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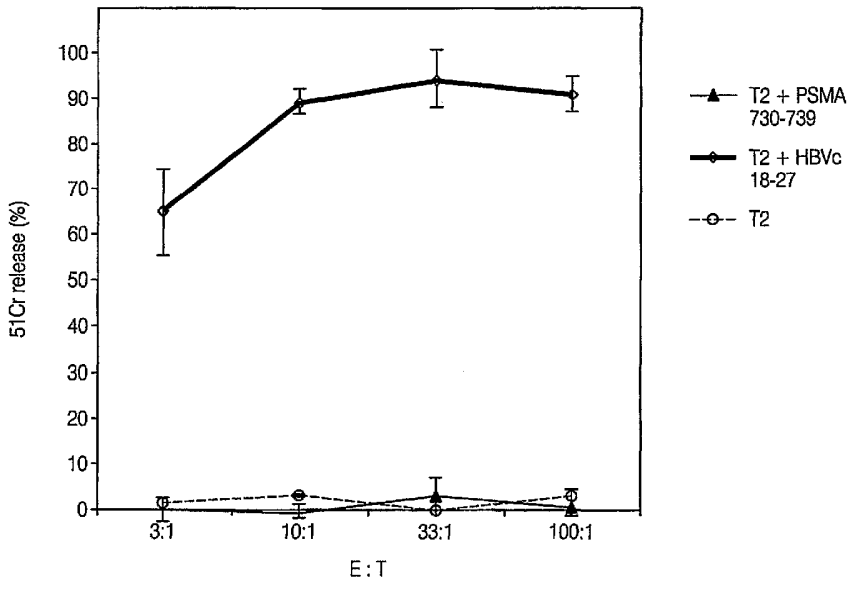
(54) **Title:** METHODS TO BYPASS CD+4 CELLS IN THE INDUCTION OF AN IMMUNE RESPONSE



(57) **Abstract:** Embodiments of the invention disclosed herein relate to methods and compositions for bypassing the involvement of CD4+ cells when generating antibody and MHC class I-restricted immune responses, controlling the nature and magnitude of the response, and promoting effective immunologic intervention in viral pathogenesis. More specifically, embodiments relate to immunogenic compositions for vaccination particularly therapeutic vaccination, against HIV and other microbial pathogens that impact functioning of the immune system, their nature, and the order, timing, and route of administration by which they are effectively used.

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METHODS TO BYPASS $CD4^+$ CELLS IN THE INDUCTION OF AN IMMUNE RESPONSE

Cross Reference to Related Applications

[0001] This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 60/640,821, filed on December 29, 2004, entitled METHODS TO BYPASS $CD4^+$ CELLS IN THE INDUCTION OF AN IMMUNE RESPONSE; the disclosure of which is incorporated herein by reference in its entirety.

Background of the Invention

Field of the Invention

[0002] Embodiments of the invention disclosed herein relate to methods and compositions for bypassing the involvement of $CD4^+$ cells when generating antibody and MHC class I-restricted immune responses, controlling the nature and magnitude of the response, and promoting effective immunologic intervention in viral pathogenesis. More specifically, embodiments relate to immunogenic compositions for vaccination particularly therapeutic vaccination, against HIV and other microbial pathogens that impact functioning of the immune system, their nature, and the order, timing, and route of administration by which they are effectively used.

Description of the Related Art

The Major Histocompatibility Complex and T Cell Target Recognition

[0003] T lymphocytes (T cells) are antigen-specific immune cells that function in response to specific antigen signals. B lymphocytes and the antibodies they produce are also antigen-specific entities. However, unlike B lymphocytes, T cells do not respond to antigens in a free or soluble form. For a T cell to respond to an antigen, it requires the antigen to be bound to a presenting molecule known as a major histocompatibility complex (MHC) antigen/protein/marker.

[0004] MHC proteins provide the means by which T cells distinguish healthy "self" cells from foreign or infected (non-self) cells. MHC molecules are a category of immune receptors that present potential peptide epitopes to be monitored subsequently by the T cells. There are two types of MHC, class I MHC and class II MHC. $CD4^+$ T cells interact with class II MHC proteins and predominately have a helper phenotype while $CD8^+$ T cells

interact with class I MHC proteins and predominately have a cytolytic phenotype, but each of them can also exhibit regulatory, particularly suppressive, function. Both classes of MHC protein are transmembrane proteins with a majority of their structure on the external surface of the cell. Additionally, both classes of MHC have a peptide binding cleft on their external portions. It is in this cleft that small fragments of proteins, native or foreign, are bound and presented to the extracellular environment.

[0005] The antigen receptor of T cells, or T cell receptor (TCR), recognizes the complex formed by peptide and MHC marker by binding to it. The MHC is highly polymorphic with the result that the specificity exhibited by a TCR is dependent on both the peptide and the MHC marker in the recognized complex. This requirement is called MHC restriction. T cell immune responses are induced when T cells recognize peptide-MHC marker complexes displayed by cells called professional antigen presenting cells (pAPCs). Effector functions, such as cytolytic activity or cytokine secretion, are actuated when T cells subsequently recognize peptide-MHC marker complexes on other cells of the body.

HIV

[0006] Human Immunodeficiency Virus (HIV) is a member of the Lentivirus genus of the *Retroviridae* family. This family of viruses is known for latency, persistent viremia, infection of the nervous system, and weak host immune responses. HIV has high affinity for $CD4^+$ T lymphocytes and monocytes. HIV binds to $CD4^+$ T-cells at the cell surface and becomes internalized. The virus replicates by generating a DNA copy by reverse transcriptase. Viral DNA becomes incorporated into the host DNA, enabling further replication. HIV is the causative agent of acquired immune deficiency syndrome, AIDS.

[0007] Despite more than 20 years of HIV related research, infection with HIV remains a major public health concern. Globally, more than 42 million people are infected, including about 5 million newly infected in the year 2003 (Garber, D. et al., *The Lancet Infectious Diseases* 4:397-413, 2004). The most common clinical manifestations of HIV are due to progressive immunodeficiency caused by a selective loss of $CD4^+$ lymphocytes (Buckland, M.S. & Pinching, A.J. *Intern. J of STD & AISA* 15:574-583, 2004). Both $CD4^+$ and $CD8^+$ T-cells are important in the control of viral, including HIV, replication. Activated $CD4^+$ T-helper cells produce cytokines and interact with cell-surface receptors that prompt B cells to produce antibodies, and they interact indirectly (via antigen presenting cells) or

directly with $CD8^+$ T lymphocytes to induce differentiation into cytotoxic cells. HIV grows far better in activated cells than in cells at rest (Roberts, J.P. *The Scientist* 18:26-27, 2004). Consequently, the very cells central in orchestrating the fight against viral pathogens, $CD4^+$ T-cells, may then be lost by apoptosis, cytolysis, or cell mediated cytotoxicity. The result is an ineffective immune response due to the prompt deletion of activated T cells, with expected repercussions on induction, expansion and differentiation of $CD8^+$ T cells and B cells recognizing viral antigens. Initially, the rate of production of $CD4^+$ T cells is greater than peripheral destruction, and so antibody production and generation of an expanded repertoire of $CD8^+$ T-cells to kill virally infected targets proceeds correctly. Over time, the rapid mutation rate of HIV, poor immunogenic characteristics of HIV proteins, and the scale of HIV replication overwhelm the host immune system. Since $CD4^+$ T cells are required to support the pool of HIV-specific $CD8^+$ T-cells, the loss of HIV-specific $CD4^+$ cells leads to a loss of HIV-specific $CD8^+$ T-cells. Immune containment of HIV infection fails and clinical progression to AIDS ensues.

[0008] Most infected patients do not exhibit overt clinical manifestations of the disease for six to ten years following initial infection. However a small group remain long-term non-progressors (LTNP), and remain free of disease for ten or more years. They exhibit lower viral loads and stable $CD4^+$ cell counts which have in part been attributed to cell-mediated immunity. The nature of viral suppression in this group has been the focus of much research. There has been a great deal of effort made to understand the characteristics of LTNP and the mechanism by which the disease-free state is achieved, so that better therapeutics and prophylactics may be designed.

Therapeutics

[0009] Morbidity and mortality associated with HIV infection have been dramatically reduced with the advent of antiretroviral therapy targeting two key enzymes: reverse transcriptase and protease. However, beneficial effects can be variable, prolonged treatment induces considerable toxicity, and effectiveness is undermined by the emergence of drug-resistant mutations. Also, the high cost of antiretroviral therapies limits access and availability in developing countries. Thus, alternative, less costly strategies capable of effecting sustained viral suppression are desperately needed.

[0010] Therapeutic immunization as a treatment for HIV infection may prove to be such an alternative. However, several critical aspects of HIV infection present novel challenges to the development of an effective vaccine. These properties include viral particles that are difficult to neutralize with antibodies; selective infection, destruction, and impaired regeneration of $CD4^+$ T-helper cells; rapid virus evolution providing escape from cellular and humoral immune responses; and high viral genetic diversity, distribution, and prevalence (Garber, D. et al., *The Lancet Infectious Diseases* 4:397-413, 2004). Also, it has been recently suggested that a vaccine-primed immune system might be more susceptible to infection. Boosting the HIV specific helper cells, an outcome of vaccination, may be giving the virus more targets to infect. Since more conventional vaccination strategies depend on co-induction of T helper (Th) cells, it is expected that their efficacy is low or the overall effect detrimental in a setting where Th cell function is impaired by HIV (Roberts, J.P. *The Scientist* 18:26-27, 2004).

[0011] The immune system may effectively eliminate virus-infected cells during the clinical course of HIV-1 infection using virus-specific major histocompatibility complex (MHC) class-I restricted CTL activity (Koup, et al.*J Exp Med.* 180(3):779-82, 1994; Koup et al. "*Nature*, 370(6489):416, 1994; and Koup et al., *J Virol.* 68(7):4650-5, 1994). There is evidence that suggests HIV-1-specific CTL activity is important for controlling viral spread during the clinical course of HIV-1 infection (Klein, 1995; Koup, 1994), for maintaining low levels of viral load during the asymptomatic phase (Musey, 1997; Rinaldo, 1995; Koup, 1994; Walker, 1987), and possibly for complete elimination of virus-infected cells, as implied from the observation of HIV-exposed, but virus-negative, children and women (Rowland-Jones, 1995; Rowland-Jones, 1993). Furthermore, observations from cross-sectional studies have shown the absence, or severely decreased levels, of HIV-1-specific CTL responses during advanced stages of HIV-1 infection (Carmichael, 1993). Taken together, recent vaccine strategy has focused on eliciting antiviral $CD8^+$ T cell responses to control the level of HIV replication *in vivo* (Garber, D. et al., *The Lancet Infectious Diseases* 4:397-413, 2004). Rationale for potential efficacy of $CD8^+$ T-cell- based AIDS vaccine is that reduction of the level of setpoint viral load may slow the rate of progression to AIDS and eliminate active reservoirs of infection.

Other Pathogens

[0012] HIV is not the only pathogen for which activation or expansion of $CD4^+$ cells is associated with pathological processes. For example corneal scarring incident to herpes simplex virus (HSV) infection is attributable to the action of $CD4^+$ T cells and the cytokines they produce. (Osorio, Y. et al., *Ocul. Immunol. Inflamm.* 10: 105-116, 2002; Altmann, D.M. & Blyth, W.A. *J. Gen. Virol.* 66:1297-1303, 1985; Xu, M. et al., *J. Immunol.* 173:1232-1239, 2004). HIV is also not the only pathogen for which impairment of the $CD4^+$ T cell response results in failure to mount a more effective immune response, persistence of infection, and greater morbidity or mortality. Failure of dendritic cells (DC) to increase class II MHC expression, and thus productively interact with $CD4^+$ T cells, contributes to the persistence of Hepatitis B virus (HBV) infection (Zheng, B.J., et al., *J. Viral Hepat.* 11:217-224, 2004; Lohr, H.F., et al., *Clin. Exp. Immunol.* 130:107-104, 2002). Similarly impaired DC-$CD4^+$ T cell interactions are involved in the poor immune responses to and persistence of infection by Hepatitis C virus (HCV) (Murakami, H., et al. *Clin. Exp. Immunol.* 137:559-565, 2004).

[0013] Embodiments described herein relate to methods and compositions that alleviate or overcome the above-described challenges associated with the treatment of microbial infections, including those associated with HIV, herpes simplex virus (HSV), HBV, HCV, hepatitis G virus (HGV), human papilloma virus (HPV), cytomegalovirus (CMV), influenza virus, human T-cell leukemia virus (HTLV), Respiratory syncytial virus (RSV), Epstein Barr virus (EBV), measles virus, and Ebola virus, for example.

Summary of the Invention

[0014] Embodiments of the present invention generally relate to a general manner of eliciting the induction, expansion and/or differentiation of the $CD8^+$ T cell population while eliciting only a modest or no $CD4^+$ T helper response (in a fashion independent of $CD4^+$ T helper response). Some embodiments include methods and compositions for inducing, entraining, and/or amplifying, the immune response to MHC class I-restricted HIV epitopes.

[0015] Some embodiments relate to methods of generating an immune response, including methods of immunization, that can include the steps of delivering to a lymphatic system of a mammal a composition that includes an immunogen, which immunogen

includes a class I MHC-restricted epitope or a B cell epitope, wherein the immunogen does not include an effective class II MHC-restricted epitope; administering an immunopotentiator to the mammal; and obtaining or detecting an epitope-specific immune response without substantial activation or expansion of $CD4^+$ cells. The term "effective class II MHC-restricted epitope" as used herein can mean a peptide sequence that can be processed from the natural antigen and presented by a class II MHC molecule expressed by the species or individual in question so as to generate a class II restricted immune response. In preferred embodiments the epitope can be an HIV, HSV, HBV, HCV, HGV, HPV, CMV, influenza virus, HTLV, respiratory syncytial virus (RSV), EBV, measles virus, and Ebola virus epitope or an epitope associated with a target antigen for any disease in which avoidance of $CD4^+$cell activation or expansion can be advantageous. Preferably, the immunogen and the immunopotentiator can be co-administered to the lymphatic system. Furthermore, in some preferred aspects the composition can include a first immunogen that includes a class I MHC-restricted epitope and a second immunogen that includes a B cell epitope, for example. Preferably, the first immunogen and the second immunogen can be the same. The method can further include co-administering a first immunogen that includes or encodes the class I MHC-restricted epitope with the immunopotentiator, and subsequently delivering a second immunogen that includes the epitope, in the form of an epitopic peptide, to the lymphatic system of the mammal. The interval between the administering step and the delivering step can be at least about seven days, for example. The first immunogen can include a nucleic acid encoding the epitope. The immunopotentiator can include, for example, a DNA molecule that includes a CpG sequence. The nucleic acid can include a DNA molecule that includes a CpG sequence which constitutes the immunopotentiator. The immunopotentiator can include, for example, a dsRNA. The first immunogen can include a polypeptide. In preferred embodiments the delivery to the lymphatic system can include delivery to a lymph node or a lymph vessel.

[0016] Some embodiments relate to methods that can include administering intranodally an adjuvant and peptide. Also, some other embodiments relate to inducing a response, and amplifying the response with peptide without requiring adjuvant for amplification. In some aspects, the amplification step can include the delivery of an immunogen along with a biological response modifier, such as an adjuvant. Preferably, in

some aspects the amplification can be accomplished without adjuvant. Also, in some instances the amplification step can be performed without the delivery of any MHC class II restricted epitopes, thereby minimizing or avoiding any $CD4^+$cells.

[0017] Also, some embodiments relate to methods of immunization, which can include, for example, a step for potentiating an immune response; a step for exposing the lymphatic system to a class I MHC-restricted epitope or a B cell epitope; and obtaining an epitope-specific immune response without substantial activation or expansion of $CD4^+$ cells.

[0018] Further embodiments relate to methods of immunization that include delivering to a mammal a first composition that includes an immunogen, which immunogen can include or encode at least a portion of a first antigen; administering a second composition that includes an amplifying peptide directly to a lymphatic system of the mammal, wherein the peptide corresponds to an epitope of said first antigen, wherein the first composition and the second composition are not the same, and inducing a cytotoxic T lymphocyte response without a T helper response. In some aspects, the immunogen can be an HIV, HSV, HBV, HCV, HPV, CMV, influenza virus, HTLV, respiratory syncytial virus (RSV), EBV, measles virus, or Ebola virus immunogen (or an immunogen that includes a sequence related to more than one of the same) and/or an immunogen associated with any disease in which avoidance of $CD4^+$cell activation or expansion can be advantageous.

[0019] Preferably, the first antigen can be an HIV antigen. The first composition can include a nucleic acid encoding the antigen, the antigen or an immunogenic fragment thereof; or a nucleic acid capable of expressing the epitope in a pAPC, for example. The nucleic acid can be delivered as a component of a protozoan, bacterium, virus, viral vector, or the like. In some aspects the first composition can include an immunogenic polypeptide and an immunopotentiator. Preferably, the immunopotentiator can be a T1 biasing cytokine, for example, IL-12, IFN-gamma, or the like. Also, the immunopotentiator can be a T1 biasing toll-like receptor ligand. The adjuvant can be an immunostimulatory sequence. The adjuvant can include RNA. The immunogenic polypeptide can be an amplifying peptide. The immunogenic polypeptide can be the first antigen. The immunogenic polypeptide can be delivered as a component of a protozoan, bacterium, virus, viral vector, virus-like particle, or the like. The adjuvant can

be delivered as a component of a protozoan, bacterium, virus, viral vector, virus-like particle, or the like. The second composition can be adjuvant-free and immunopotentiator-free. The delivering step can include direct administration to the lymphatic system of the mammal. In some aspects the direct administration to the lymphatic system of the mammal can include direct administration to a lymph node or lymph vessel. The direct administration can be to two or more lymph nodes or lymph vessels. The lymph node can be, for example, inguinal, axillary, cervical, or tonsilar lymph nodes. The method can further include obtaining an effector T cell response to the first antigen, and the effector T cell response can include production of a pro-inflammatory cytokine, including, for example, gamma-IFN or TNFα (alpha). The effector T cell response can include the production of a T cell chemokine, for example, RANTES or MIP-1α. The epitope can be a housekeeping epitope or an immune epitope, for example. The terms "housekeeping epitope" and "immune epitope" are defined in U.S. Publication No. 2003-0215425, which is incorporated herein by reference in its entirety. The delivering step or the administering step can include a single bolus injection, repeated bolus injections, or a continuous infusion, for example. The infusion can have a duration of between about 8 hours to about 7 days, for example. The method can include an interval between termination of the delivering step and beginning the administering step, wherein the interval is at least about seven days, between about 7 and about 14 days, or from about 14 to about 75 days, or over about 75 days for example. The method can be used for treating AIDS. The first antigen can be a target-associated antigen. The target can be an HIV infected cell, for example. Additionally, the method can be used to treat other viral infections or any disease where avoidance of $CD4^+$cell activation or expansion can be advantageous. Examples of viral infections include those caused by HSV, HBV, HCV, HPV, CMV, influenza virus, HTLV, respiratory syncytial virus (RSV), EBV, measles virus, or Ebola virus. The effector T cell response can be detected by at least one indicator, for example, a cytokine assay, an Elispot assay, a cytotoxicity assay, a tetramer assay, a DTH-response, a clinical response, decrease pathogen titre, pathogen clearance, amelioration of a disease symptom, or the like. The effector T cell response can be a cytotoxic T cell response.

[0020] Some embodiments relate to methods of generating an immune response, including methods of immunization against HIV, HSV, HBV, HCV, HPV, CMV, influenza virus, HTLV, EBV, respiratory syncytial virus (RSV), measles virus, Ebola virus, or any

disease where avoidance of $CD4^+$cell activation or expansion can be advantageous. The methods can include, for example, delivering to a mammal a first composition that includes a a first antigen, an immunogenic fragment thereof, or a nucleic acid encoding the either of the same; and administering a second composition that includes a peptide, directly to the lymphatic system of the mammal, wherein the peptide corresponds to an epitope of the first antigen. The method can further include obtaining an effector T cell response to the antigen.

[0021] Some embodiments relate to methods of augmenting an existing antigen-specific immune response that includes administering a composition that includes a peptide, directly to the lymphatic system of a mammal, wherein the peptide corresponds to an epitope of said antigen, and wherein said composition was not used to induce the immune response; and obtaining augmentation of an HIV antigen-specific immune response. The augmentation can include sustaining the response over time, reactivating quiescent T cells, including $CD8^+$ cells. The augmentation can include expanding the population of HIV antigen-specific T cells. In some aspects the composition does not include an immunopotentiator, while in others it does include an immunopotentiator. The antigen-specific immune response can be, for example, an HIV, HSV, HBV, HCV, HPV, CMV, influenza virus, HTLV, respiratory syncytial virus (RSV), EBV, measles virus, or Ebola virus antigen-specific immune response, or antigen-specific immune response associated with any other disease where avoidance of $CD4^+$cell activation or expansion can be advantageous. Preferably, it is an HIV antigen-specific immune response.

[0022] Further embodiments relate to methods of immunization that include, for example, delivering to a mammal a first composition that includes an HIV immunogen, which immunogen includes or encodes at least a portion of a first antigen and at least a portion of a second antigen; and administering a second composition that includes a first peptide, and a third composition that includes a second peptide, directly to the lymphatic system of the mammal, wherein the first peptide corresponds to an epitope of said first antigen, and wherein the second peptide corresponds to an epitope of said second antigen, wherein the first composition is not the same as the second or third compositions. The method can further include obtaining an effector T cell response to the antigen. The second and third compositions each can include the first and the second peptides. It should be understood that in some embodiments the HIV immunogen mentioned above can be

replaced by an HSV immunogen, an HBV immunogen, an HCV immunogen, an HPV immunogen, a CMV immunogen, an influenza virus immunogen, an HTLV immunogen, an RSV immunogen, an EBV immunogen, or a measles virus immunogen, an Ebola virus immunogen, or an immunogen associated with any other disease where avoidance or minimization of $CD4^+$activation or expansion can be advantageous.

[0023] Some embodiments relate to methods of immunization against HIV that include, for example, administering a series of immunogenic doses directly into the lymphatic system of a mammal wherein the series includes at least 1 entraining dose and at least 1 amplifying dose, and wherein the entraining dose includes a nucleic acid encoding an immunogen and wherein the amplifying dose is free of any virus, viral vector, replication-competent vector, or the like. The method can include about 1-6 entraining doses,for example, or even more than 6, for example, 1-7, 1-8, 1-9, 1-10 or more. The method can include administering a plurality of entraining doses, wherein the doses are administered over a course of one to about seven days. The entraining doses, amplifying doses, or entraining and amplifying doses can be delivered in multiple pairs of injections, wherein a first member of a pair is administered within about 1, 2, 3, 4 or 5 days and preferably within about 4 days of a second member of the pair, and wherein an interval between first members of different pairs is at least about, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 days, for example. Preferably, an interval between first members of different pairs is at least about 14 days, for example. The interval between a last entraining dose and a first amplifying dose can be between about 1 and about 150 days, about 3 and about 125 days, and preferably about 7 and about 100 days, for example. The method can further include obtaining an antigen-specific immune response. It should be understood that in some embodiments, the above-mentioned methods of immunization against HIV can be modified to generate an immune response, including immunization against HSV, HBV, HCV, HPV, CMV, influenza virus, HTLV, RSV, EBV, measles virus, Ebola virus, or against any other disease where avoidance or minimization of $CD4^+$activation or expansion can be advantageous.

[0024] Also, some embodiments relate to sets of immunogenic compositions for inducing an immune response in a mammal that includes about 1-6 or more entraining doses and at least one amplifying dose, wherein the entraining doses includes a nucleic acid encoding an HIV immunogen, and wherein the amplifying dose includes a peptide epitope,

and wherein the epitope is presented by pAPC expressing the nucleic acid. In some aspects, the sets of compositions can include more than 6 entraining doses, for example, about 1-7, 1-8, 1-9, 1-10 or more. In some aspects at least one dose further can include an adjuvant, for example, RNA. The entraining and amplifying doses can be in a carrier suitable for direct administration to the lymphatic system, for example a lymph node. The nucleic acid can be a plasmid. The epitope can be a class I MHC epitope. The MHC can be any MHC, including, for example, those listed in Tables 1-4, including combinations of the same, while other embodiments specifically exclude any one or more of the MHCs or combinations thereof. Tables 3-4 include frequencies for the listed HLA antigens. Preferably, the HLA can be, for example, HLA-A2, HLA-B7, and the like. The immunogen can include an epitope array, which can include, for example, a liberation sequence. The term "liberation sequence" as used herein is defined in U.S. Publication No. 2003-0228634, published on December 11, 2003, which is incorporated herein by reference in its entirety. The immunogen can be a target-associated antigen, for example, an antigen from an HIV infected cell. The immunogen can be a fragment of a target-associated antigen that includes an epitope cluster. It should be understood that in some embodiments the nucleic acid encoding the HIV immunogen mentioned above, can be replaced by a nucleic acid encoding one or more of the following: an HSV immunogen, an HBV immunogen, an HCV immunogen, an HPV immunogen, a CMV immunogen, an influenza virus immunogen, an HTLV immunogen, an RSV immunogen, an EBV immunogen, or a measles virus immunogen, an Ebola virus immunogen, or an immunogen associated with any other disease where avoidance or minimization of $CD4^+$ activation or expansion can be advantageous.

[0025] Further embodiments relate to sets of immunogenic compositions for inducing a class I MHC-restricted immune response in a mammal, which methods can include, for example, 1-6, or more, entraining doses and at least one amplifying dose, wherein the entraining doses include an HIV immunogen (or other immunogen as described above or elsewhere herein) or a nucleic acid encoding an immunogen (or encoding another immunogen as described above or elsewhere herein) and an immunopotentiator, and wherein the amplifying dose includes a peptide epitope, and wherein the epitope is presented by pAPC. In some aspects, the sets of compositions can include more than 6 entraining doses, for example, about 1-7, 1-8, 1-9, 1-10 or more. The nucleic acid encoding

the HIV immunogen further can include an immunostimulatory sequence which serves as the immunopotentiating agent. The immunogen can be, for example, a virus or replication competent vector that includes or induces an immunopotentiating agent. The immunogen can be, for example, a bacterium, bacterial lysate, purified cell wall component, or the like, wherein the bacterial cell wall component is capable of functioning as the immunopotentiating agent. The immunopotentiating agent can be, for example, a TLR ligand, an immunostimulatory sequence, a CpG-containing DNA, a dsRNA, an endocytic-Pattern Recognition Receptor (PRR) ligand, a lipopolysacharide (LPS), a quillaja saponin, tucaresol, a pro-inflammatory cytokine, and the like.

[0026] Some embodiments relate to methods of immunization, which methods can include the step of delivering to a mammal a first composition that includes a first immunogen, the first immunogen including or encoding at least a portion of a first antigen; and subsequently administering a second composition that includes an epitopic peptide directly to the lymphatic system of the mammal, wherein the peptide corresponds to a class I MHC-restricted epitope of the first antigen, wherein the second composition is not the same as the first composition such that an epitope-specific immune response is amplified without substantial activation or expansion of $CD4^+$ T cells. In some aspects, the delivering step can further include the delivery of an immunopotentiator or adjuvant.

[0027] Also, some embodiments relate to methods of generating an immune response against a disease-related antigen in which it is advantageous to minimize the expansion of $CD4^+$ lymphocytes. The methods can include delivering to an animal a first immunogen and an immunopotentiator, the first immunogen including or encoding at least a first portion of a first antigen, wherein the at least a portion of a first antigen does not include a class II MHC restricted epitope for an MHC expressed by the animal; and administering, preferably after the delivering step, an epitopic peptide directly to a lymphatic system of the animal, wherein the peptide corresponds to a class I MHC-restricted epitope of the first antigen, wherein the epitopic peptide is not the same as the first immunogen. The animal can be, for example, a human or a non human, preferably a mammal. In some aspects the animal can be, for example, a feline, a canine, an avian such as for example, a chicken or a turkey, a bovine, an equine, other livestock or farm animals, or any other animal. In some embodiments, the at least a portion of a first antigen can include a class II restricted epitope.

[0028] In some embodiments the term "corresponds" can mean that the peptide has the wild-type or native epitope sequence from the antigen or that the peptide is cross-reactive or an analog of the wild-type epitope sequence. Examples of such cross-reactive and analogs, including how to make the same, are found in U.S. Patent Publication No. 2003-0220239, published on December November 27, 2003; U.S. Patent Application No. 11/155,929, filed on June 17, 2005, entitled NY-ESO-1 PEPTIDE ANALOGS; and U.S. Patent Application No. 11/156,253, filed on June 17, 2005, entitled SSX-2 PEPTIDE ANALOGS; each of which is incorporated herein by reference in its entirety.

[0029] The disease is caused by, for example, HIV, HSV, HBV, HCV, HGV, EBV, HPV, CMV, influenza virus, HTLV, RSV, EBV, measles virus, Ebola virus, and the like. The first immunogen and the immunopotentiator can be delivered to a lymphatic system of the animal, for example to a lymph node or a lymph vessel. The first immunogen and the immunopotentiator can be delivered to a same location on or in the animal. Also, they can be delivered simultaneously, for example at the same time or within about 1-2 minutes of each other or over a period of time together. They can be delivered within more than 2 minutes, for example, within about 3, 4, 5, 6, 7, 8, 9, or 10 minutes, within 15, 30, 45 or 60 minutes of each other, within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 hours, or within the same day. Further, the epitopic peptide can be delivered to a lymphatic system of the animal, for example to a lymph node or a lymph vessel. The first immunogen and the immunopotentiator can be delivered as part of a same composition.

[0030] The at least a portion of a first antigen can include, for example, a whole antigen, less than the full-length of a whole antigen, a contiguous fragment of less than 80%, 70%, 60%., 50, %, 40%, 30%, 20% or 10% of the whole antigen, one or more class I T cell epitope, B cell epitope, or combinations thereof, a chimeric molecule that includes more than one class I T cell epitope, B cell epitope, or combinations thereof, and the like. The first immunogen can encode the at least a portion of a first antigen and can include an immunostimulatory sequence that serves as the immunopotentiator. The first immunogen can encode one or more epitopes, wherein the one or more epitopes are class I restricted T cell epitopes or B cell epitopes. The first immunogen can encode a chimeric nucleic acid sequence that includes more than one class I T cell epitope, B cell epitope, or a combination thereof. The immunogen as used in the delivering step can also be any other immunogen, including those described elsewhere herein and in the listed and incorporated references.

[0031] The administering step can be performed subsequent to the delivering step, for example, about 1, 2, 3, 4, 5, or 6 days after, preferably about 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30 days or more after the delivering step.

[0032] Also, in some instances, the at least a first portion of a first antigen does not include or encode any MHC class II restricted epitope for the species of the animal, or does not include or encode any human class II restricted epitope. The first immunogen further can include or encodes at least a second portion of the first antigen, wherein the at least a second portion of the first antigen does not include a class II MHC restricted epitope for an MHC expressed by the animal. The first immunogen can encode the at least a first portion a first antigen and the at least a second portion of the first antigen. The first immunogen further can include or encode one or more additional portions of the first antigen, wherein the one or more additional portions of the first antigen do not include a class II MHC restricted epitope for an MHC expressed by the animal. The first immunogen further can include or encode at least a first portion of a second antigen. The delivering step further can include delivering a second immunogen that includes or encoding at least a first portion of a second antigen, wherein the at least a first portion of a second antigen does not include a class II MHC restricted epitope for an MHC expressed by the animal. The methods further can include detecting or obtaining an epitope-specific immune response without substantial activation or expansion of $CD4^+$ T cells. The detection or determination of obtaining can be done by any suitable method.

[0033] In some embodiments, "substantial" can be used in the context of "substantial activation or expansion" of, for example, CD4+T cells. In this context, "substantial activation or expansion" generally indicates a level of activation or expansion that would reach a level of physiological significance or disease significance beyond mere detectability. For example, if a population of CD4+T cells activated or expanded at a level above the detection cutoff, but below a level that would change the characteristics of the population to function as a target population for infectious agents or to fulfill other functions of the population, but below a level that would produce a clinically relevant change in the characteristics, it is understood that the population has not experienced substantial activation or expansion.

[0034] Also, some embodiments relate to methods of generating an immune response against an HIV infection. The methods can include the steps of delivering to an

animal a composition that includes a nucleic acid encoding first immunogen and that includes an immunostimulatory sequence that can serve as an immunopotentiator, the nucleic acid encoding at least a first portion of a first HIV antigen, wherein the at least a portion of a first antigen does not include a class II MHC restricted epitope for an MHC expressed by the animal; and administering, preferably subsequent to the delivering step, an epitopic peptide directly to a lymphatic system of the animal, wherein the peptide corresponds to a class I MHC-restricted epitope of the at least a first portion of a first HIV antigen, wherein the epitopic peptide is not the same as the first immunogen. In some embodiments, the at least a portion of a first HIV antigen can include a class II restricted epitope. The first HIV antigen can be, for example, gag, pol, env, tat, gp120, gp160, gp41, nef, gag p, gp, gag p24, rt, and the like. The nucleic acid can encode one or more of SEQ ID NOs:1-531, preferably one or more of SEQ ID NOs:1-6, or any other HIV epitope.

[0035] Further, some embodiments relate to methods of generating an immune response against a cell infected by an HIV. The methods can include delivering to patient a composition that includes a nucleic acid encoding one or more of SEQ ID NOs:1-531, preferably one or more of SEQ ID NOs:1-531, and an adjuvant, the nucleic acid can encode at least a first portion of a first HIV antigen, wherein the at least a portion of a first antigen does not include a class II MHC restricted epitope for an MHC expressed by the patient, wherein the adjuvant can any adjuvant, preferably a CpG, a dsRNA poly IC, or a TLR mimic; and administering, preferably after the delivering step, one or more epitopic peptides directly to a lymph node of the patient, wherein the peptide is one that was encoded by the nucleic acid or is an analog thereof. In some embodiments, the at least a portion of a first HIV antigen can include a class II restricted epitope.

[0036] Some embodiments relate to sets of immunogenic compositions, which can include, for example, any of the compositions described herein, including as exemplified in the examples. Some embodiments relate to one or more immunogenic products, which can include, for example, one or more immunogens, viruses, vectors, antigens, peptides, epitopes, or combinations thereof: Also, some embodiments relate to kits that include one or more of the following: an immunogenic composition as described or exemplified herein, sets of such compositions, products, or sets of products, any other material, substance or composition of matter described herein, instructions for use, delivery vehicles, and combinations of any of the same.

[0037] Other embodiments relate to sets of immunogenic compositions for inducing an immune response in a mammal including 1 to 6 or more entraining doses and at least one amplifying dose, wherein the entraining doses can include a nucleic acid encoding an immunogen, and wherein the amplifying dose can include a peptide epitope, and wherein the epitope can be presented or is presentable by pAPC expressing the nucleic acid. The one dose further can include an adjuvant, for example, RNA. The entraining and amplifying doses can be in a carrier suitable for direct administration to the lymphatic system, a lymph node and the like. The nucleic acid can be a plasmid. The epitope can be a class I HLA epitope, for example, one listed in Tables 1-4. The HLA preferably can be HLA-A2. The immunogen can include an epitope array, which array can include a liberation sequence. The immunogen can consist essentially of a target-associated antigen. The target-associated antigen can be a tumor-associated antigen, a microbial antigen, any other antigen, and the like. The immunogen can include a fragment of a target-associated antigen that can include an epitope cluster.

[0038] Further embodiments can include sets of immunogenic compositions for inducing a class I MHC-restricted immune response in a mammal including 1-6 entraining doses and at least one amplifying dose, wherein the entraining doses can include an immunogen or a nucleic acid encoding an immunogen and an immunopotentiator, and wherein the amplifying dose can include a peptide epitope, and wherein the epitope can be presented by pAPC. The nucleic acid encoding the immunogen further can include an immunostimulatory sequence which can be capable of functioning as the immunopotentiating agent. The immunogen can be a virus or replication-competent vector that can include or can induce an immunopotentiating agent. The immunogen can be a bacterium, bacterial lysate, or purified cell wall component. Also, the bacterial cell wall component can be capable of functioning as the immunopotentiating agent. The immunopotentiating agent can be, for example, a TLR ligand, an immunostimulatory sequence, a CpG-containing DNA, a dsRNA, an endocytic-Pattern Recognition Receptor (PRR) ligand, an LPS, a quillaja saponin, tucaresol, a pro-inflammatory cytokine, and the like. In some preferred embodiments for promoting multivalent responses the sets can include multiple entraining doses and/or multiple amplification doses corresponding to various individual antigens, or combinations of antigens, for each administration. The multiple entrainment doses can be administered as part of a single composition or as part of

more than one composition. The amplifying doses can be administered at disparate times and/or to more than one site, for example.

Brief Description of the Drawings

[0039] Figure 1: shows the cytolytic response to an HBV epitope induced by intranodal administration of peptide plus poly(IC).

[0040] Figure 2: shows the cytolytic response to a PSMA model epitope induced by intranodal administration of peptide plus poly(IC).

[0041] Figure 3: shows the cytolytic response to a pair of PRAME model epitopes induced by intranodal administration of peptide plus poly(IC).

[0042] Figure 4: shows the specific susceptibility of $CD8^{\pm}$-lymphocytes to in vitro activation subsequent to immunization.

Detailed Description of the Preferred Embodiment

[0043] Some embodiments disclosed herein relate to methods and compositions for bypassing the involvement of $CD4^+$ cells when generating antibody and MHC class I-restricted immune responses, controlling the nature and magnitude of the response, and promoting effective immunologic intervention in viral pathogenesis, or in other settings in which avoidance of CD4+ cell activation or expansion can be advantageous. . More specifically, some embodiments relate to immunogenic compositions for vaccination, particularly therapeutic vaccination against HIV, HSV, HBV, HCV, HPV, CMV, influenza virus, HTLV, RSV, EBV, measles virus, Ebola virus and other microbial pathogens that impact functioning of the immune system, their nature, and the order, timing, and route of administration by which they are effectively used. Some embodiments relate to methods of generating an immune response against HIV, HSV, HBV, HCV, HPV, CMV, influenza virus, HTLV, RSV, EBV, measles virus, Ebola virus and other microbial pathogens while minimizing, limiting or preventing adverse effects associated with the activation or expansion of $CD4^+$ cells. Various examples of viruses and microbes are provided in Tables 5-7 and in the other references mentioned herein which are incorporated by reference in their entirety. Other embodiments relate to methods for expanding the $CD8^+$ and/or antibody response while mitigating any ill effect caused by the activation or expansion of the $CD4^+$ subset. Further embodiments relate to methods for expanding the $CD8^+$ and/or

antibody response while overcoming any impairment of $CD4^+$ T cell responses. Also, some embodiments relate to methods of amplifying an anti-HIV $CD8^+$ T cell and/or antibody response while causing little or no effect on, or expansion of $CD4^+$ cells. Such embodiments can also be particularly useful in situations where an antigen is prone to generating undesired $CD4^+$ T regulatory cells, or in which $CD4^+$ T cells contribute to an immunopathology.

[0044] Methods and compositions disclosed herein are useful in the generation of an immune response or a therapeutic response to HIV, HSV, HBV, HCV, HPV, CMV, influenza virus, HTLV, RSV, EBV, measles virus, Ebola virus which can be accomplished in a manner that avoids concerns that the immunization process itself will promote spread of the infection and exacerbate progression of related disease and in addition, in a manner that does not require a fully functional Th cell population.

[0045] Some embodiments relate to a two-stage immunization protocol for the generation of a CTL response. In the first stage an immune response comprising a memory CTL response to one or more class I MHC-restricted epitopes of the target antigen can be established. Typically this can be accomplished by intranodal administration of a naked DNA plasmid capable of expression of an appropriate antigen in a professional antigen presenting cell (pAPC). In other preferred embodiments immunogen can be combined with an appropriate toll-like receptor (TLR) ligand such as a CpG oligonucleotide or synthetic dsRNA (polyI:C). Intralymphatic administration however, is not an essential feature of this first stage of the protocol, and more conventional routes of administration can be used. In preferred embodiments the term "immunogen" can be defined as a molecule capable of inducing an immune response against an antigen, a vector expressing such a molecule, or a composition comprising one or more such molecules or vectors.

[0046] The magnitude of the response at this stage is not crucial. Quite modest responses suffice, although moderate and strong responses can also occur, and thus, the involvement of $CD4^+$ responses can be tolerated at this stage of the procedure without destroying the usefulness of the complete protocol. $CD4^+$ responses can generally be avoided by use of immunogens that do not comprise or express class II MHC-restricted epitopes, or at least not ones that can be presented by the class II MHC alleles expressed by a particular subject.

[0047] Immunogens that do contain potentially problematic class II MHC-restricted epitopes can be modified, for example, by deletion, mutation, or any other modification of the epitope(s) so as to inhibit or prevent processing, transport, and/or MHC-binding of the class II epitope. The immunopotentiator used in the first stage generally can be one that acts primarily on pAPC, for example, dendritic cells, and not directly on lymphocytes. Thus, they will not be a major cause of activation or proliferation of $CD4^+$ lymphocytes. Intranodal administration can further reduce any generalized effects of these agents.

[0048] In the second stage of this protocol one or more epitopic peptides, corresponding to the epitope(s) immunized against in the first stage, can be administered directly to the lymphatic system. In preferred embodiments the term "epitopic peptide" can mean a peptide that comprises, consists essentially of, or consists of an epitope. The peptide(s) can be used without any immunopotentiator or other adjuvant, although immunopotentiators or other adjuvants optionally can be used. In preferred embodiments administration can be directly to a lymph node, and in the case of multiple peptides it can be preferred that only a single peptide be administered to any particular lymph node on any particular occasion, although in some aspects the more than one or all peptides can be administered to the same lymph node. In some embodiments, for example those promoting a multivalent response and in which multiple amplifying peptides are used, it can be advantageous that only a single peptide be administered to any particular lymph node on any particular occasion. Thus, one peptide can be administered to the right inguinal lymph node and a second peptide to the left inguinal lymph node at the same time, for example. Additional peptides can be administered to other lymph nodes even if they were not sites of induction as it is not essential that initiating and amplifying doses be administered to the same site due to migration of T lymphocytes. Alternatively any additional peptides can be administered about one or two days later to the same lymph node(s), preferably, about three, four, five or six days later to the same lymph node(s) used for the previously administered amplifying peptides since the time interval between induction and amplification is not a crucial parameter, although in preferred embodiments the time interval can be greater than about a week, for example, about seven, eight, nine, ten, eleven, twelve, thirteen, fourteen or more days later. Segregation of administration of amplifying peptides generally can be of less importance if their MHC-binding affinities are similar, but

grows in importance as the affinities become more disparate. Incompatible formulations of various peptides can also make segregated administration preferable. Where the MHC-binding affinities of the various peptides are similar, it can be less preferred that the administration of only a single peptide be administered to a particular lymph node during a particular occasion. Where the MHC-binding affinities of the various peptides are or become more disparate, it can be more preferred that only a single peptide be administered to any particular lymph node on any particular occasion. Such peptides will generally not bind to class II MHC markers, so typically only the class I-restricted, $CD8^+$ response will be amplified. Further details related to this methodology are described in U.S. Patent Application No. 10/871,707 (Pub. No. 2005-0079152 A1), filed on June 17, 2004, Publication No. 20050079152A1, entitled METHODS TO ELICIT, ENHANCE AND SUSTAIN IMMUNE RESPONSES AGAINST MHC CLASS I-RESTRICTED EPITOPES, FOR PROPHYLACTIC OR THERAPEUTIC PURPOSES, which is incorporated herein by reference in its entirety. Additional methodology related to generating immune responses, including multivalent responses, is disclosed in U.S. Provisional Patent Application No. 60/640,402, filed on December 29, 2004 and in nonprovisional U.S. Patent Application No. __/___,___ (Pub. No. _____) (Attorney Docket No. MANNK.047A), filed on the same date as this application, both entitled METHODS TO ELICIT, ENHANCE AND SUSTAIN IMMUNE RESPONSES AGAINST MHC CLASS I-RESTRICTED EPITOPES, FOR PROPHYLACTIC OR THERAPEUTIC PURPOSES, each of which is incorporated herein by reference in its entirety. Intralymphatic immunization for the induction of CTL responses is taught in U.S. Patent Application Nos. 09/380,534; 09/776,232 (Publication No. 20020007173), now U.S. Patent No. 6,977,074, filed on February 6, 2001, entitled METHOD OF INDUCING A CTL RESPONSE each of which is incorporated herein by reference in its entirety.

[0049] In some embodiments, an immunogen can be co-administered intralymphatically with an immunopotentiating biological response modifier (BRM). In one aspect the immunogen can include one or more class I MHC-restricted peptide epitopes and a CTL response can be generated. In another aspect the immunogen can include one or more B cell epitopes and an antibody response can be generated. In either aspect, it can be preferred that the immunogen lacks class II MHC-restricted epitopes, or at least ones that can be presented by the class II MHC alleles expressed by a particular subject. This feature

can be ensured by using *in silico* predictive methods and standard characterization of peptide binding to MHC class I and II molecules, using well known experimental methods. The use and advantages of intralymphatic administration of BRMs are disclosed in co-pending provisional U.S. Patent Application No. 60/640,727, filed on December 29, 2004, and in nonprovisional U.S. Patent Application No. __/_____ (Attorney Docket No. MANNK.046A), filed on the same date as this application, both entitled METHODS TO TRIGGER, MAINTAIN AND MANIPULATE IMMUNE RESPONSES BY TARGETED ADMINISTRATION OF BIOLOGICAL RESPONSE MODIFIERS INTO LYMPHOID ORGANS, each of which is incorporated herein by reference in its entirety.

[0050] In some embodiments, preferred immunogens for class I MHC-restricted epitopes are epitopic peptides. As in the immunization protocol above, it can be preferred to administer only a single peptide to any particular lymph node on any particular occasion. For B cell epitopes, free peptides are not ideal immunogens. Preferably the target epitope is multivalent in the immunogen. Examples include multiple conjugation to a carrier protein; recombinant proteins and polypeptides comprising the epitope, for example, IgG with the epitope grafted into the CDR3 position; Ig-peptide fusion proteins (with peptide at N or C terminal position); and iterative chains of the epitope with or without spacer sequences; and dendrimers. It is preferred that any carrier protein, whether monovalent or multivalent for the epitope, be a self-protein so that the recipient will have at least a degree of tolerance for any presentable class II MHC-restricted epitopes in the carrier. In humans, human serum albumin and immunoglobulins are potential choices as carriers. $CD8^+$ cells secrete various cytokines in addition to having cytolytic activity. Thus immunization with both types of immunogen can improve the response to the B cell immunogen, especially in the case that the B cell immunogen is monovalent for the target epitope.

[0051] The disclosed methods are advantageous over many protocols in HIV vaccine therapeutics. Current vaccines commonly rely on interaction with, or result in expansion of the $CD4^+$ population in an attempt to control viral infection, but can in fact detrimentally provide new targets for viral infection. The disclosed DNA prime-peptide boost method is itself advantageous over other protocols that use only peptide or do not follow the entrain-and-amplify methodology. The peptide based immunization or immune amplification strategy has advantages over other methods, particularly certain microbial vectors, for example. This is due to the fact that more complex vectors, such as live

attenuated viral or bacterial vectors, may induce deleterious side-effects, for example, *in vivo* replication or recombination; or become ineffective upon repeated administration due to generation of neutralizing antibodies against the vector itself. Additionally, when harnessed in such a way to become strong immunogens, peptides can circumvent the need for proteasome-mediated processing (as with protein or more complex antigens, in context of "cross-processing" or subsequent to cellular infection). That is because cellular antigen processing for MHC-class I restricted presentation is a phenomenon that inherently selects dominant (favored) epitopes over subdominant epitopes, potentially interfering with the immunogenicity of epitopes corresponding to valid targets. Thus, if antigen presenting cells are defective, use of peptides may circumvent the need for competent processing that is a prerequisite for effectiveness of complex vectors. Finally, effective peptide based immunization simplifies and shortens the process of development of immunotherapeutics.

[0052] Thus, effective peptide-based immune amplification methods, particularly including those described herein, can be of considerable benefit to prophylactic and/or therapeutic generation of an immune response against HIV, HSV, EBV, HBV or HCV, including benefit for vaccination against the same. Additional benefits can be achieved by avoiding simultaneous use of cumbersome, unsafe, or complex adjuvant techniques, although such techniques can be utilized in various embodiments described herein.

[0053] Previous HIV immunization methods displayed certain important limitations including that the high mutation rate of HIV creates immune escape mutants with ease, and viral surface protein gp120 is a poor immunogen inherently resistant to antibody attack. Immunization methods in general also displayed certain limitations: very often, conclusions regarding the potency of vaccines were extrapolated from immunogenicity data generated from one or from a very limited panel of ultrasensitive read-out assays. Frequently, despite the inferred potency of a vaccination regimen, the clinical response was not significant or was at best modest. Secondly, subsequent to immunization, T regulatory cells, along with more conventional T effector cells, can be generated and/or expanded, and such cells can interfere with the function of the desired immune response. The importance of such mechanisms in active immunotherapy has been recognized only recently.

[0054] Intranodal administration of immunogens provides a basis for the control of the magnitude and profile of immune responses. The effective *in vivo* loading of pAPC accomplished as a result of such administration, enables a substantial magnitude of immunity, even by using an antigen in its most simple form—a peptide epitope—otherwise generally associated with poor pharmacokinetics. The quality of response can be further controlled via the nature of immunogens, vectors, and protocols of immunization. Such protocols can be applied for enhancing/modifying the response in infections such as HIV. Further, intranodal administration of BRMs allows one to take advantage of their immunopotentiating activity while avoiding the toxicity commonly associated with otherwise required dosages.

[0055] Immunization has traditionally relied on repeated administration of antigen to augment the magnitude of the immune response. The use of DNA vaccines has resulted in high quality responses, but it has been difficult to obtain high magnitude responses using such vaccines, even with repeated booster doses. Both characteristics of the response, high quality and low magnitude, are likely due to the relatively low levels of epitope loading onto MHC achieved with these vectors. Instead it has become more common to boost such vaccines using antigen encoded in a live virus vector in order to achieve the high magnitude of response needed for clinical usefulness. However, the use of live vectors can entail several drawbacks including potential safety issues, decreasing effectiveness of later boosts due to a humoral response to the vector induced by the prior administrations, and the costs of creation and production. Thus, use of live vectors or DNA alone, although eliciting high quality responses, may result in a limited magnitude or sustainability of response due to a reduced *in vivo* transfection rate (former) or generation of anti-vector neutralizing responses (the latter).

[0056] Disclosed herein are embodiments that relate to protocols and to methods that, when applied to peptides, rendered them effective as immune therapeutic tools. Such methods circumvent the poor PK of peptides, and if applied in context of specific, and often more complex regimens, result in robust amplification and/or control of immune response. In preferred embodiments, direct administration of peptide into lymphoid organs results in unexpectedly strong amplification of immune responses, following a priming agent that induces a strong, moderate or even mild (at or below levels of detection by conventional techniques) immune response consisting of Tc1 cells. While

preferred embodiments can employ intralymphatic administration of antigen at all stages of immunization, intralymphatic administration of adjuvant-free peptide can be most preferred. Peptide amplification utilizing intralymphatic administration can be applied to existing immune responses that may have been previously induced. Previous induction can occur by means of natural exposure to the antigen or by means of commonly used routes of administration, including without limitation subcutaneous, intradermal, intraperitoneal, intramuscular, and mucosal.

[0057] Also as shown herein, optimal initiation, resulting in subsequent expansion of specific T cells, can be better achieved by exposing the naive T cells to limited amounts of antigen (as can result from the often limited expression of plasmid-encoded antigen) in a rich co-stimulatory context (such as in a lymph node). That can result in activation of T cells carrying T cell receptors that recognize with high affinity the MHC - peptide complexes on antigen presenting cells and can result in generation of memory cells that are more reactive to subsequent stimulation. The beneficial co-stimulatory environment can be augmented or ensured through the use of immunopotentiating agents and thus intralymphatic administration, while advantageous, is not in all embodiments required for initiation of the immune response.

[0058] While the poor pharmacokinetics of free peptides has prevented their use in most routes of administration, direct administration into secondary lymphoid organs, particularly lymph nodes, has proven effective when the level of antigen is maintained more or less continuously by continuous infusion or frequent injection (for example, daily). Such intranodal administration for the generation of CTL is taught in U.S. Patent Application Nos. 09/380,534; 09/776,232 (Pub. No. 20020007173A1), now U.S. Patent No. 6,977,074; in PCT Application No. PCTUS98/14289 (Pub. No. WO 99/02183 A2) each entitled METHOD OF INDUCING A CTL RESPONSE and in U.S. Application No. 10/871,707 (Pub. No. 2005-0079152 A1), filed on June 17, 2004, entitled METHODS TO ELICIT, ENHANCE AND SUSTAIN IMMUNE RESPONSES AGAINST MHC CLASS I-RESTRICTED EPITOPES, FOR PROPHYLACTIC OR THERAPEUTIC PURPOSES, each of which is hereby incorporated by reference in its entirety. Intranodal administration of peptide was effective in amplifying a response initially induced with a plasmid DNA vaccine. Moreover, the cytokine profile was distinct, with plasmid DNA induction/peptide amplification generally resulting in greater chemokine (chemoattractant

cytokine) and lesser immunosuppressive cytokine production than either DNA/DNA or peptide/peptide protocols.

[0059] Thus, such DNA induction/peptide amplification protocols can improve the effectiveness of compositions, including therapeutic vaccines for cancer and chronic infections. Beneficial epitope selection principles for such immunotherapeutics are disclosed in U.S. Patent Application Nos. 09/560,465, 10/026,066 (Pub. No. 20030215425 A1), 10/005,905, filed November 7, 2001, 10/895,523 (Pub. No. 2005-0130920 A1), filed July 20, 2004, and 10/896,325 (Pub No. _____), filed July 20, 2004, all entitled EPITOPE SYNCHRONIZATION IN ANTIGEN PRESENTING CELLS; 09/561,074, now U.S. Patent No. 6,861,234, and 10/956,401 (Pub. No. 2005-0069982 A1), filed on October 1, 2004, both entitled METHOD OF EPITOPE DISCOVERY; 09/561,571, filed April 28, 2000, entitled EPITOPE CLUSTERS; 10/094,699 (Pub. No. 20030046714 A1), filed March 7, 2002, 11/073,347, (Pub. No. _____), filed June 30, 2005, each entitled ANTI-NEOVASCULATURE PREPARATIONS FOR CANCER; and 10/117,937 (Pub. No. 20030220239 A1), filed April 4, 2002, 11/067,159 (Pub. No. 2005-0221440A1), filed February 25, 2005, 10/067,064 (Pub. No. 2005-0142114 A1), filed February 25, 2005, and 10/657,022 (Publication No. 2004-0180354 A1), and PCT Application No. PCT/US2003/027706 (Pub. No. WO 04/022709 A2), each entitled EPITOPE SEQUENCES, and each of which is hereby incorporated by reference in its entirety. Aspects of the overall design of vaccine plasmids are disclosed in U.S. Patent Application Nos. 09/561,572, filed April 28, 2000, and 10/225,568 (Pub. No. 2003-0138808 A1), filed August 20, 2002, both entitled EXPRESSION VECTORS ENCODING EPITOPES OF TARGET-ASSOCIATED ANTIGENS and U.S. Patent Application Nos. 10/292,413 (Pub. No.20030228634 A1), 10/777,053 (Pub. No. 2004-0132088 A1), filed on February 10, 2004, and 10/837,217 (Pub. No. _____), filed on April 30, 2004, all entitled EXPRESSION VECTORS ENCODING EPITOPES OF TARGET-ASSOCIATED ANTIGENS AND METHODS FOR THEIR DESIGN; 10/225,568 (Pub No. 2003-0138808 A1), PCT Application No. PCT/US2003/026231 (Pub. No. WO 2004/018666) and U.S. Patent No. 6,709,844 and U.S. Patent Application No. 10/437,830 (Pub. No. 2003-0180949 A1), filed on May 13, 2003, each entitled AVOIDANCE OF UNDESIRABLE REPLICATION INTERMEDIATES IN PLASMID PROPAGATION, each of which is hereby incorporated by reference in its entirety. Specific antigenic combinations of

particular benefit in directing an immune response against particular cancers are disclosed in provisional U.S. Provisional Application No. 60/479,554, filed on June 17, 2003, U.S. Patent Application No. 10/871,708 (Pub. No. 2005-0118186 A1), filed on June 17, 2004, PCT Patent Application No. PCT/US2004/019571 (Pub. No. WO 2004/112825), U.S. Provisional Application No. 60/640,598, filed December 29, 2005, and U.S. Patent Application No __/___,___ (Pub. No. _____), (Attorney Docket No. MANNK.049A), filed on the same date as this application, all entitled COMBINATIONS OF TUMOR-ASSOCIATED ANTIGENS IN VACCINES FOR VARIOUS TYPES OF CANCERS, each of which is also hereby incorporated by reference in its entirety. Specific antigenic combinations of particular benefit in directing an immune response against HIV are disclosed in an article by Kiepiela et al., ("Dominant influence of HLA-B in mediating the potential co-evolution of HIV and HLA," *Nature*, vol. 432, pages 769-775 (December 9, 2004)), which is also hereby incorporated by reference in its entirety.

[0060] The use and advantages of intralymphatic administration of BRMs are disclosed in provisional U.S. Patent Application No. 60/640,727, filed December 29, 2005 and U.S. Patent Application No. __/___,___ (Pub. No. _____) (Attorney Docket No. MANNK.046A), filed on the same date as this application, both entitled Methods to trigger, maintain and manipulate immune responses by targeted administration of biological response modifiers into lymphoid organs, each of which is incorporated herein by reference in it entirety. Additional methodology, compositions, peptides, and peptide analogues are disclosed in U.S. Patent Application No. 09/999,186, filed November 7, 2001, entitled METHODS OF COMMERCIALIZING AN ANTIGEN; and U.S. Provisional U.S. Patent Application No. 60/640,402, filed December 29, 2005 and Application No. __/___,___ (Pub. No. _____) (Attorney Docket No. MANNK.047A), filed on the same date as this application, both entitled METHODS TO ELICIT, ENHANCE AND SUSTAIN IMMUNE RESPONSES AGAINST MHC CLASS I-RESTRICTED EPITOPES, FOR PROPHYLACTIC OR THERAPEUTIC PURPOSES, each of which is hereby incorporated by reference in its entirety.

[0061] The integration of diagnostic techniques to assess and monitor immune responsiveness with methods of immunization is discussed more fully in Provisional U.S. Patent Application No. 60/580,964, filed June 17, 2004, and 11/155,928 (Pub. No. _____), filed June 17, 2005, both entitled IMPROVED EFFICACY OF ACTIVE

IMMUNOTHERAPY BY INTEGRATING DIAGNOSTIC WITH THERAPEUTIC METHODS, each of which is hereby incorporated by reference in its entirety. Additional methodology, compositions, peptides, and peptide analogues are disclosed in U.S. Provisional Patent Application No. 60/581,001, filed on June 17, 2004 and U.S. Patent Application No. 11/156,253 (Pub. No. _____), filed on June 17, 2005, both entitled SSX-2 PEPTIDE ANALOGS; and U.S. Provisional Patent Application No. 60/580,962, filed on June 17, 2004, and U.S. Patent Application No. 11/155,929 (Pub. No. _____), filed on June 17, 2005, both entitled NY-ESO PEPTIDE ANALOGS; U.S. Patent Application No. 09/999,186, filed November 7, 2001, entitled METHODS OF COMMERCIALIZING AN ANTIGEN; each of which is hereby incorporated by reference in its entirety. Various viruses, viral antigens, and viral antigen epitopes that can be used in the embodiments described herein are disclosed in U.S. Patent Application No. 20020007173A1 (now U.S. Patent No. 6,977,074). In some aspects, one or more, including any combination of the listed viruses, viral antigens, or viral epitopes can be specifically included or excluded from the an embodiment of a method.

[0062] Other relevant disclosures are present in U.S. Patent Application No. 11/156,369 (Pub. No. _____), and U.S. Provisional Patent Application No. 60/691,889, both filed on June 17, 2005, both entitled EPITOPE ANALOGS, and each of which is incorporated herein by reference in its entirety. Also relevant are, U.S. Provisional Patent App. Nos. 60/691,579, filed on June 17, 2005, entitled METHODS AND COMPOSITIONS TO ELICIT MULTIVALENT IMMUNE RESPONSES AGAINST DOMINANT AND SUBDOMINANT EPITOPES, EXPRESSED ON CANCER CELLS AND TUMOR STROMA, 60/691,581, filed on June 17, 2005, entitled MULTIVALENT ENTRAIN-AND-AMPLIFY IMMUNOTHERAPEUTICS FOR CARCINOMA, and U.S. Patent Application No. 11/155,288 (Pub. No. _____), filed June 17, 2005, entitled COMBINATIONS OF TUMOR-ASSOCIATED ANTIGENS IN DIAGNOSTICS FOR VARIOUS TYPES OF CANCERS, each of which is incorporated herein by reference in its entirety.

[0063] Induction with an agent such as non-replicating recombinant DNA (plasmid) can have, and have shown, an impact on the subsequent doses, enabling robust amplification of immunity to epitopes expressed by the recombinant DNA and peptide, and entraining its cytolytic nature. In fact, when single or multiple administrations of

recombinant DNA vector or peptide separately achieved modest immune or no responses, inducing with DNA and amplifying with peptide achieved substantially higher responses, both as a rate of responders and as a magnitude of response. As shown in U.S. Patent Application Nos. 10/871,707, filed on June 17, 2004, entitled METHODS TO ELICIT, ENHANCE AND SUSTAIN IMMUNE RESPONSES AGAINST MHC CLASS I-RESTRICTED EPITOPES, FOR PROPHYLACTIC OR THERAPEUTIC PURPOSES, (Publication No. 2005-0079152-A1); 60/640,727, filed December 29, 2004, entitled METHODS TO TRIGGER, MAINTAIN AND MANIPULATE IMMUNE RESPONSES BY TARGETED ADMINISTRATION OF BIOLOGICAL RESPONSE MODIFIERS INTO LYMPHOID ORGANS; __/___,___ (Atty. Docket No. 047A) cited above; and WO05002621 (each of which is incorporated herein by reference in its entirety) the rate of response was at least doubled and the magnitude of response (mean and median) was at least tripled by using a recombinant DNA induction / peptide –amplification protocol. Thus, preferred protocols result in induction of immunity (Tc1 immunity) that is able to deal with antigenic cells *in vivo*, within lymphoid and non-lymphoid organs.

[0064] Such induce-and-amplify protocols involving specific sequences of recombinant DNA entrainment doses, followed by peptide boosts administered to lymphoid organs, are thus useful for the purpose of induction, amplification and maintenance of strong T cell responses, for example for prophylaxis or therapy of infectious diseases Target diseases can include those caused by prions, for example. Exemplary diseases, organisms and antigens and epitopes associated with target organisms, cells and diseases are described in U.S. Application No. 09/776,232 (filed on February 2, 2001; Pub. No. 20020007173 A1) entitled METHODS OF INDUCING A CTL RESPONSE. Among the infectious diseases that can be addressed are those caused by agents that tend to establish chronic infections (HIV, herpes simplex virus, CMV, Hepatitis B and C viruses, papilloma virus and the like) and/or those that are connected with acute infections (for example, influenza virus, measles, RSV, Ebola virus). All these infectious agents have defined or definable antigens that can be used as basis for designing compositions such as peptide epitopes.

[0065] Practice of various of the methodological embodiments can require use of at least two different compositions and, especially when there is more than a single target antigen, can involve several compositions to be administered together and/or at different

times. Thus, some embodiments can relate to sets and subsets of immunogenic compositions and individual doses thereof. Multivalency can be achieved using compositions that include multivalent immunogens, combinations of monovalent immunogens, coordinated use of compositions that include a monovalent immunogen or various combinations thereof. Multiple compositions, manufactured for use in a particular treatment regimen or protocol according to such methods, can define an immunotherapeutic product. In some embodiments all or a subset of the compositions of the product can be packaged together in a kit. In some instances the inducing and amplifying compositions targeting a single epitope, or set of epitopes, can be packaged together. In other instances multiple inducing compositions can be assembled in one kit and the corresponding amplifying compositions assembled in another kit. Alternatively compositions may be packaged and sold individually along with instructions, in printed form or on machine-readable media, describing how they can be used in conjunction with each other to achieve the beneficial results of the methods. Further variations will be apparent to one of skill in the art. The use of various packaging schemes comprising less than all of the compositions that might be used in a particular protocol or regimen facilitates the personalization of the treatment, for example based on observed response to the immunotherapeutic or its various components, as described in Provisional U.S. Patent Application No. 60/580,964, and U.S. Patent Application No. 11/155,928 both entitled IMPROVED EFFICACY OF ACTIVE IMMUNOTHERAPY BY INTEGRATING DIAGNOSTIC WITH THERAPEUTIC METHODS, each of which is incorporated by reference in its entirety.

[0066] Embodiments are directed to methods, uses, therapies and compositions related to epitopes and compositions with specificity for MHC, including, for example, those listed in Tables 1-4. Other embodiments include one or more of the MHCs listed in Tables 1-4, including combinations of the same, while other embodiments specifically exclude any one or more of the MHCs or combinations thereof. Tables 3-4 include frequencies for the listed HLA antigens.

Table 1

Class I MHC Molecules

Class I

Human

HLA-A1
HLA-A*0101
HLA-A*0201
HLA-A*0202
HLA-A*0203
HLA-A*0204
HLA-A*0205
HLA-A*0206
HLA-A*0207
HLA-A*0209
HLA-A*0214
HLA-A3
HLA-A*0301
HLA-A*1101
HLA-A23
HLA-A24
HLA-A25
HLA-A*2902
HLA-A*3101
HLA-A*3302
HLA-A*6801
HLA-A*6901
HLA-B7
HLA-B*0702
HLA-B*0703
HLA-B*0704
HLA-B*0705
HLA-B8
HLA-B13
HLA-B14
HLA-B*1501 (B62)
HLA-B17
HLA-B18
HLA-B22
HLA-B27
HLA-B*2702
HLA-B*2704
HLA-B*2705
HLA-B*2709
HLA-B35
HLA-B*3501
HLA-B*3502
HLA-B*3701

HLA-B*3801
HLA-B*39011
HLA-B*3902
HLA-B40
HLA-B*40012 (B60)
HLA-B*4006 (B61)
HLA-B44
HLA-B*4402
HLA-B*4403
HLA-B*4501
HLA-B*4601
HLA-B51
HLA-B*5101
HLA-B*5102
HLA-B*5103
HLA-B*5201
HLA-B*5301
HLA-B*5401
HLA-B*5501
HLA-B*5502
HLA-B*5601
HLA-B*5801
HLA-B*6701
HLA-B*7301
HLA-B*7801
HLA-Cw*0102
HLA-Cw*0301
HLA-Cw*0304
HLA-Cw*0401
HLA-Cw*0601
HLA-Cw*0602
HLA-Cw*0702
HLA-Cw8
HLA-Cw*1601 M
HLA-G

Murine

$H2\text{-}K^{d}$
$H2\text{-}D^{d}$
$H2\text{-}L^{d}$
$H2\text{-}K^{b}$
$H2\text{-}D^{b}$
$H2\text{-}K^{k}$
$H2\text{-}K^{kml}$
$Qa\text{-}1^{a}$
Qa-2
H2-M3

Rat
RT1.A[a]
RT1.A[l]

Bovine
Bota-A11
Bota-A20

Chicken
B-F4
B-F12
B-F15
B-F19

Chimpanzee
Patr-A*04
Patr-A*11
Patr-B*01
Patr-B*13
Patr-B*16

Baboon
Papa-A*06

Macaque
Mamu-A*01

Swine
SLA (haplotype d/d)

Virus homolog
hCMV class I homolog UL18

Table 2

Class I MHC Molecules

Class I

Human

HLA-A1
HLA-A*0101
HLA-A*0201
HLA-A*0202
HLA-A*0204
HLA-A*0205
HLA-A*0206
HLA-A*0207
HLA-A*0214
HLA-A3
HLA-A*1101
HLA-A24
HLA-A*2902
HLA-A*3101
HLA-A*3302
HLA-A*6801
HLA-A*6901
HLA-B7
HLA-B*0702
HLA-B*0703
HLA-B*0704
HLA-B*0705
HLA-B8
HLA-B14
HLA-B*1501 (B62)
HLA-B27
HLA-B*2702
HLA-B*2705
HLA-B35
HLA-B*3501
HLA-B*3502
HLA-B*3701
HLA-B*3801
HLA-B*39011
HLA-B*3902
HLA-B40
HLA-B*40012 (B60)
HLA-B*4006 (B61)
HLA-B44
HLA-B*4402
HLA-B*4403
HLA-B*4601

HLA-B51
HLA-B*5101
HLA-B*5102
HLA-B*5103
HLA-B*5201
HLA-B*5301
HLA-B*5401
HLA-B*5501
HLA-B*5502
HLA-B*5601
HLA-B*5801
HLA-B*6701
HLA-B*7301
HLA-B*7801
HLA-Cw*0102
HLA-Cw*0301
HLA-Cw*0304
HLA-Cw*0401
HLA-Cw*0601
HLA-Cw*0602
HLA-Cw*0702
HLA-G

Murine

$H2\text{-}K^{d}$
$H2\text{-}D^{d}$
$H2\text{-}L^{d}$
$H2\text{-}K^{b}$
$H2\text{-}D^{b}$
$H2\text{-}K^{k}$
$H2\text{-}K^{km1}$
Qa-2

Rat

$RT1.A^{a}$
$RT1.A^{l}$

Bovine

Bota-A11
Bota-A20

Chicken

B-F4
B-F12
B-F15
B-F19

Virus homolog

hCMV class I homolog UL18

Table 3

Estimated gene frequencies of HLA-A antigens

Antigen	CAU		AFR		ASI		LAT		NAT	
	Gf[a]	SE[b]	Gf	SE	Gf	SE	Gf	SE	Gf	SE
A1	15.1843	0.0489	5.7256	0.0771	4.4818	0.0846	7.4007	0.0978	12.0316	0.2533
A2	28.6535	0.0619	18.8849	0.1317	24.6352	0.1794	28.1198	0.1700	29.3408	0.3585
A3	13.3890	0.0463	8.4406	0.0925	2.6454	0.0655	8.0789	0.1019	11.0293	0.2437
A28	4.4652	0.0280	9.9269	0.0997	1.7657	0.0537	8.9446	0.1067	5.3856	0.1750
A36	0.0221	0.0020	1.8836	0.0448	0.0148	0.0049	0.1584	0.0148	0.1545	0.0303
A23	1.8287	0.0181	10.2086	0.1010	0.3256	0.0231	2.9269	0.0628	1.9903	0.1080
A24	9.3251	0.0395	2.9668	0.0560	22.0391	0.1722	13.2610	0.1271	12.6613	0.2590
A9 unsplit	0.0809	0.0038	0.0367	0.0063	0.0858	0.0119	0.0537	0.0086	0.0356	0.0145
A9 total	11.2347	0.0429	13.2121	0.1128	22.4505	0.1733	16.2416	0.1382	14.6872	0.2756
A25	2.1157	0.0195	0.4329	0.0216	0.0990	0.0128	1.1937	0.0404	1.4520	0.0924
A26	3.8795	0.0262	2.8284	0.0547	4.6628	0.0862	3.2612	0.0662	2.4292	0.1191
A34	0.1508	0.0052	3.5228	0.0610	1.3529	0.0470	0.4928	0.0260	0.3150	0.0432
A43	0.0018	0.0006	0.0334	0.0060	0.0231	0.0062	0.0055	0.0028	0.0059	0.0059
A66	0.0173	0.0018	0.2233	0.0155	0.0478	0.0089	0.0399	0.0074	0.0534	0.0178
A10 unsplit	0.0790	0.0038	0.0939	0.0101	0.1255	0.0144	0.0647	0.0094	0.0298	0.0133
A10 total	6.2441	0.0328	7.1348	0.0850	6.3111	0.0993	5.0578	0.0816	4.2853	0.1565
A29	3.5796	0.0252	3.2071	0.0582	1.1233	0.0429	4.5156	0.0774	3.4345	0.1410
A30	2.5067	0.0212	13.0969	0.1129	2.2025	0.0598	4.4873	0.0772	2.5314	0.1215
A31	2.7386	0.0221	1.6556	0.0420	3.6005	0.0761	4.8328	0.0800	6.0881	0.1855
A32	3.6956	0.0256	1.5384	0.0405	1.0331	0.0411	2.7064	0.0604	2.5521	0.1220
A33	1.2080	0.0148	6.5607	0.0822	9.2701	0.1191	2.6593	0.0599	1.0754	0.0796
A74	0.0277	0.0022	1.9949	0.0461	0.0561	0.0096	0.2027	0.0167	0.1068	0.0252
A19 unsplit	0.0567	0.0032	0.2057	0.0149	0.0990	0.0128	0.1211	0.0129	0.0475	0.0168
A19 total	13.8129	0.0468	28.2593	0.1504	17.3846	0.1555	19.5252	0.1481	15.8358	0.2832
AX	0.8204	0.0297	4.9506	0.0963	2.9916	0.1177	1.6332	0.0878	1.8454	0.1925

[a]Gene frequency.
[b]Standard error.

Table 4

Estimated gene frequencies for HLA-B antigens

Antigen	CAU		AFR		ASI		LAT		NAT	
	Gf[a]	SE[b]	Gf	SE	Gf	SE	Gf	SE	Gf	SE
B7	12.1782	0.0445	10.5960	0.1024	4.2691	0.0827	6.4477	0.0918	10.9845	0.2432
B8	9.4077	0.0397	3.8315	0.0634	1.3322	0.0467	3.8225	0.0715	8.5789	0.2176
B13	2.3061	0.0203	0.8103	0.0295	4.9222	0.0886	1.2699	0.0416	1.7495	0.1013
B14	4.3481	0.0277	3.0331	0.0566	0.5004	0.0287	5.4166	0.0846	2.9823	0.1316
B18	4.7980	0.0290	3.2057	0.0582	1.1246	0.0429	4.2349	0.0752	3.3422	0.1391
B27	4.3831	0.0278	1.2918	0.0372	2.2355	0.0603	2.3724	0.0567	5.1970	0.1721
B35	9.6614	0.0402	8.5172	0.0927	8.1203	0.1122	14.6516	0.1329	10.1198	0.2345
B37	1.4032	0.0159	0.5916	0.0252	1.2327	0.0449	0.7807	0.0327	0.9755	0.0759
B41	0.9211	0.0129	0.8183	0.0296	0.1303	0.0147	1.2818	0.0418	0.4766	0.0531
B42	0.0608	0.0033	5.6991	0.0768	0.0841	0.0118	0.5866	0.0284	0.2856	0.0411
B46	0.0099	0.0013	0.0151	0.0040	4.9292	0.0886	0.0234	0.0057	0.0238	0.0119
B47	0.2069	0.0061	0.1305	0.0119	0.0956	0.0126	0.1832	0.0159	0.2139	0.0356
B48	0.0865	0.0040	0.1316	0.0119	2.0276	0.0575	1.5915	0.0466	1.0267	0.0778
B53	0.4620	0.0092	10.9529	0.1039	0.4315	0.0266	1.6982	0.0481	1.0804	0.0798
B59	0.0020	0.0006	0.0032	0.0019	0.4277	0.0265	0.0055	0.0028	0[c]	———
B67	0.0040	0.0009	0.0086	0.0030	0.2276	0.0194	0.0055	0.0028	0.0059	0.0059
B70	0.3270	0.0077	7.3571	0.0866	0.8901	0.0382	1.9266	0.0512	0.6901	0.0639
B73	0.0108	0.0014	0.0032	0.0019	0.0132	0.0047	0.0261	0.0060	0[c]	———
B51	5.4215	0.0307	2.5980	0.0525	7.4751	0.1080	6.8147	0.0943	6.9077	0.1968
B52	0.9658	0.0132	1.3712	0.0383	3.5121	0.0752	2.2447	0.0552	0.6960	0.0641
B5 unsplit	0.1565	0.0053	0.1522	0.0128	0.1288	0.0146	0.1546	0.0146	0.1307	0.0278
B5 total	6.5438	0.0435	4.1214	0.0747	11.1160	0.1504	9.2141	0.1324	7.7344	0.2784
B44	13.4838	0.0465	7.0137	0.0847	5.6807	0.0948	9.9253	0.1121	11.8024	0.2511
B45	0.5771	0.0102	4.8069	0.0708	0.1816	0.0173	1.8812	0.0506	0.7603	0.0670
B12 unsplit	0.0788	0.0038	0.0280	0.0055	0.0049	0.0029	0.0193	0.0051	0.0654	0.0197
B12 total	14.1440	0.0474	11.8486	0.1072	5.8673	0.0963	11.8258	0.1210	12.6281	0.2584
B62	5.9117	0.0320	1.5267	0.0404	9.2249	0.1190	4.1825	0.0747	6.9421	0.1973
B63	0.4302	0.0088	1.8865	0.0448	0.4438	0.0270	0.8083	0.0333	0.3738	0.0471
B75	0.0104	0.0014	0.0226	0.0049	1.9673	0.0566	0.1101	0.0123	0.0356	0.0145
B76	0.0026	0.0007	0.0065	0.0026	0.0874	0.0120	0.0055	0.0028	0	
B77	0.0057	0.0010	0.0119	0.0036	0.0577	0.0098	0.0083	0.0034	0[c]	———
B15 unsplit	0.1305	0.0049	0.0691	0.0086	0.4301	0.0266	0.1820	0.0158	0.0059	0.0059
B15 total	6.4910	0.0334	3.5232	0.0608	12.2112	0.1344	5.2967	0.0835	0.0715	0.0206
									7.4290	0.2035
B38	2.4413	0.0209	0.3323	0.0189	3.2818	0.0728	1.9652	0.0517	1.1017	0.0806
B39	1.9614	0.0188	1.2893	0.0371	2.0352	0.0576	6.3040	0.0909	4.5527	0.1615
B16 unsplit	0.0638	0.0034	0.0237	0.0051	0.0644	0.0103	0.1226	0.0130	0.0593	0.0188
B16 total	4.4667	0.0280	1.6453	0.0419	5.3814	0.0921	8.3917	0.1036	5.7137	0.1797
B57	3.5955	0.0252	5.6746	0.0766	2.5782	0.0647	2.1800	0.0544	2.7265	0.1260
B58	0.7152	0.0114	5.9546	0.0784	4.0189	0.0803	1.2481	0.0413	0.9398	0.0745
B17 unsplit	0.2845	0.0072	0.3248	0.0187	0.3751	0.0248	0.1446	0.0141	0.2674	0.0398
B17 total	4.5952	0.0284	11.9540	0.1076	6.9722	0.1041	3.5727	0.0691	3.9338	0.1503
B49	1.6452	0.0172	2.6286	0.0528	0.2440	0.0200	2.3353	0.0562	1.5462	0.0953
B50	1.0580	0.0138	0.8636	0.0304	0.4421	0.0270	1.8883	0.0507	0.7862	0.0681
B21 unsplit	0.0702	0.0036	0.0270	0.0054	0.0132	0.0047	0.0771	0.0103	0.0356	0.0145
B21 total	2.7733	0.0222	3.5192	0.0608	0.6993	0.0339	4.3007	0.0755	2.3680	0.1174
B54	0.0124	0.0015	0.0183	0.0044	2.6873	0.0660	0.0289	0.0063	0.0534	0.0178
B55	1.9046	0.0185	0.4895	0.0229	2.2444	0.0604	0.9515	0.0361	1.4054	0.0909
B56	0.5527	0.0100	0.2686	0.0170	0.8260	0.0368	0.3596	0.0222	0.3387	0.0448
B22 unsplit	0.1682	0.0055	0.0496	0.0073	0.2730	0.0212	0.0372	0.0071	0.1246	0.0272
B22 total	2.0852	0.0217	0.8261	0.0297	6.0307	0.0971	1.3771	0.0433	1.9221	0.1060

Antigen	CAU		AFR		ASI		LAT		NAT	
	Gf[a]	SE[b]	Gf	SE	Gf	SE	Gf	SE	Gf	SE
B60	5.2222	0.0302	1.5299	0.0404	8.3254	0.1135	2.2538	0.0553	5.7218	0.1801
B61	1.1916	0.0147	0.4709	0.0225	6.2072	0.0989	4.6691	0.0788	2.6023	0.1231
B40 unsplit	0.2696	0.0070	0.0388	0.0065	0.3205	0.0230	0.2473	0.0184	0.2271	0.0367
B40 total	6.6834	0.0338	2.0396	0.0465	14.8531	0.1462	7.1702	0.0963	8.5512	0.2168
BX	1.0922	0.0252	3.5258	0.0802	3.8749	0.0988	2.5266	0.0807	1.9867	0.1634

[a]Gene frequency. [b]Standard error. [c]The observed gene count was zero.

[0067] Some embodiments relate to methods, uses, therapies, kits, products and compositions related to generating immune responses against diseases, such as viral diseases and other microbial diseases, including without limitation the microbes and viruses listed in Tables 5-7. Furthermore, some embodiments relate to or can utilize antigens from various animals, as well as B cell and class I T cell epitopes from the antigens. Without being limited thereto, examples of some antigens and epitopes are listed in Tables 5-7 and in the other references cited herein which are incorporated herein by reference in their entirety. It should be understood that in some embodiments, one ore more or combinations of the viruses, antigens, and epitopes listed and referenced herein can be specifically excluded, while in some embodiments one or more or combinations thereof can be included. The B cell epitopes and class I T cell epitopes that can be used with the various embodiments are not limited to those that are specifically listed, as additional epitopes can be easily determined by the skilled artisan using any suitable technique. As one example, additional class I T cell epitopes can be identified using a suitable method such as epitopes with binding specificity for any MHC molecule. Any B cell epitope for any microbe or antigen listed here can be easily determined using any suitable technique by one of skill in the art. For example, such epitopes can be identified using homology modeling techniques and using the Bcipep database alone or in combination with predictive techniques. Also, The identification of B cell epitopes which are able to elicit an antibody response can be readily accomplished using techniques well known in the art. See, e.g., Geysen et al. (1984) Proc. Natl. Acad. Sci. USA 81:3998-4002 (general method of rapidly synthesizing peptides to determine the location of immunogenic epitopes in a given antigen); U.S. Pat. No. 4,708,871 (procedures for identifying and chemically synthesizing epitopes of antigens); and Geysen et al. (1986) Molecular Immunology 23:709-715 (technique for identifying peptides with high affinity for a given antibody); each of which is incorporated herein by reference in its entirety.

Table 5

Virus	Protein	AA Position	T cell epitope MHC ligand (Antigen)	MHC molecule
EBV	EBNA-3	325-333	AYPLHEQHG (SEQ ID NO:12)	HLA-B8
EBV	EBNA-3	158-166	YlKSFVSDA (SEQ ID NO:13)	HLA-B8
EBV	LMP-2	236-244	RRRWRRLTV (SEQ ID NO:14)	HLA-B*2704
EBV	EBNA-6	258-266	RRIYDLIEL (SEQ ID NO:15)	HLA-B*2705
EBV	EBNA-3	458-466	YPLHEQHGM (SEQ ID NO:16)	HLA-B*3501
EBV	EBNA-3	458-466	YPLHEQHGM (SEQ ID NO:17)	HLA-B*3503
EBV	LMP-2	426-434	CLGGLLTMV (SEQ ID NO:18)	HLA-A*0201
EBV	EBNA-l	480-484	NIAEGLRAL (SEQ ID NO:19)	HLA-A*0201
EBV	EBNA-1	519-527	NLRRGTALA (SEQ ID NO:20)	HLA-A*0201
EBV	EBNA-l	525-533	ALAIPQCRL (SEQ ID NO:21)	HLA-A*0201
EBV	EBNA-1	575-582	VLKDAIKDL (SEQ ID NO:22)	HLA-A*0201
EBV	EBNA-1	562-570	FMVFLQTHI (SEQ ID NO:23)	HLA-A*0201
EBV	EBNA-2	15-23	HLIVDTDSL (SEQ ID NO:24)	HLA-A*0201
EBV	EBNA-2	22-30	SLGNPSLSV (SEQ ID NO:25)	HLA-A*0201
EBV	EBNA-2	126-134	PLASAMRML (SEQ ID NO:26)	HLA-A*0201
EBV	EBNA-2	132-140	RMLWMANYl (SEQ ID NO:27)	HLA-A*0201
EBV	EBNA-2	133-141	MLWMANYIV (SEQ ID NO:28)	HL.A-A*0201
EBV	EBNA-2	151-159	ILPQGPQTA (SEQ ID NO:29)	HLA-A*0201
EBV	EBNA-2	171-179	PLRPTAPTI (SEQ ID NO:30)	HLA-A*0201
EBV	EBNA-2	205-213	PLPPATLTV (SEQ ID NO:31)	HLA-A*0201
EBV	EBNA-2	246-254	RMHLPVLHV	HLA-A*0201

			(SEQ ID NO:32)	
EBV	EBNA-2	287-295	PMPLPPSQL	HLA-A*0201
			(SEQ ID NO:33)	
EBV	EBNA-2	294-302	QLPPPAAPA	HLA-A*0201
			(SEQ ID NO:34)	
EBV	EBNA-2	381-389	SMPELSPVL	HLA-A*0201
			(SEQ ID NO:35)	
EBV	EBNA-2	453-461	DLDESWDYI	HLA-A*0201
			(SEQ ID NO:36)	
EBV	BZLF1	43-51	PLPCVLWPV	HLA-A*0201
			(SEQ ID NO:37)	
EBV	BZLF1	167-175	SLEECDSEL	HLA-A*0201
			(SEQ ID NO:38)	
EBV	BZLF1	176-184	EIKRYKNRV	HLA-A*0201
			(SEQ ID NO:39)	
EBV	BZLF1	195-203	QLLQHYREV	HLA-A*0201
			(SEQ ID NO:40)	
EBV	BZLF1	196-204	LLQHYREVA	HLA-A*0201
			(SEQ ID NO:41)	
EBV	BZLFI	217-225	LLKQMCPSL	HLA-A*0201
			(SEQ ID NO:42)	
EBV	BZLF1	229-237	SIIPRTPDV	HLA-A*0201
			(SEQ ID NO:43)	
EBV	EBNA-6	284-293	LLDFVRFMGV	HLA-A*0201
			(SEQ ID NO:44)	
EBV	EBNA-3	464-472	SVRDRLARL	HLA-A*0203
			(SEQ ID NO:45)	
EBV	EBNA-4	416-424	IVTDFSVIK	HLA-A*1101
			(SEQ ID NO:46)	
EBV	EBNA-4	399-408	AVFDRKSDAK	HLA-A*0201
			(SEQ ID NO:47)	
EBV	EBNA-3	246-253	RYSIFFDY	HLA-A24
			(SEQ ID NO:48)	
EBV	EBNA-6	881-889	QPRAPIRPI	HLA-B7
			(SEQ ID NO:49)	
EBV	EBNA-3	379-387	RPPIFIRRI.	HLA-B7
			(SEQ ID NO:50)	
EBV	EBNA-1	426-434	EPDVPPGAI	HLA-B7
			(SEQ ID NO:51)	
EBV	EBNA-1	228-236	IPQCRLTPL	HLA-B7
			(SEQ ID NO:52)	
EBV	EBNA-1	546-554	GPGPQPGPL	HLA-B7
			(SEQ ID NO:53)	
EBV	EBNA-1	550-558	QPGPLRESI	HLA-B7
			(SEQ ID NO:54)	
EBV	EBNA-1	72-80	R.PQKRPSCI	HLA-B7
			(SEQ ID NO:55)	
EBV	EBNA-2	224-232	PPTPLLTVL	HLA-B7

			(SEQ ID NO:56)	
EBV	EBNA-2	241-249	TPSPPRMHL	HLA-B7
			(SEQ ID NO:57)	
EBV	EBNA-2	244-252	PPRMHLPVL	HLA-B7
			(SEQ ID NO:58)	
EBV	EBNA-2	254-262	VPDQSMHPL	HLA-B7
			(SEQ ID NO:59)	
EBV	EBNA-2	446-454	PPSIDPADL	HLA-B7
			(SEQ ID NO:60)	
EBV	BZLFI	44-52	LPCVLWPVL	HLA-B7
			(SEQ ID NO:61)	
EBV	BZLF1	222-231	CPSLDVDSII	HLA-B7
			(SEQ ID NO:62)	
EBV	BZLFI	234-242	TPDVLHEDL	HLA-B7
			(SEQ ID NO:63)	
EBV	EBNA-3	339-347	FLRGRAYGL	HLA-B8
			(SEQ ID NO:64)	
EBV	EBNA-3	26-34	QAKWRLQTL	HLA-B8
			(SEQ ID NO:65)	
HCV	NS3	389-397	HSKKKCDEL	HLA-B8
			(SEQ ID NO:66)	
HCV	env E	44-51	ASRCWVAM	HLA-B*3501
			(SEQ ID NO:67)	
HCV	core protein	27-35	GQIVGGVYL	HLA-B*40012
			(SEQ ID NO:68)	
HCV	NSI	77-85	PPLTDFDQGW	HLA-B*5301
			(SEQ ID NO:69)	
HCV	core protein	18-27	LMGYIPLVGA	H2-Dd
			(SEQ ID NO:70)	
HCV	core protein	16-25	ADLMGYIPLV	H2-Dd
			(SEQ ID NO:71)	
HCV	NS5	409-424	MSYSWTGALVT PCAEE	H2-Dd
			(SEQ ID NO:72)	
HCV	NS1	205-213	KHPDATYSR	Papa-A06
			(SEQ ID NO:73)	
HCV-1	NSI	159-167	TRPPLGNWF	Patr-B13
			(SEQ ID NO:74)	
HCV-1	NS3	351-359	VPHPNIEEV	Patr-B13
			(SEQ ID NO:75)	
HCV-1	NS3	438-446	YTGDFDSVI	Patr-B01
			(SEQ ID NO:76)	
HCV-1	NS4	328-335	SWAIKWEY	Patr-Al 1
			(SEQ ID NO:77)	
HCV-1	NSI	205-213	KHPDATYSR	Patr-A04
			(SEQ ID NO:78)	
HCV-1	NS3	440-448	GDFDSVIDC	Patr-A04

			(SEQ ID NO:79)	
HCV-1	NS3	400-409	KLVALGINAV	HLA-A*0201
			(SEQ ID NO:80)	
HCV-1	NS3	440-448	GDFDSVIDC	Patr-B16
			(SEQ ID NO:81)	
HCV-1	env E	118-126	GNASRCWVA	Patr-BI6
			(SEQ ID NO:82)	
HIV	nef	117-125	TQGYFPQWQ	HLA-B*3701
			(SEQ ID NO:83)	
HIV	gagp24	143-151	HQAISPRTI,	HLA-Cw*0301
			(SEQ ID NO:84)	
HIV	gagp24	140-151	QMVHQAISPRTL	HLA-Cw*0301
			(SEQ ID NO:85)	
HIV	gpl20	431-440	MYAPPIGGQI	H2-Kd
			(SEQ ID NO:86)	
HIV	gpl60	318-327	RGPGRAFVTI	H2-Dd
			(SEQ ID NO:87)	
HIV	gp120	17-29	MPGRAFVTI	H2-Ld
			(SEQ ID NO:88)	
HIV	gp4l	583-591	RYLKDQQLL	HLA_A24
			(SEQ ID NO:89)	
HIV	gagp24	267-275	IVGLNKIVR	HLA-A*3302
			(SEQ ID NO:90)	
HIV	gagp24	262-270	EIYKRWIIL	HLA-B8
			(SEQ ID NO:91)	
HIV	gagp24	261-269	GEIYKRWI1	HLA-B8
			(SEQ ID NO:92)	
HIV	gagp17	93-101	EIKDTKEAL	HLA-B8
			(SEQ ID NO:93)	
HIV	gp4l	586-593	YLKDQQLL	HLA-B8
			(SEQ ID NO:94)	
HIV	gagp24	267-277	ILGLNKIVRMY	HLA-B* 1501
			(SEQ ID NO:95)	
HIV	gp4l	584-592	ERYLKDQQL	HLA-B14
			(SEQ ID NO:96)	
HIV	nef	115-125	YHTQGYFPQWQ	HLA-B17
			(SEQ ID NO:97)	
HIV	nef	117-128	TQGYFPQWQNY T	HLA-B17
			(SEQ ID NO:98)	
HIV	gpl20	314-322	GRAFVTIGK	HLA-B*2705
			(SEQ ID NO:99)	
HIV	gagp24	263-271	KRWIILGLN	HLA-B*2702
			(SEQ ID NO:100)	
HIV	nef	72-82	QVPLRPMTYK	HLA-B*3501

			(SEQ ID NO:101)	
HIV-1	gagp 17	24-31	GGKKKYKL	HLA-B8
			(SEQ ID NO:102)	
HIV-1	gpl20	2-10	RVKEKYQHL	HLA-B8
			(SEQ ID NO:103)	
HIV-1	gagp24	298-306	DRFYKTLRA	HLA-B 14
			(SEQ ID NO:104)	
HIV-1	NEF	132-147	GVRYPLTFGWC YKLVP	HLA-B18
			(SEQ ID NO:105)	
HIV-1	gagp24	265-24	KRWIILGLNK	HLA-B*2705
			(SEQ ID NO:106)	
HIV-1	nef	190-198	AFHHVAREL	HLA-B*5201
			(SEQ ID NO:107)	
HIV-1	RT	476-484	ILKEPVHGV	HLA-A*0201
			(SEQ ID NO:108)	
HIV-1	nef	190-198	AFHHVAREL	HLA-A*0201
			(SEQ ID NO:109)	
HIV-1	gpI60	120-128	KLTPLCVTL	HLA-A*0201
			(SEQ ID NO:110)	
HIV-1	Gp160	814-823	SLLNATDIAV	HLA-A*0201
			(SEQ ID NO:111)	
HIV-1	RT	179-187	VIYQYMDDL	HLA-A*0201
			(SEQ ID NO:112)	
HIV-1	gagp 17	77-85	SLYNTVATL	HLA-A*0201
			(SEQ ID NO:113)	
HIV-1	gp160	315-329	RGPGRAFVT1	HLA-A*0201
			(SEQ ID NO:114)	
HIV-1	gp4l	768-778	RLRDLLLIVTR	HLA-A3
			(SEQ ID NO:115)	
HIV-1	nef	73-82	QVPLRPMTYK	HLA-A3
			(SEQ ID NO:116)	
HIV-1	gp120	36-45	TVYYGVPVWK	HLA-A3
			(SEQ ID NO:117)	
HIV-1	gagp17	20-29	RLRPGGKKK	HLA-A3
			(SEQ ID NO:118)	
HIV-1	gp120	38-46	VYYGVPVWK	HLA-A3
			(SEQ ID NO:119)	
HIV-1	nef	74-82	VPLRPMTYK	HLA-a*1101
			(SEQ ID NO:120)	
HIV-1	gagp24	325-333	AIFQSSMTK	HLA-A*1101
			(SEQ ID NO:121)	
HIV-1	nef	73-82	QVPLRPMTYK	HLA-A*1101
			(SEQ ID NO:122)	
HIV-1	nef	83-94	AAVDLSHFLKEK	HLA-A*1101
			(SEQ ID NO:123)	
HIV-1	gagp24	349-359	ACQGVGGPGGH K	HLA-A*1101

			(SEQ ID NO:124)	
HIV-1	gagp24	203-212	ETINEEAAEW	HLA-A25
			(SEQ ID NO:125)	
HIV-1	nef	128-137	TPGPGVRYPL	HLA-B7
			(SEQ ID NO:126)	
Mv	HA	343-351	DPVIDRLYL	H2-Ld
			(SEQ ID NO:127)	
MV	HA	544-552	SPGRSFSYF	H2-Ld
			(SEQ ID NO:128)	
Pseudorabies virus gp	G111	455-463	IAGIGILAI	HLA-A*0201
			(SEQ ID NO:129)	
Rabiesvirus	NS	197-205	VEAEIAHQI	H2-Kk
			(SEQ ID NO:130)	
RSV	M2	82-90	SYIGSINNI	H2-Kd
			(SEQ ID NO:131)	
SIV	gagp11C	179-190	EGCTPYDTNQML	Mamu-A*01
			(SEQ ID NO:132)	
EBV	EBNA-6	335-343	KEHVIQNAF	HLA-B44
			(SEQ ID NO:133)	
EBV	EBNA-6	130-139	EENLLDFVRF	HLA-B*4403
			(SEQ ID NO:134)	
EBV	EBNA-2	42-51	DTPLIPLTIF	HLA-B51
			(SEQ ID NO:135)	
EBV	EBNA-6	213-222	QNGALAINTF	HLA-1362
			(SEQ ID NO:136)	
EBV	EBNA-3	603-611	RLRAEAGVK	HLA-A3
			(SEQ ID NO:137)	
HBV	sAg	348-357	GLSPTVWLSV	HLA-A*0201
			(SEQ ID NO:138)	
HBV	SAg	335-343	WLSLLVPFV	HLA-A*0201
			(SEQ ID NO:139)	
HBV	cAg	18-27	FLPSDFFPSV	HLA-A*0201
			(SEQ ID NO:140)	
HBV	cAg	18-27	FLPSDFFPSV	HLA-A*0202
			(SEQ ID NO:141)	
HBV	cAg	18-27	FLPSDFFPSV	HLA-A*0205
			(SEQ ID NO:142)	
HBV	cAg	18-27	FLPSDFFPSV	HLA-A*0206
			(SEQ ID NO:143)	
HBV	pol	575-583	FLLSLGIHL	HLA-A*0201
			(SEQ ID NO:144)	
HBV	pol	816-824	SLYADSPSV	HLA-A*0201
			(SEQ ID NO:145)	
HBV	pol	455-463	GLSRYVARL	HLA-A*0201
			(SEQ ID NO:146)	
HBV	env	338-347	LLVPFVQWFV	HLA-A*0201

			(SEQ ID NO:147)	
HBV	pol	642-650	ALMPLYACI	HLA-A*0201
			(SEQ ID NO:148)	
HBV	env	378-387	LLPIFFCLWV	HLA-A*0201
			(SEQ ID NO:149)	
HBV	pol	538-546	YMDDVVLGA	HLA-A*0201
			(SEQ ID NO:150)	
HBV	env	250-258	LLLCLIFLL	HLA-A*0201
			(SEQ ID NO:151)	
HBV	env	260-269	LLDYQGMLPV	HLA-A*0201
			(SEQ ID NO:152)	
HBV	env	370-379	SIVSPFIPLL	HLA-A*0201
			(SEQ ID NO:153)	
HBV	env	183-191	FLLTRILTI	HLA-A*0201
			(SEQ ID NO:154)	
HBV	cAg	88-96	YVNVNMGLK	HLA-A* 1101
			(SEQ ID NO:155)	
HBV	cAg	141-151	STLPETTVVRR	HLA-A*3101
			(SEQ ID NO:156)	
HBV	cAg	141-151	STLPETTVVRR	HLA-A*6801
			(SEQ ID NO:157)	
HBV	cAg	18-27	FLPSDFFPSV	HLA-A*6801
			(SEQ ID NO:158)	
HBV	sAg	28-39	IPQSLDSWWTSL	H2-Ld
			(SEQ ID NO:159)	
HBV	cAg	93-100	MGLKFRQL	H2-Kb
			(SEQ ID NO:160)	
HBV	preS	141-149	STBXQSGXQ	HLA-A*0201
			(SEQ ID NO:161)	
HCMV	gp B	618-628	FIAGNSAYEYV	HLA-A*0201
			(SEQ ID NO:162)	
HCMV	E1	978-989	SDEEFAIVAYTL	HLA-B18
			(SEQ ID NO:163)	
HCMV	pp65	397-411	DDVWTSGSDSD EELV	HLA-b35
			(SEQ ID NO:164)	
HCMV	pp65	123-131	IPSINVHHY	HLA-B*3501
			(SEQ ID NO:165)	
HCMV	pp65	495-504	NLVPMVATVO	HLA-A*0201
			(SEQ ID NO:166)	
HCMV	pp65	415-429	RKTPRVTOGGA MAGA	HLA-B7
			(SEQ ID NO:167)	
HCV	MP	17-25	DLMGYIPLV	HLA-A*0201
			(SEQ ID NO:168)	
HCV	MP	63-72	LLALLSCLTV	HLA-A*0201
			(SEQ ID NO:169)	

HCV	MP	105-112	ILHTPGCV (SEQ ID NO:170)	HLA-A*0201
HCV	env E	66-75	QLRRHIDLLV (SEQ ID NO:171)	HLA-A*0201
HCV	env E	88-96	DLCGSVFLV (SEQ ID NO:172)	HLA-A*0201
HCV	env E	172-180	SMVGNWAKV (SEQ ID NO:173)	HLA-A*0201
HCV	NSI	308-316	HLIIQNIVDV (SEQ ID NO:174)	HLA-A*0201
HCV	NSI	340-348	FLLLADARV (SEQ ID NO:175)	HLA-A*0201
HCV	NS2	234-246	GLRDLAVAVEP VV (SEQ ID NO:176)	HLA-A*0201
HCV	NSI	18-28	SLLAPGAKQNV (SEQ ID NO:177)	HLA-A*0201
HCV	NSI	19-28	LLAPGAKQNV (SEQ ID NO:178)	HLA-A*0201
HCV	NS4	192-201	LLFNILGGWV (SEQ ID NO:179)	HLA-A*0201
HCV	NS3	579-587	YLVAYQATV (SEQ ID NO:180)	HLA-A*0201
HCV	core protein	34-43	YLLPRRGPRL (SEQ ID NO:181)	HLA-A*0201
HCV	MP	63-72	LLALLSCLTI (SEQ ID NO:182)	HLA-A*0201
HCV	NS4	174-182	SLMAFTAAV (SEQ ID NO:183)	HLA-A*0201
HCV	NS3	67-75	CINGVCWTV (SEQ ID NO:184)	HLA-A*0201
HCV	NS3	163-171	LLCPAGHAV (SEQ ID NO:185)	HLA-A*0201
HCV	NS5	239-247	ILDSFDPLV (SEQ ID NO:186)	HLA-A*0201
HCV	NS4A	236-244	ILAGYGAGV (SEQ ID NO:187)	HLA-A*0201
HCV	NS5	714-722	GLQDCTMLV (SEQ ID NO:188)	HLA-A*0201
HCV	NS3	281-290	TGAPVTYSTY (SEQ ID NO:189)	HLA-A*0201
HCV	NS4A	149-157	HMWNFISGI (SEQ ID NO:190)	HLA-A*0201
HCV	NS5	575-583	RVCEKMALY (SEQ ID NO:191)	HLA-A*0201-A3
HCV	NS1	238-246	TINYTIFK (SEQ ID NO:192)	HLA-A*1101

HCV	NS2	109-116	YISWCLWW (SEQ ID NO:193)	HLA-A23
HCV	core protein	40-48	GPRLGVRAT (SEQ ID NO:194)	HLA-B7
HIV-1	gp120	380-388	SFNCGGEFF (SEQ ID NO:195)	HLA-Cw*0401
HIV-1	RT	206-214	TEMEKEGKI (SEQ ID NO:196)	H2-Kk
HIV-1	p17	18-26	KIRLRPGGK (SEQ ID NO:197)	HLA-A*0301
HIV-1	P17	20-29	RLRPGGKKKY (SEQ ID NO:198)	HLA-A*0301
HIV- I	RT	325-333	AIFQSSMTK (SEQ ID NO:199)	HLA-A*0301
HIV-1	p17	84-92	TLYCVHQRI (SEQ ID NO:200)	HLA-A11
HIV-1	RT	508-517	IYQEPFKNLK (SEQ ID NO:201)	HLA-Al1
HIV-1	p17	28-36	KYKLKHIVW (SEQ ID NO:202)	HLA-A24
HIV-1	gp120	53-62	LFCASDAKAY (SEQ ID NO:203)	HLA-A24
HIV-1	gagp24	145-155	QAISPRTLNAW (SEQ ID NO:204)	HLA-A25
HIV-1	gagp24	167-175	EVIPMFSAL (SEQ ID NO:205)	HLA-A26
HIV-1	RT	593-603	ETFYVDGAANR (SEQ ID NO:206)	HLA-A26
HIV-1	gp4l	775-785	RLRDLLLIVTR (SEQ ID NO:207)	HLA-A31
HIV-1	RT	559-568	PIQKETWETW (SEQ ID NO:208)	HLA-A32
HIV-1	gpl20	419-427	RIKQIINMW (SEQ ID NO:209)	HLA-A32
HIV-1	RT	71-79	ITLWQRPLV (SEQ ID NO:210)	HLA-A*6802
HIV-1	RT	85-93	DTVLEEMNL (SEQ ID NO:211)	HLA-A*6802
HIV-1	RT	71-79	ITLWQRPLV (SEQ ID NO:212)	HLA-A*7401
HIV-1	gag p24	148-156	SPRTLNAWV (SEQ ID NO:213)	HLA-B7
HIV-1	gagp24	179-187	ATPQDLNTM (SEQ ID NO:214)	HLA-B7
HIV-1	gpl20	303-312	RPNNNTRKSI (SEQ ID NO:215)	HLA-B7
HIV-1	gp4l	843-851	IPRRIRQGL	HLA-B7

			(SEQ ID NO:216)	
HIV-1	p17	74-82	ELRSLYNTV	HLA-B8
			(SEQ ID NO:217)	
HIV-1	nef	13-20	WPTVRERM	HLA-B8
			(SEQ ID NO:218)	
HIV-1	nef	90-97	FLKEKGGL	HLA-B8
			(SEQ ID NO:219)	
HIV-1	gag p24	183-191	DLNTMLNTV	HLA-B14
			(SEQ ID NO:220)	
HIV-1	P17	18-27	KIRLRPGGKK	HLA-B27
			(SEQ ID NO:221)	
HIV-1	p17	19-27	IRLRPGGKK	HLA-B27
			(SEQ ID NO:222)	
HIV-1	gp4l	791-799	GRRGWEALKY	HLA-B27
			(SEQ ID NO:223)	
HIV-1	nef	73-82	QVPLRPMTYK	HLA-B27
			(SEQ ID NO:224)	
HIV-1	GP41	590-597	RYLKDQQL	HLA-B27
			(SEQ ID NO:225)	
HIV-1	nef	105-114	RRQDILDLWI	HLA-B*2705
			(SEQ ID NO:226)	
HIV-1	nef	134-141	RYPLTFGW	HLA-B*2705
			(SEQ ID NO:227)	
HIV-1	p17	36-44	WASRELERF	HLA-B35
			(SEQ ID NO:228)	
HIV-1	GAG P24	262-270	TVLDVGDAY	HLA-B35
			(SEQ ID NO:229)	
HIV-1	gp120	42-52	VPVWKEATTTL	HLA-B35
			(SEQ ID NO:230)	
HIV-1	P17	36-44	NSSKVSQNY	HLA-B35
			(SEQ ID NO:231)	
HIV-1	gag p24	254-262	PPIPVGDIY	HLA-B35
			(SEQ ID NO:232)	
HIV-1	RT	342-350	HPDIVIYQY	HLA-B35
			(SEQ ID NO:233)	
HIV-1	gp41	611-619	TAVPWNASW	HLA-B35
			(SEQ ID NO:234)	
HIV-1	gag	245-253	NPVPVGNlY	HLA-B35
			(SEQ ID NO:235)	
HIV-1	nef	120-128	YFPDWQNYT	HLA-B37
			(SEQ ID NO:236)	
HIV-1	gag p24	193-201	GHQAAMQML	HLA-B42
			(SEQ ID NO:237)	
HIV-1	p17	20-29	RLRPGGKKKY	HLA-B42
			(SEQ ID NO:238)	
HIV-1	RT	438-446	YPGIKVRQL	HLA-B42
			(SEQ ID NO:239)	
HIV-1	RT	591-600	GAETFYVDGA	HLA-B45

			(SEQ ID NO:240)	
HIV-1	gag p24	325-333	NANPDCKTI	HLA-B51
			(SEQ ID NO:241)	
HIV-1	gag p24	275-282	RMYSPTSI	HLA-B52
			(SEQ ID NO:242)	
HIV-1	gp120	42-51	VPVWKEATTT	HLA-B*5501
			(SEQ ID NO:243)	
HIV-1	gag p24	147-155	ISPRTLNAW	HLA-B57
			(SEQ ID NO:244)	
HIV-1	gag p24	240-249	TSTLQEQIGW	HLA-B57
			(SEQ ID NO:245)	
HIV-1	gag p24	162-172	KAFSPEVIPMF	HLA-B57
			(SEQ ID NO:246)	
HIV-1	gag p24	311-319	QASQEVKNW	HLA-B57
			(SEQ ID NO:247)	
HIV-1	gag p24	311-319	QASQDVKNW	HLA-B57
			(SEQ ID NO:248)	
HIV-1	nef	116-125	HTQGYFPDWQ	HLA-B57
			(SEQ ID NO:249)	
HIV-1	nef	120-128	YFPDWQNYT	HLA-B57
			(SEQ ID NO:250)	
HIV-1	gag p24	240-249	TSTLQEQIGW	HLA-B58
			(SEQ ID NO:251)	
HIV-1	p17	20-29	RLRPGGKKKY	HLA-B62
			(SEQ ID NO:252)	
HIV-1	p24	268-277	LGLNKJVRMY	HLA-B62
			(SEQ ID NO:253)	
HIV-1	RT	415-426	LVGKLNWASQI Y	HLA-B62
			(SEQ ID NO:254)	
HIV-1	RT	476-485	ILKEPVHGVY	HLA-B62
			(SEQ ID NO:255)	
HIV-1	nef	117-127	TQGYFPDWQNY	HLA-B62
			(SEQ ID NO:256)	
HIV-1	nef	84-91	AVDLSHFL	HLA-B62
			(SEQ ID NO:257)	
HIV-1	gag p24	168-175	VIPMFSAL	HLA-Cw*0102
			(SEQ ID NO:258)	
HIV-1	gp120	376-384	FNCGGEFFY	HLA-A29
			(SEQ ID NO:259)	
HIV-1	gp120	375-383	SFNCGGEFF	HLA-B15
			(SEQ ID NO:260)	
HIV-1	nef	136-145	PLTFGWCYKL	HLA-A*0201
			(SEQ ID NO:261)	
HIV-1	nef	180-189	VLEWRFDSRL	HLA-A*0201
			(SEQ ID NO:262)	
HIV-1	nef	68-77	FPVTPQVPLR	HLA-B7

			(SEQ ID NO:263)	
HIV-1	nef	128-137	TPGPGVRYPL	HLA-B7
			(SEQ ID NO:264)	
HIV-1	gag p24	308-316	QASQEVKNW	HLA-Cw*0401
			(SEQ ID NO:265)	
HIV-1 IIIB	RT	273-282	VPLDEDFRKY	HLA-B35
			(SEQ ID NO:266)	
HIV-1 IIIB	RT	25-33	NPDIVIYQY	HLA-B35
			(SEQ ID NO:267)	
HIV-1 IIIB	gp41	557-565	RAIEAQAHL	HLA-B51
			(SEQ ID NO:268)	
HIV-1 IIIB	RT	231-238	TAFTIPSI	HLA-B51
			(SEQ ID NO:269)	
HIV- I IIIB	p24	215-223	VHPVHAGPIA	HLA-B*5501
			(SEQ ID NO:270)	
HIV-1 IIIB	gp120	156-165	NCSFNISTSI	HLA-Cw8
			(SEQ ID NO:271)	
HIV- I IIIB	gp120	241-249	CTNVSTVQC	HLA-Cw8
			(SEQ ID NO:272)	
HIV-1 5F2	gp120	312-320	IGPGRAFHT	H2-Dd
			(SEQ ID NO:273)	
HIV-1 5F2	pol	25-33	NPDIVIYQY	HLA-B*3501
			(SEQ ID NO:274)	
HIV-15F2	pol	432-441	EPIVGAETFY	HLA-B*3501
			(SEQ ID NO:275)	
HIV-1 5F2	pol	432-440	EPIVGAETF	HLA-B*3501
			(SEQ ID NO:276)	
HIV-1 5F2	pol	6-14	SPAIFQSSM	HLA-B*3501
			(SEQ ID NO:277)	
HIV-1 5F2	pol	59-68	VPLDKDFRKY	HLA-B*3501
			(SEQ ID NO:278)	
HIV-1 5F2	pol	6-14	IPLTEEAEL	HLA-B*3501
			(SEQ ID NO:279)	
HIV-1 5F2	nef	69-79	RPQVPLRPMTY	HLA-B*3501
			(SEQ ID NO:280)	
HIV-1 5F2	nef	66-74	FPVRPQVPL	HLA-B*3501
			(SEQ ID NO:281)	
HIV-1 5F2	env	10-18	DPNPQEVVL	HLA-B*3501
			(SEQ ID NO:282)	
HIV-1 5F2	env	7-15	RPIVSTQLL	HLA-B*3501
			(SEQ ID NO:283)	
HIV-1 5F2	pol	6-14	IPLTEEAEL	HLA-B51
			(SEQ ID NO:284)	
HIV-1 5F2	env	10-18	DPNPQEVVL	HLA-B51
			(SEQ ID NO:285)	
HIV-1 5F2	gagp24	199-207	AMQMLKETI	H2-Kd
			(SEQ ID NO:286)	

HIV-2	gagp24	182-190	TPYDrNQML (SEQ ID NO:287)	HLA-B*5301
HIV-2	gag	260-269	RRWIQLGLQKV (SEQ ID NO:288)	HLA-B*2703
HIV-1 5F2	gp41	593-607	GIWGCSGKLICT TAV (SEQ ID NO:289)	HLA-B17
HIV-1 5F2	gp41	753-767	ALIWEDLRSLCL FSY (SEQ ID NO:290)	HLA-B22
HPV 6b	E7	21-30	GLHCYEQLV (SEQ ID NO:291)	HLA-A*0201
HPV 6b	E7	47-55	PLKQHFQIV (SEQ ID NO:292)	HLA-A*0201
HPV11	E7	4-12	RLVTLKDIV (SEQ ID NO:293)	HLA-A*0201
HPV16	E7	86-94	TLGIVCPIC (SEQ ID NO:294)	HLA-A*0201
HPV16	E7	85-93	GTLGIVCPI (SEQ ID NO:295)	HLA-A*0201
HPV16	E7	12-20	MLDLQPETT (SEQ ID NO:296)	HLA-A*0201
HPV16	E7	11-20	YMLDLQPETT (SEQ ID NO:297)	HLA-A*0201
HPV16	E6	15-22	RPRKLPQL (SEQ ID NO:298)	HLA-B7
HPV16	E6	49-57	RAHYNIVTF (SEQ ID NO:299)	HW-Db
HSV	gp B	498-505	SSIEFARL (SEQ ID NO:300)	H2-Kb
HSV-1	gp C	480-488	GIGIGVLAA (SEQ ID NO:301)	HLA-A*0201
HSV-1	ICP27	448-456	DYATLGVGV (SEQ ID NO:302)	H2-Kd
HSV-1	ICP27	322-332	LYRTFAGNPRA (SEQ ID NO:303)	H2-Kd
HSV-1	UL39	822-829	QTFDFGRL (SEQ ID NO:304)	H2-Kb
HSV-2	gpC	446-454	GAGIGVAVL (SEQ ID NO:305)	HLA-A*0201
HLTV-1	TAX	11-19	LLFGYPVYV (SEQ ID NO:306)	HLA-A*0201
Influenza	MP	58-66	GILGFVFTL (SEQ ID NO:307)	HLA-A*0201
Influenza	MP	59-68	ILGFVFTLTV (SEQ ID NO:308)	HLA-A*0201
Influenza	NP	265-273	ILRGSVAHK (SEQ ID NO:309)	HLA-A3

Influenza	NP	91-99	KTGGPIYKR (SEQ ID NO:310)	HLA-A*6801
Influenza	NP	380-388	ELRSRYWAI (SEQ ID NO:311)	HLA-B8
Influenza	NP	381-388	LRSRYWAI (SEQ ID NO:312)	HLA-B*2702
Influenza	NP	339-347	EDLRVLSFI (SEQ ID NO:313)	HLA-B*3701
Influenza	NSI	158-166	GEISPLPSL (SEQ ID NO:314)	HLA-B44
Influenza	NP	338-346	FEDLRVLSF (SEQ ID NO:315)	HLA-B44
Influenza	NSI	158-166	GEISPLPSL (SEQ ID NO:316)	HLA-B*4402
Influenza	NP	338-346	FEDLRVLSF (SEQ ID NO:317)	HLA-B*4402
Influenza	PBI	591-599	VSDGGPKLY (SEQ ID NO:318)	HLA-Al
Influenza A	NP	44-52	CTELKLSDY (SEQ ID NO:319)	HLA-Al
Influenza	NSI	122-130	AIMDKNIIL (SEQ ID NO:320)	HLA-A*0201
Influenza A	NSI	123-132	IMDKNIILKA (SEQ ID NO:321)	HLA-A*0201
Influenza A	NP	383-391	SRYWAIRTR (SEQ ID NO:322)	HLA-B*2705
Influenza A	NP	147-155	TYQRTRALV (SEQ ID NO:323)	H2-Kd
Influenza A	HA	210-219	TYVSVSTSTL (SEQ ID NO:324)	H2-Kd
Influenza A	HA	518-526	IYSTVASSL (SEQ ID NO:325)	H2-Kd
Influenza A	HA	259-266	FEANGNLI (SEQ ID NO:326)	H2-Kk
Influenza A	HA	10-18	IEGGWTGMl (SEQ ID NO:327)	H2-Kk
Influenza A	NP	50-57	SDYEGRLI (SEQ ID NO:328)	H2-Kk
Influenza a	NSI	152-160	EEGAIVGEI (SEQ ID NO:329)	H2-Kk
Influenza A34	NP	336-374	ASNENMETM (SEQ ID NO:330)	H2Db
Influenza A68	NP	366-374	ASNENMDAM (SEQ ID NO:331)	H2Db
Influenza B	NP	85-94	KLGEFYNQMM (SEQ ID NO:332)	HLA-A*0201
Influenza B	NP	85-94	KAGEFYNQMM (SEQ ID NO:333)	HLA-A*0201

Influenza JAP	HA	204-212	LYQNVGTYV (SEQ ID NO:334)	H2Kd
Influenza JAP	HA	210-219	TYVSVGTSTL (SEQ ID NO:335)	H2-Kd
Influenza JAP	HA	523-531	VYQILATYA (SEQ ID NO:336)	H2-Kd
Influenza JAP	HA	529-537	IYATVAGSL (SEQ ID NO:337)	H2-Kd
Influenza JAP	HA	210-219	TYVSVGTSTI(L>I) (SEQ ID NO:338)	H2-Kd
Influenza JAP	HA	255-262	FESTGNLI (SEQ ID NO:339)	H2-Kk
JHMV	cAg	318-326	APTAGAFFF (SEQ ID NO:340)	H2-Ld
LCMV	NP	118-126	RPQASGVYM (SEQ ID NO:341)	H2-Ld
LCMV	NP	396-404	FQPQNGQFI (SEQ ID NO:342)	H2-Db
LCMV	GP	276-286	SGVENPGGYCL (SEQ ID NO:343)	H2-Db
LCMV	GP	33-42	KAVYNFATCG (SEQ ID NO:344)	H2-Db
MCMV	pp89	168-176	YPHFMPTNL (SEQ ID NO:345)	H2-Ld
MHV	spike protein	510-518	CLSWNGPHL (SEQ ID NO:346)	H2-Db
MV	F protein	437-447	SRRYPDAVYLH (SEQ ID NO:347)	HLA-B*2705
Mv	F protein	438-446	RRYPDAVYL (SEQ ID NO:348)	HLA-B*2705
Mv	NP	281-289	YPALGLHEF (SEQ ID NO:349)	H2-Ld

Table 6

HLA Class I Motifs

Sequence (Antigen)	Source	Ref.
IVTDFSVIK	EBNA-4 416-424	115,117
(SEQ ID NO:350)		
VPLRPMTYK	HIV- 1 NEF 74-82	115
(SEQ ID NO:351)		
AIFQSSMTK	HIV- I gag p24 325-333	115
(SEQ ID NO:352)		

QVPLRPMTYK	HIV-1 nef 73-82	118
(SEQ ID NO:353)		
AAVDLSHFLKEK	HIV-1 nef 83-94	120
(SEQ ID NO:354)		
ACQ G V G G P G G H K	HIV-1 I I 1B p24 349-359	122
(SEQ ID NO:355)		
RYLKDQQLL	HIV GP 41 583-591	124
(SEQ ID NO:356)		
RYSIFFDY	Ebna-3 246-253	101
(SEQ ID NO:357)		
ETINEEAAEW	HIV- 1 gag p24 203-212	127
(SEQ ID NO:358)		
STLPETTVVRR	HBV cAg 141 -151	129
(SEQ ID NO:359)		
MSLQRQFLR	ORF 3P-gp75 294-321 (bp)	130
(SEQ ID NO:360)		
IVGLNKIVR	HIV gag p24 267-267-275	132, 133
(SEQ ID NO:361)		
TIHDIILEC	HPV16 E6 29-37	97
(SEQ ID NO:362)		
LGIVCPICS	HPV16 E7 87-95	97
(SEQ ID NO:363)		
FLPSDFFPSV	HBV cAg 18-27	51
(SEQ ID NO:364)		
SVRDRLARL	EBNA-3 464-472	101
(SEQ ID NO:365)		
FLPSDFFPSV	HBV cAg 18-27	51
(SEQ ID NO:366)		
FLPSDFFPSV	HBV cAg 18-27	51
(SEQ ID NO:367)		
R L R D L L L I V T R	HIV-1 gp41 768-778	108
(SEQ ID NO:368)		
QVPLRPMTYK	HIV-1 nef 73-82	109

(SEQ ID NO:369)		
TVYYGVPVWK	HIV-1 gp120-36-45	110
(SEQ ID NO:370)		
RLRPGGKKK	HIV- 1 gag p 17 20-29	110
(SEQ ID NO:371)		
ILRGSVAHK	Influenza NP 265-273	21
(SEQ ID NO:372)		
RLRAEAGVK	EBNA-3 603-611	111
(SEQ ID NO:373)		
RLRDLLLIVTR	HIV-1 gp41 770-780	112
(SEQ ID NO:374)		
VYYGVPVWK	HIV- I GP 120 38-46	113
(SEQ ID NO:375)		
RVCEKMALY	HCV NS5 575-583	114
(SEQ ID NO:376)		
VSDGGPNLY	Influenza A PB 1591-599	21,23
(SEQ ID NO:377)		
CTELKLSDY	Influenza A NP 44-52	23
(SEQ ID NO:378)		
STBXQSGXQ	HBV PRE-S PROTEIN 141-149	43
(SEQ ID NO:379)		
ILKEPVHGV	HIV- I RT 476-484	4,31,47
(SEQ ID NO:380)		
LLGFVFTLTV	Influenza MP 59-68	4,39
(SEQ ID NO:381)		
LLFGYPVYVV	HTLV-1 tax 11-19	40
(SEQ ID NO:382)		
GLSPTVWLSV	HBV sAg 348-357	48
(SEQ ID NO:383)		
WLSLLVPFV	HBV sAg 335-343	49,50.51
(SEQ ID NO:384)		
FLPSDFFPSV	HBV cAg 18-27	52

(SEQ ID NO:385)		
C L G 0 L L T M V	EBV LMP-2 426-434	48
(SEQ ID NO:386)		
FLAGNSAYEYV	HCMV gp 618-628B	53
(SEQ ID NO:387)		
KLGEFYNQMM	Influenza BNP 85-94	54
(SEQ ID NO:388)		
KLVALGINAV	HCV-1 NS3 400-409	55
(SEQ ID NO:389)		
DLMGYIPLV	HCV MP 17-25	56
(SEQ ID NO:390)		
RLVTLKDIV	HPV 11 EZ 4-12	34,35
(SEQ ID NO:391)		
AFHIIVAREL	HIV- I nef 190-198	63
(SEQ ID NO:392)		
KAGEFYNQMM	Influenza BNP 85-94	65
(SEQ ID NO:395)		
NIAEGLRAL	EBNA-1 480-488	66
(SEQ ID NO:396)		
NLRRGTALA	EBNA-1 519-527	66
(SEQ ID NO:397)		
ALAIPQCRL	EBNA-1 525-533	66
(SEQ ID NO:398)		
VLKDAIKDL	EBNA-1 575-582	66
(SEQ ID NO:399)		
FMVFLQTHI	EBNA-1 562-570	66
(SEQ ID NO:400)		
HLIVDTDSL	EBNA-2 15-23	66
(SEQ ID NO:401)		
SLGNPSLSV	EBNA-2 22-30	66
(SEQ ID NO:402)		
PLASAMRML	EBNA-2 126-134	66
(SEQ ID NO:403)		
RMLWMANYI	EBNA-2 132-140	66

(SEQ ID NO:404)		
MLWMANYIV	EBNA-2 133-141	66
(SEQ ID NO:405)		
ILPQGPQTA	EBNA-2 151-159	66
(SEQ ID NO:406)		
PLRPTAPTTI	EBNA-2 171-179	66
(SEQ ID NO:407)		
PLPPATLTV	EBNA-2 205-213	66
(SEQ ID NO:408)		
R M H L P V L H V	EBNA-2 246-254	66
(SEQ ID NO:409)		
PMPLPPSQL	EBNA-2 287-295	66
(SEQ ID NO:410)		
QLPPPAAPA	EBNA-2 294-302	66
(SEQ ID NO:411)		
SMPELSPVL	EBNA-2 381-389	66
(SEQ ID NO:412)		
DLDESWDYl	EBNA-2 453-461	66
(SEQ ID NO:413)		
P L P C V L W P VV	BZLFl 43-51	66
(SEQ ID NO:414)		
SLEECDSEL	BZLFl 167-175	66
(SEQ ID NO:415)		
EIKRYKNRV	BZLFI 176-184	66
(SEQ ID NO:416)		
QLLQFIYREV	BZLF1 195-203	66
(SEQ ID NO:417)		
LLQHYREVA	BZLFI 196-204	66
(SEQ ID NO:418)		
LLKQMCPSL	BZLFI 217-225	66
(SEQ ID NO:419)		
SIIPRTPDV	BZLFI 229-237	66
(SEQ ID NO:420)		
AIMDKNIIL	Influenza A NS1 122-130	67

(SEQ ID NO:421)		
IMDKNIILKA	Influenza A NS1 123-132	67
(SEQ ID NO:422)		
LLALLSCLTV	HCV MP 63-72	69
(SEQ ID NO:423)		
ILHTPGCV	HCV MP 105-112	69
(SEQ ID NO:424)		
QLRRHIDLLV	HCV env E 66-75	69
(SEQ ID NO:425)		
DLCGSVFLV	HCV env E 88-96	69
(SEQ ID NO:426)		
SMVGNWAKV	HCV env E 172-180	69
(SEQ ID NO:427)		
HLHQNIVDV	HCV NSI 308-316	69
(SEQ ID NO:428)		
FLLLADARV	HCV NSI 340-348	69
(SEQ ID NO:429)		
GLRDLAVAVEPVV	HCV NS2 234-246	69
(SEQ ID NO:430)		
SLLAPGAKQNV	HCV NS1 18-28	69
(SEQ ID NO:431)		
LLAPGAKQNV	HCV NS1 19-28	69
(SEQ ID NO:432)		
FLLSLGIHL	HBV pol 575-583	70
(SEQ ID NO:433)		
SLYADSPSV	HBV pol 816-824	70
(SEQ ID NO:434)		
GLSRYVARL	HBV POL 455-463	70
(SEQ ID NO:435)		
KLTPLCVTL	HIV- I gp 160 120-128	72
(SEQ ID NO:438)		
SLLNATDIAV	HIV- I GP 160 814-823	72
(SEQ ID NO:439)		
LLFNILGGWV	HCV NS4 192-201	74

(SEQ ID NO:441)		
LLVPFVQWFW	HBV env 338-347	74
(SEQ ID NO:442)		
ALMPLYACI	HBV pol 642-650	74
(SEQ ID NO:443)		
YLVAYQATV	HCV NS3 579-587	74
(SEQ ID NO:444)		
YLLPRRGPRL	HCV core protein 34-43	74
(SEQ ID NO:446)		
LLPIFFCLWV	HBV env 378-387	74
(SEQ ID NO:447)		
YMDDVVLGA	HBV Pol 538-546	74
(SEQ ID NO:448)		
GTLGIVCPI	HPV16 E7 85-93	74
(SEQ ID NO:449)		
LLALLSCLTI	HCV MP 63-72	74
(SEQ ID NO:450)		
MLDLQPETT	HPV 16 E7 12-20	74
(SEQ ID NO:451)		
SLMAFTAAV	HCV NS4 174-182	75
(SEQ ID NO:452)		
CINGVCWTV	HCV NS3 67-75	75
(SEQ ID NO:453)		
L L C P A G H A V	HCV NS3 163-171	54
(SEQ ID NO:455)		
ILDSFDPLV	HCV NSS 239-247	54
(SEQ ID NO:456)		
LLLCLIFLL	HBV env 250-258	79
(SEQ ID NO:457)		
LIDYQGMLPV	HBV env 260-269	79
(SEQ ID NO:458)		
SIVSPFIPLL	HBV env 370-379	79
(SEQ ID NO:459)		
FLLTRILTI	HBV env 183-191	80

(SEQ ID NO:460)		
ILAGYGAGV	HCV NS S4A 236-244	82
(SEQ ID NO:463)		
GLQDCTMLV	HCV NS5 714-722	82
(SEQ ID NO:464)		
TGAPVTYSTY	HCV NS3 281-290	83
(SEQ ID NO:465)		
VIYQYMDDLV	HIV-1RT 179-187	84
(SEQ ID NO:466)		
(SEQ ID NO:468)		
GIGIGVLAA	HSV- I gp C 480-488	86
(SEQ ID NO:472)		
GAGIGVAVL	HSV-2 gp C 446-454	86
(SEQ ID NO:473)		
IAGIGILAI	Pseudorabies gpGIN 455-463	86
(SEQ ID NO:474)		
SLYNTVATL	HIV- I gag p 17 77-85	99
(SEQ ID NO:484)		
RGPGRAFVTI	HIV- I gp 160 315-329	90
(SEQ ID NO:486)		
HMWNFISGI	HCV NS4A 149-157	91
(SEQ ID NO:487)		
NLVPMVATVQ	HCMV pp65 495-504	92
(SEQ ID NO:488)		
GLHCYEQLV	HPV 6b E7 21-30	93
(SEQ ID NO:489)		
PLKQHFQIV	HPV 6b E7 47-55	93
(SEQ ID NO:490)		
LLDFVRFMGV	EBNA-6 284-293	95
(SEQ ID NO:491)		
AIMEKNIML	Influenza Alaska NS 1 122-130	67
(SEQ ID NO:492)		
YMLDLQPETT	HPV 16 E7 11-20*	97
(SEQ ID NO:495)		

LLMGTLGIV	HPV16 E7 82-90**	97
(SEQ ID NO:496)		
TLGIVCPI	HPV 16 E7 86-93	97
(SEQ ID NO:497)		
TLTSCNTSV	HIV-1 gp120 197-205	98
(SEQ ID NO:498)		
KLPQLCTEL	HPV 16 E6 18-26	97
(SEQ ID NO:499)		
YVNVNMGLK*	HBV cAg 88-96	116
(SEQ ID NO:337)		

* Ref numbers refer to the references listed in Han-Georg Rammensee, Jutta Bachmann, and Stefan Stevanovic entitled "MHC Ligands and Peptide Motifs," Springer-Verlag, Germany, 1997 Landes Bioscience, Austin, Texas); which is incorporated herein by reference in its entirety for any purpose, including for example, epitope prediction technique, epitope, antigen, microbe or cell type.

Table 7

Listing of Various Viruses, Viral Antigens, and Epitopes

HIV	**herpes simplex virus (HSV),**	**HBV**	**HCV**	**human papilloma virus (HPV)**	**cytomegalovirus (CMV)**
Antigens: Env, Gag, Nef and Pol Tat proteins, including gp120, gp160, gp41, p24gag and p55gag envelope proteins, derived from HIV such as, including members of the various	(HSV) types 1 and 2, such as HSV-1 and HSV-2 glycoproteins gB, gD and gH	HBc-IgG Ag HBV core IgG Ag HBc-IgG antigen Hepatitis B e antigen (HBeAg)			CMV gB and gH

genetic subtypes of HIV isolates HIV.sub.IIIb, HIV.sub.SF2, HIV.sub.LAV, HIV.sub.LAI, HIV.sub.MN, HIV-1.sub.CM235, HIV-1.sub.US4, HIV-2; proteins derived from simian immunodeficiency virus (SIV)					
Epitopes: HIV-1 RT 476-484		HBV pre-S protein 141-149			
Respiratory syncytial virus (RSV)	**Epstein Barr virus (EBV)**	**measles virus**	**human T-cell leukemia virus (HTLV)**	**Ebola virus**	**influenza virus**
				EBO-Z viral antigens EBO-S and EBO-Z virus antigens	Influenza A PB1 591-599 Influenza A NP 44-52 Influenza MP 58-66 Influenza MP 59-68

[0068] The following examples are for illustrative purposes only and are not intended to limit the scope of the various embodiments in any way.

Example 1. Immunogenic Compositions (*e.g.*, Viral Vaccines).

[0069] Six groups (n=6) of HLA-A2 transgenic mice are injected with 25 ug of plasmid vector bilaterally in the inguinal lymph nodes, according to the following schedule: day 0, 3, 14 and 17. The vector encodes three A2 restricted epitopes from HIV *gag* (SLYNTVATL (SEQ ID NO:1), VLAEAMSQV (SEQ ID NO:2), MTNNPPIPV (SEQ ID NO:3)), two from *pol* (KLVGKLNWA (SEQ ID NO:4), ILKEPVHGV (SEQ ID NO:5)) and one from *env* (KLTPLCVTL (SEQ ID NO:6)). Two weeks after the last cycle of entrainment, mice are injected with mixtures encompassing all of these five peptides (5ug/peptide/node bilaterally three days apart). In parallel, five groups of mice are injected with individual peptides (5ug/peptide/node bilaterally three days apart). Seven days later the mice are bled and response is assessed by tetramer staining against each peptide. Afterwards, half of the mice are challenged with recombinant *Vaccinia* viruses expressing *env, gag* or *pol* (10^3 $TCID_{50}$/mouse) and at 7 days, the viral titer is measured in the ovaries by using a conventional plaque assay. The other half are sacrificed, the splenocytes are stimulated with peptides for 5 days and the cytotoxic activity is measured against target cells coated with peptides. As controls, mice are injected with plasmid or peptides alone. Mice entrained with plasmid and amplified with peptides show stronger immunity against all five peptides, by tetramer staining and cytotoxicity.

[0070] Thus, cytotoxic immunity can be generated in various cases, by the methodology described, without including epitopes that bind to MHC class II and thus have the possibility to interact with, activate and/or expand Th cells. It results then that cytotoxic immunity can be generated in absence of functional Th cells (conditions in which Th cells are affected by a pathologic process – such as resulting from HIV infection; or conditions that affect Th cell function indirectly, due to abnormalities of antigen presenting cells – caused by viruses such as HBV, HCV and EBV). Further, use of peptides corresponding to MHC class I–restricted epitopes in context of the methodology mentioned above, overcomes the need for antigen processing and thus may deal with situations mentioned above, in which the function of APC is diminished. In addition, bypassing the employment of Th cells in inducing a therapeutic response comprising CTL, can circumvent potential immunopathology mediated by expanded $CD4^+$ Th response in viral infections such as those caused by HSV.

[0071] More generally, in order to break tolerance, restore immune responsiveness or induce immunity against non-self antigens such as viral, bacterial, parasitic or microbial, subjects, such as mice, humans, or other mammals, are immunized with: vectors such as plasmids; viruses; peptide plus adjuvant (CpG, dsRNA, TLR mimics); recombinant protein plus an adjuvant (CpG, dsRNA, TLR mimics); killed microbes or purified antigens, such as cell wall components; and are boosted by intranodal injection with peptide (corresponding to a target epitope for which they were immunized) without adjuvant. The immune response measured before and after boost by tetramer staining and other methods shows a substantial increase in the magnitude of immune response. Such a strategy can be used to protect against infection or treat chronic infections caused by agents such as HBV, HCV, HPV, CMV, influenza virus, HIV, HTLV, RSV, etc. It should be noted that the above methodology and the other methodologies described elsewhere herein can be used to treat non-human animals, where avoidance or minimization of CD4+ cells is advantageous. For example the methods can be used to treat infections by viruses in felines and canines, avians such as for example, chickens and turkeys, bovines, equines, other livestock and farm animals, and any other animal. See Table 7, above.

Example 2. Induction of immune response to MHC class I –restricted epitopes by intranodal administration of peptides corresponding to such defined epitopes and adjuvant (synthetic dsRNA)

[0072] A*0201 transgenic mice (n=4/group) were immunized with the following known MHC class I restricted peptide epitopes: HBVc 18-27 (FLPSDFFPSD; SEQ ID NO:7), PSMA 730-739 (RQIYVAAFTV; SEQ ID NO:8), PRAME 300-309 (SLLQHLIGL; SEQ ID NO:9) or PRAME 425-433 (ALYVDSLFFL; SEQ ID NO:10) admixed with synthetic dsRNA (poly(IC), by direct inoculation into the inguinal lymph nodes using 12.5μg peptide + 12.5μg of adjuvant, in 25μl of PBS / each inguinal lymph node at day 0, 3, 14 and 17).

[0073] One week after the final administration, splenocytes were stimulated *ex vivo* with 10μg/ml of the same peptide in presence of 5U/ml of rIL-2 and tested in a standard cytotoxic assay, against: ^{51}Cr-labeled target cells (T2 cells) uncoated, coated with cognate peptide or negative control peptide, at various Effector:Target ratios (Figures 1-2);

or similarly labeled MCF-7 cells coated with PRAME 730-739, PRAME 425-433 or uncoated.

[0074] The radioactivity released in the supernatant over 4 hours was measured using a γ (gamma)-counter. The response was quantified as % lysis = (sample signal – background) / (maximal signal – background) x 100, where background represents radioactivity released by target cells alone when incubated in assay medium, and the maximal signal is the radioactivity released by target cells lysed with detergent.

Example 3. Specific activation in the $CD8^+$ lymphocyte subset

[0075] Splenocytes isolated from pSEM plasmid primed, Melan-A 26-35 (A27L; SEQ ID NO:11) peptide boosted HHD-1 transgenic mice were stimulated with a Melan-A specific tetramer reagent for 4hrs. A fuller description of pSEM can be found in U.S. Patent Publication No. 2003-0228634, published on December 11, 2003, and in U.S. Patent Publication No. 2005-0079152, published on April 14, 2005 each of which is incorporated herein by reference in its entirety. Cells were then washed and stained with a rat anti-mouse CD8 antibody for 30 minutes. Cells were washed, permeablized, and then stained intracellularly with anti-mouse-IFN-γ antibody for 30 minutes. Cells were washed, fixed and analyzed on a FACS Calibur flowcytometer. A gate (R1) was drawn around the total lymphocyte population (figure 4A) and the percentage of Melan-A antigen specific CTLs was determined by co-staining with CD8 (figure 4B). It is seen in figure 4C that only CD8 positive cells within the total lymphocyte population were activated following antigen stimulation with 15.7% of these cells capable of producing IFN-g compared to 0.2% of the CD8 negative fraction. This demonstrates that following Melan-A immunization, only cytotoxic T cells were activated upon additional antigen stimulation, reflecting the predominant effect of this immunization protocol on the $CD8^+$ population rather than the $CD4^{\pm}$ population.

[0076] The results showed in Figures 1-4, demonstrate successful induction of peptide specific cytotoxic immunity against target cells expressing MHC class I (known to be mediated by CTL – or cytotoxic lymphocytes that are MHC class I-restricted $CD8^+$ T cells), by intranodal administration of peptides corresponding to known MHC class I epitopes, together with poly IC.

[0077] The scientific literature is rich with descriptions of both antibody and CTL (class I MHC-restricted) epitopes from viral target proteins, including HIV, HSV, HBV, HCV, and EBV, which can be used in the various embodiments described herein. Advantageous choices of particular target proteins will be apparent to one of skill in the art pertaining to the individual viruses (or other pathogens). In some cases CTL or antibody epitopes, or their use, are described in the literature along with class II-MHC restricted epitopes. Indeed, inclusion of class II MHC-restricted epitopes in some cases in the literature is reported as being preferred or essential. Nonetheless, when such CTL or antibody epitopes are utilized in some preferred embodiments, preferably they can be used without inclusion of such class II MHC-restricted epitopes. Examples of CTL epitopes for HIV are disclosed in U.S. Patent No. 6,656,471; and in Wilson, C.C., et al., *J. Immunol.* 171:5611-5623, 2003. Many other epitopes are known by those of skill in the art.

[0078] The term "consists essentially of" as used herein means that the scope of what is included is limited to the specified materials or steps and those that do not materially affect the basic and novel characteristic(s) of the embodiment.

[0079] In some embodiments, the numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth used to describe and claim certain embodiments of the invention are to be understood as being modified in some instances by the term "about." Accordingly, in some embodiments, the numerical parameters set forth in the written description and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by a particular embodiment. In some embodiments, the numerical parameters should be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of some embodiments of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as practicable. The numerical values presented in some embodiments of the invention may contain certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

[0080] In some embodiments, the numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth used to describe and claim certain embodiments of the invention are to be understood as being modified in some instances by the term "about." Accordingly, in some embodiments, the numerical

parameters set forth in the written description and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by a particular embodiment. In some embodiments, the numerical parameters should be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of some embodiments of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as practicable. The numerical values presented in some embodiments of the invention may contain certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

[0081] In some embodiments, the terms "a" and "an" and "the" and similar referents used in the context of describing a particular embodiment of the invention (especially in the context of certain of the following claims) may be construed to cover both the singular and the plural. The recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g. "such as") provided with respect to certain embodiments herein is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the invention.

[0082] Groupings of alternative elements or embodiments of the invention disclosed herein are not to be construed as limitations. Each group member may be referred to and claimed individually or in any combination with other members of the group or other elements found herein. It is anticipated that one or more members of a group may be included in, or deleted from, a group for reasons of convenience and/or patentability. When any such inclusion or deletion occurs, the specification is herein deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

[0083] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations on those

preferred embodiments will become apparent to those of ordinary skill in the art upon reading the foregoing description. It is contemplated that skilled artisans may employ such variations as appropriate, and the invention may be practiced otherwise than specifically described herein. Accordingly, many embodiments of this invention include all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

[0084] Furthermore, numerous references have been made to patents and printed publications throughout this specification. Each of the above cited references and printed publications are herein individually incorporated by reference in their entirety for any of the materials, substances, compositions of matter, methodologies, and devices described therein.

[0085] In closing, it is to be understood that the embodiments of the invention disclosed herein are illustrative of the principles of the present invention. Other modifications that may be employed may be within the scope of the invention. Thus, by way of example, but not of limitation, alternative configurations of the present invention may be utilized in accordance with the teachings herein. Accordingly, the present invention is not limited to that precisely as shown and described.

What is claimed:

1. A method of immunization, the method comprising the steps of:

delivering to a lymphatic system of a mammal a composition comprising an immunogen, the immunogen comprising a class I MHC-restricted epitope or a B cell epitope, wherein the immunogen does not comprise an effective class II MHC-restricted epitope; and

administering an immunopotentiator to the mammal

such that an epitope-specific immune response is induced without substantial activation or expansion of $CD4^+$ T cells.

2. The method of claim 1 where in the epitope is an HIV epitope.

3. The method of claim 1 wherein the immunogen and the immunopotentiator are co-administered to the lymphatic system.

4. The method of claim 1 wherein the composition comprises a first immunogen comprising a class I MHC-restricted epitope and a second immunogen comprising a B cell epitope.

5. The method of claim 4 wherein the first immunogen and the second immunogen are the same.

6. The method of claim 1 comprising

co-administering a first immunogen comprising or encoding the class I MHC-restricted epitope with the immunopotentiator, and

subsequently delivering a second immunogen comprising the epitope, in the form of an epitopic peptide, to the lymphatic system of the mammal.

7. The method of claim 6 wherein the interval between the administering step and the delivering step is at least about seven days.

8. The method of claim 6 wherein the first immunogen comprises a nucleic acid encoding the epitope.

9. The method of claim 6 wherein the immunopotentiator comprises a DNA molecule comprising a CpG sequence.

10. The method of claim 8 wherein the nucleic acid comprises a DNA molecule comprising a CpG sequence which constitutes the immunopotentiator.

11. The method of claim 6 wherein the immunopotentiator comprises dsRNA.

12. The method of claim 6 wherein the first immunogen comprises a polypeptide.

13. The method of claim 1 wherein delivery to the lymphatic system comprises delivery to a lymph node or lymph vessel.

14. A method of immunization comprising:

a step for potentiating an immune response,

a step for exposing the lymphatic system to a class I MHC-restricted epitope or a B cell epitope,

wherein an epitope-specific immune response is induced without substantial activation or expansion of $CD4^+$ T cells.

15. A method of immunization comprising:

delivering to a mammal a first composition comprising a first immunogen, the first immunogen comprising or encoding at least a portion of a first antigen; and subsequently

administering a second composition comprising an epitopic peptide directly to the lymphatic system of the mammal, wherein the peptide corresponds to a class I MHC-restricted epitope of said first antigen, wherein said second composition is not the same as the first composition such that an epitope-specific immune response is amplified without substantial activation or expansion of $CD4^+$ T cells.

16. A method of generating an immune response against a disease-related antigen in which it is advantageous to minimize the expansion of $CD4^+$ lymphocytes, comprising:

delivering to an animal a first immunogen and an immunopotentiator, the first immunogen comprising or encoding at least a first portion of a first antigen, wherein said at least a portion of a first antigen does not comprise a class II MHC restricted epitope for an MHC expressed by said animal; and

administering subsequent to said delivering step an epitopic peptide directly to a lymphatic system of the animal, wherein the peptide corresponds to a class I MHC-restricted epitope of said first antigen, wherein said epitopic peptide is not the same as the first immunogen.

17. The method of Claim 16, wherein the disease is caused by HIV.

18. The method of Claim 16, wherein the disease is caused by a virus selected from the group consisting of HSV, HBV, HCV, EBV, HPV, CMV, influenza virus, HTLV, RSV, EBV, measles virus, and Ebola virus.

19. The method of Claim 16, wherein the animal is a human.

20. The method of Claim 16, wherein the animal is a non-human animal.

21. The method of Claim 20, wherein said non-human animal is a mammal.

22. The method of Claim 16, wherein said first immunogen and said immunopotentiator are delivered to a lymphatic system of the animal.

23. The method of Claim 22, wherein said first immunogen and said immunopotentiator are delivered to a lymph node.

24. The method of Claim 16, wherein said epitopic peptide is delivered to a lymph node.

25. The method of Claim 16, wherein said first immunogen and said immunopotentiator are delivered to a same location on or in said animal.

26. The method of Claim 16, wherein said first immunogen and said immunopotentiator are delivered simultaneously.

27. The method of Claim 16, wherein said first immunogen and said immunopotentiator are delivered on the same day.

28. The method of Claim 16, wherein said first immunogen and said immunopotentiator are delivered as part of a same composition.

29. The method of Claim 16, wherein said at least a portion of a first antigen comprises a whole antigen.

30. The method of Claim 16, wherein said at least a portion of a first antigen comprises less than the full-length of whole antigen.

31. The method of Claim 30, wherein said at least a portion of a first antigen comprises a contiguous fragment of less than 80%, 70%, 60%., 50, %, 40%, 30%, 20% or 10% of the whole antigen.

32. The method of Claim 16, wherein said first immunogen encodes said at least a portion of a first antigen and comprises an immunostimulatory sequence that serves as said immunopotentiator.

33. The method of Claim 31, wherein said first immunogen encodes one or more epitopes, wherein the one or more epitopes are class I restricted T cell epitopes or B cell epitopes.

34. The method of Claim 33, wherein said administering step is performed about 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 25, 30 days or more after the delivering step.

35. The method of Claim 16, wherein said at least a first portion of a first antigen does not comprise or encode any MHC class II restricted epitope for the species of said animal or does not comprise or encode any human class II restricted epitope.

36. The method of Claim 16, wherein said first immunogen further comprises or encodes at least a second portion of said first antigen, wherein said at least a second portion of said first antigen does not comprise a class II MHC restricted epitope for an MHC expressed by said animal.

37. The method of Claim 36, wherein said first immunogen encodes said at least a first portion a first antigen and said at least a second portion of said first antigen.

38. The method of Claim 36, wherein said first immunogen further comprises or encodes one or more additional portions of said first antigen, wherein said one or more additional portions of said first antigen do not comprise a class II MHC restricted epitope for an MHC expressed by said animal.

39. The method of Claim 16, wherein said first immunogen further comprises or encodes at least a first portion of a second antigen.

40. The method of Claim 16, wherein said delivering step further comprises delivering a second immunogen comprising or encoding at least a first portion of a second antigen, wherein said at least a first portion of a second antigen does not comprise a class II MHC restricted epitope for an MHC expressed by said animal.

41. The method of Claim 16, further comprising detecting or obtaining an epitope-specific immune response without substantial activation or expansion of $CD4^+$ T cells.

42. A method of generating an immune response against an HIV infection, comprising:

delivering to an animal a composition comprising a nucleic acid encoding first immunogen and an immunopotentiator, the nucleic acid encoding at least a first portion of a first HIV antigen, wherein said at least a portion of a first antigen does

not comprise a class II MHC restricted epitope for an MHC expressed by said animal; and

administering subsequent to said delivering step an epitopic peptide directly to a lymphatic system of the animal, wherein the peptide corresponds to a class I MHC-restricted epitope of said at least a first portion of a first HIV antigen, wherein said epitopic peptide is not the same as the first immunogen.

43. The method of Claim 42, wherein said first HIV antigen is selected from the group consisting of gag, pol, env, tat, gp120, gp160, gp41, nef, gag p, gp, gag p24, and rt.

44. The method of Claim 42, wherein said nucleic acid encodes one or more of SEQ ID NOs:1-6.

45. A method of generating an immune response against a cell infected by an HIV, comprising:

delivering to patient a composition comprising a nucleic acid encoding one or more of SEQ ID NOs:1-6 and an adjuvant, the nucleic acid encoding at least a first portion of a first HIV antigen, wherein said at least a portion of a first antigen does not comprise a class II MHC restricted epitope for an MHC expressed by said patient, wherein said adjuvant is a CpG, a dsRNA poly IC, or a TLR mimic; and

administering one or more epitopic peptides directly to a lymph node of the patient, wherein the peptide is one that was encoded by said nucleic acid or is an analog thereof.

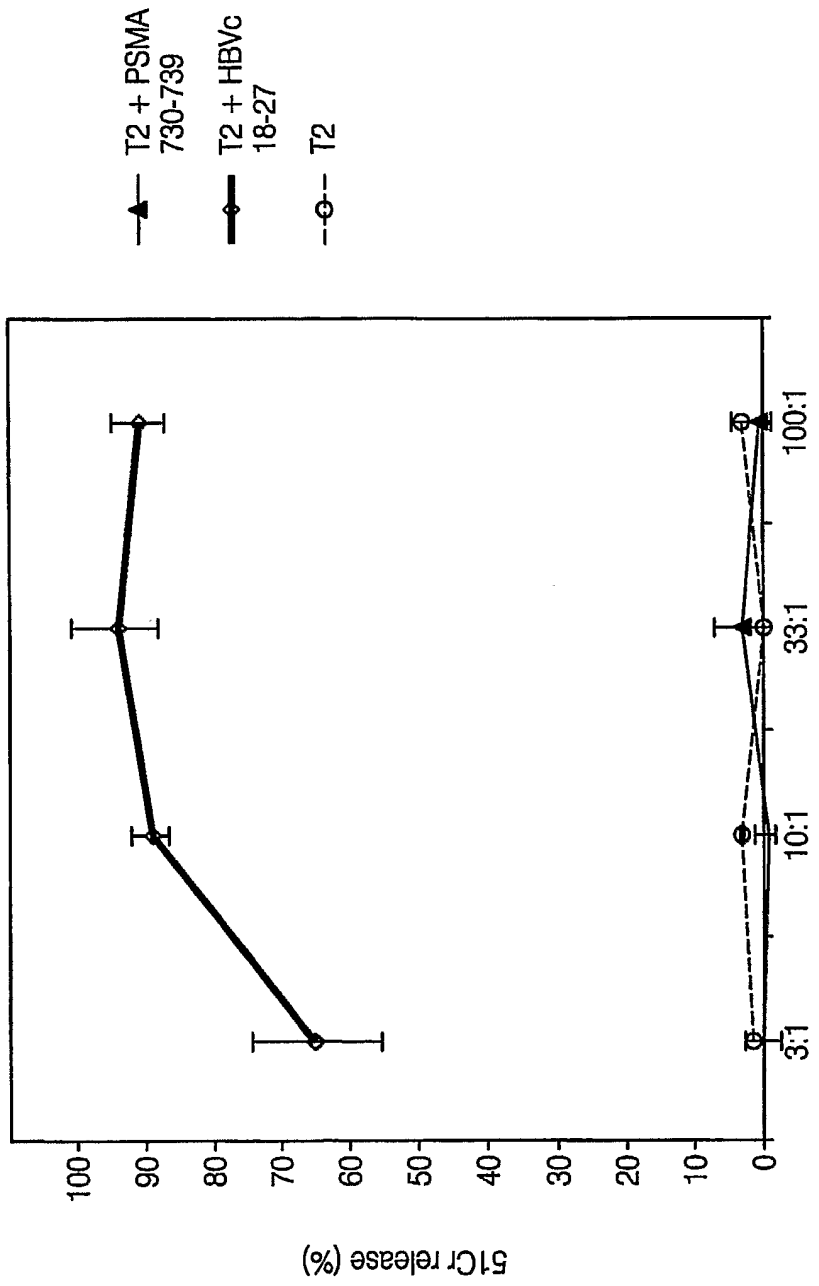
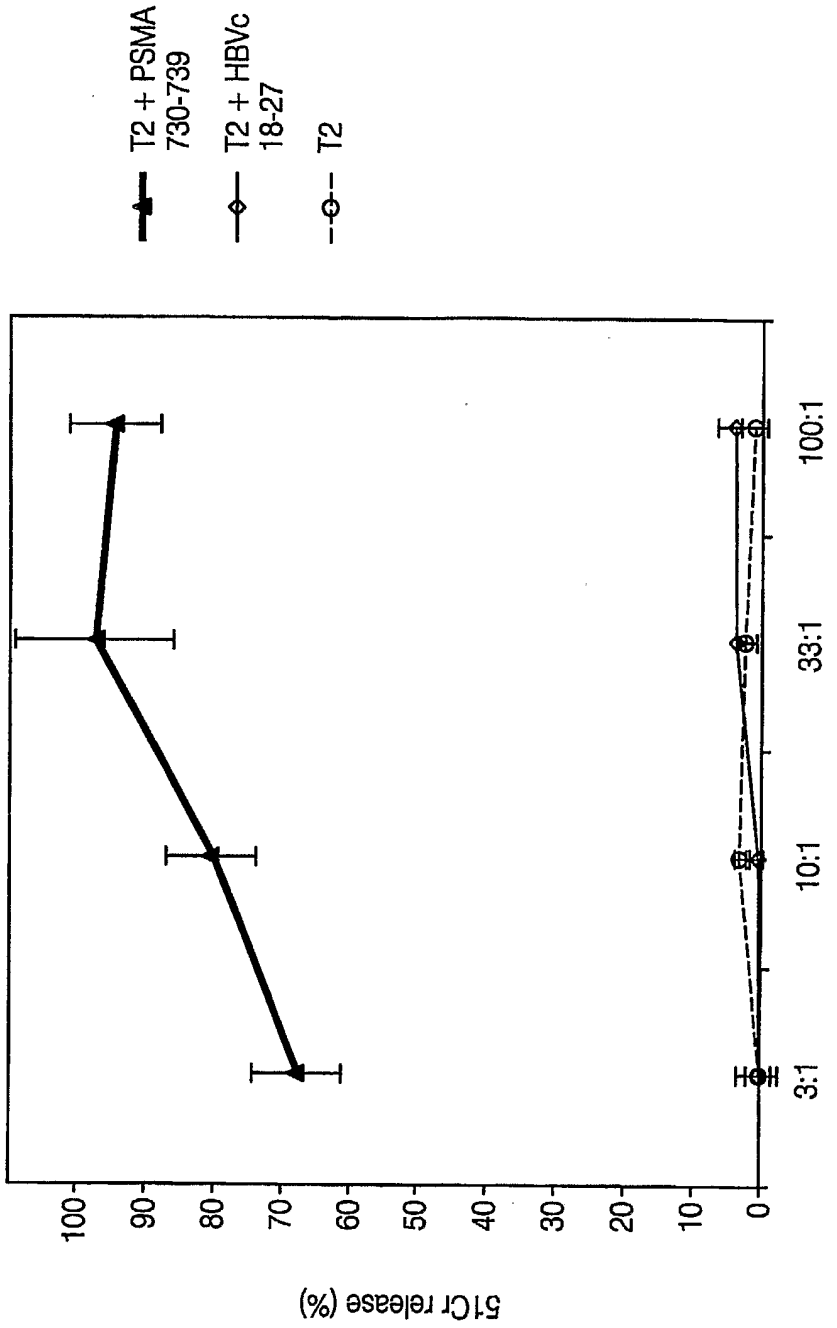

T2 + PSMA 730-739
T2 + HBVc 18-27
T2
51Cr release (%)
100
90
80
70
60
50
40
30
20
10
0
3:1
10:1
33:1
100:1
E:T

FIG. 1


T2 + PSMA 730-739
T2 + HBVc 18-27
T2
51Cr release (%)
100
90
80
70
60
50
40
30
20
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3:1
10:1
33:1
100:1
E:T

FIG. 2

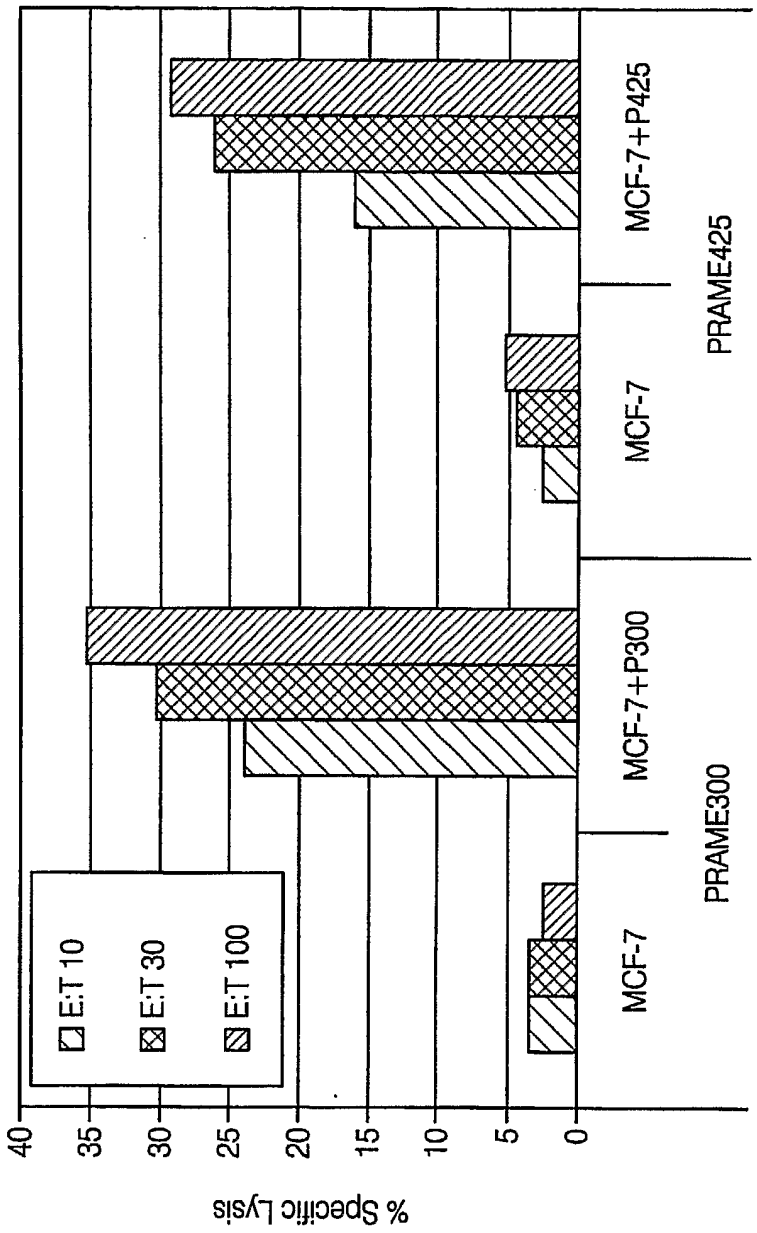
% Specific Lysis
40
35
30
25
20
15
10
5
0
E:T 10
E:T 30
E:T 100
MCF-7
MCF-7+P300
PRAME300
MCF-7
MCF-7+P425
PRAME425

FIG. 3

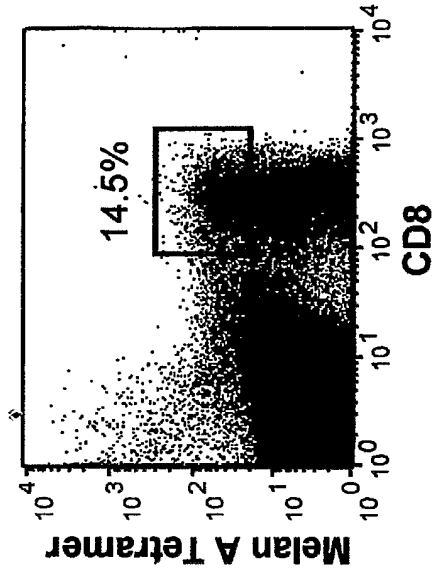

FIG. 4B

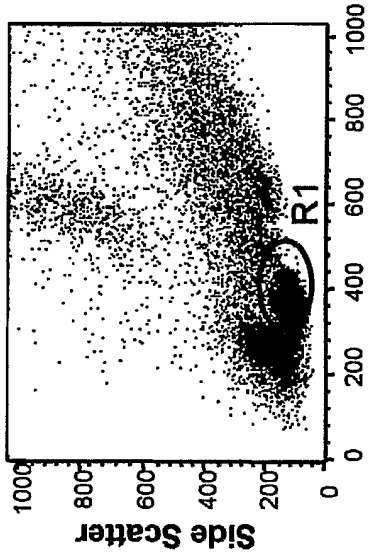

FIG. 4A

FIG. 4C