tool for glycoproteomics and a potent modulator of the proliferation response in CD3<+>, CD4<+>, and CD8<+> populations of human PBMCs", INTERNATIONAL JOURNAL OF BIOCHEMISTRY AND CELL BIOLOGY, PERGAMON, GB, vol. 40, no. 5, 13 November 2007 (2007-11-13), pages 929-941, XP022514440, ISSN: 1357-2725, DOI: 10.1016/J.BIOCEL.2007.10.033 abstract	1-13

X	GILLJAM GUSTAV: "Envelope glycoproteins of HIV-1, HIV-2, and SIV purified with Galanthus nivalis agglutinin induce strong immune responses", AIDS RESEARCH AND HUMAN RETROVIRUSES, vol. 9, no. 5, 1993, pages 431-438, XP002740958, ISSN: 0889-2229	10,11
Y	abstract; figures 3-5	1-13

X	WO 2007/149490 A1 (PROGENICS PHARM INC [US]; CORNELL RES FOUNDATION INC [US]; OLSON WILLI) 27 December 2007 (2007-12-27)	10,11
Y	pages 1-3; claims 5, 14, 44	1-13

Y	NOBUYUKI MATOBA ET AL: "HIV-1 Neutralization Profile and Plant-Based Recombinant Expression of Actinohivin, an Env Glycan-Specific Lectin Devoid of T-Cell Mitogenic Activity", PLOS ONE, vol. 5, no. 6, 15 June 2010 (2010-06-15), page e11143, XP055196039, DOI: 10.1371/journal.pone.0011143 page 2, column 1; figure 2	1-13

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2015/029815

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2011130145 A1	20-10-2011	CN 103025756 A	03-04-2013
		EP 2558488 A1	20-02-2013
		US 2013096051 A1	18-04-2013
		US 2015080293 A1	19-03-2015
		WO 2011130145 A1	20-10-2011
WO 2007149490 A1	27-12-2007	CA 2655929 A1	27-12-2007
		EP 2040746 A1	01-04-2009
		WO 2007149490 A1	27-12-2007