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(71) Applicants (for all designated States except US):

BIOVAXIM LIMITED [GB/GB]; Finsgate 5-7 Cran-
wood Street, London EC1 V9EE (GB). **UNIVERSITE**
PARIS DESCARTES [FR/FR]; 12, rue de l'Ecole de Mé-
decine, F-75006 PARIS (FR). **INSTITUT DE RECHER-**
CHE POUR LE DEVELOPPEMENT (IRD) [FR/FR];
Le Sextant - 44, bd de Dunkerque, CS 90009, F-13000
MARSEILLE (FR).

(72) Inventors; and

(75) **Inventors/Applicants** (for US only): **ANDRIEU, Jean-**
Marie [FR/FR]; 95, boulevard Saint Michel, F-75005
PARIS (FR). **LU, Louis** [FR/CN]; Room 3201, Building
13, Dong Hu Wan, 8 Li Ze Xi Jie, Wang Jing Area, Chaoy-
ang District, BEIJING 100102 (CN).

(74) **Agent: FAIVRE PETIT, Frédérique**; Cabinet Regim-
beau, 20, rue de Chazelles, F-75847 Paris Cedex 17 (FR).

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(54) **Title:** THERAPEUTIC VACCINE COMPOSITIONS INDUCING TOLERANCE TO HIV FOR TREATING HIV INFECTIONS IN HUMANS

(57) **Abstract:** The present invention is related to a therapeutic mucosal or intradermal or intraepithelial vaccine composition for use in a method for treating an infection by a HIV virus in a human in need thereof, comprising: - an antigen made of inactivated auto-
logous HIV virus; and - a tolerogenic adjuvant which, when combined to said antigen in said therapeutic vaccine composition, induces and, preferably, maintains immunotolerance to said HIV virus in said human, thereby treating said infection in said human. More precisely, the therapeutic vaccine compositions of the present invention produce a virus-specific non-cytotoxic MHC-Ib/E-restricted suppressive CD8⁺ T cell- induced immunotolerance ("Ts" immunotolerance for "T suppressive" immunotolerance) upon mucosal or intradermal or intraepithelial administration to humans. "Ts" immunotolerance is associated with the suppression of viral replication. The present invention also relates to a method for treating an infection by a HIV virus in a human in need thereof, comprising at least the step of mucosally or intradermally or intraepithelial administering a therapeutic vaccine composition as defined above to said human.



THERAPEUTIC VACCINE COMPOSITIONS INDUCING TOLERANCE TO HIV FOR TREATING HIV INFECTIONS IN HUMANS

The present invention relates to a novel therapy against HIV infections in humans.

More precisely, the present invention is directed to a therapeutic mucosal or intradermal or intraepithelial vaccine composition for use in a method for treating an infection by a HIV virus in a human in need thereof, comprising:

- an antigen made of inactivated autologous HIV virus; and
- a tolerogenic adjuvant which, when combined to said antigen in said therapeutic vaccine composition, induces and, preferably, maintains immunotolerance to said HIV virus in said human, thereby treating said infection in said human.

The present invention also relates to a method for treating an infection by a HIV virus in a human in need thereof, comprising at least the step of mucosally or intradermally or intraepithelially administering a therapeutic vaccine composition as defined above to said human.

Background to the invention

More than twenty five years after the discovery of human immunodeficiency virus (HIV), recent projections from the World Health Organization and the Joint United Nations Program on HIV/AIDS indicate that if the pandemic progresses at its current rate, there will be more than 30 million infections by 2011.

However, no efficient definitive and non toxic therapy exists so far. New therapeutic strategies are therefore awaited to combat the worldwide AIDS pandemic.

Most scientists involved in HIV pathogenesis and prevention feel that before testing HIV preventive vaccines or other biological compositions for preventing or treating HIV infection in human beings, it would be more constructive to test their counterparts in non-human primates (Morgan C, et al., 2008). The non-human primate of choice is the macaque rhesus and among macaques, it has now been conclusively shown that macaques of Chinese origin infected by the Simian Immunodeficiency Virus (SIV) 239 are the best model mimicking most of the clinical, virologic and immunologic aspects of the evolution of HIV infection in humans (Marcondes MC, et al. 2006; Stahl-Hennig C, et al. 2007; Chen S, et al. 2008).

Tolerance is the physiological capacity of the immune system to recognize antigens taken in through the mucosal system and to develop anergy generally

associated with other immunological modifications to a subsequent encounter with the same antigens. Tolerance had been frequently shown to elicit mucosal sIgA permitting antibody containment of mucosal antigens without stimulating the systemic immune compartment. TGF- β , a regulatory cytokine had also been sometime involved in the development of tolerance. The active suppression by CD25+ regulatory T cells had also been frequently suggested as a potential mechanism of mucosal tolerance (Faria and Weiner, 2005; Mestecky et al., 2007). However, none of these immunological modifications was observed in the vaccine composition-induced immunotolerance described here, which is principally characterized by the powerful activity of non-cytolytic, MHC-Ib/E-restricted CD8+ "regulatory" T cells which suppress the activation of HIV Gag- and Pol-derived epitope-presenting CD4+T cells, a type of immune reaction so far unrecognized and, more specifically, a completely new type of immune tolerance.

Summary of the invention

As detailed in the Examples below, the Inventors were able to show for the first time that, surprisingly, original vaccine compositions were capable of inducing a new type of SIV-specific tolerance. Moreover, when said SIV-specific tolerance was induced, the Inventors showed that it prevented SIV replication/dissemination and the subsequent establishment of the infection *in vivo*.

Indeed, the Inventors have surprisingly shown that upon administering a vaccine composition as disclosed here either mucosally (preferably orally) or by the intradermal or intraepithelial route, virus replication was significantly inhibited, or even abrogated or prevented.

Thus, a new type of virus-specific immunotolerance is unexpectedly induced by the vaccine compositions disclosed here, said immunotolerance being characterized by a strong non-cytotoxic, MHC (for "Major Histocompatibility Complex") -Ib/E-restricted CD8+T cell response suppressing the early activation of HIV antigen-presenting CD4+T cells. More precisely, the vaccine compositions disclosed here produce a HIV Gag and/or Pol antigen-specific MHC-Ib/E-restricted non-cytotoxic suppressive CD8+T cell-induced immunotolerance (also named herein "Ts" immunotolerance for "T suppressive" immunotolerance) upon mucosal or intradermal or intraepithelial administration to subjects.

In the light of these results, it is provided by the present invention a novel therapeutic vaccine composition achieving a "Ts" immunotolerance as defined above for treating a HIV infection in humans.

An object of the present invention is to provide a therapeutic mucosal (preferably oral) or intradermal or intraepithelial vaccine composition for use in a method for treating an infection by a HIV virus in a human in need thereof, comprising:

- an antigen made of inactivated autologous HIV virus; and
- a tolerogenic adjuvant which, when combined to said antigen in said therapeutic vaccine composition, induces and, preferably, maintains immunotolerance to said HIV virus in said human, thereby treating said infection in said human.

It is a further object of the present invention to provide a therapeutic tolerogenic vaccine composition as described herein, for use as a medicament.

Another object of the present invention is to provide a method for treating an infection by a HIV virus in a human in need thereof, comprising at least the step of mucosally or intradermally or intraepithelially administering an effective amount of a therapeutic vaccine composition as defined above to said human.

Yet another object of the present invention is to provide products containing:

- an appropriate adjuvant, that is to say a tolerogenic adjuvant as defined above; and
 - an antigen made of inactivated autologous HIV virus as defined above,
- as a combined therapeutic tolerogenic vaccine composition for simultaneous, separate or sequential use in mucosally or intradermally or intraepithelially vaccinating a human in need thereof, thereby treating a HIV infection in said human.

Brief description of the drawings

The present invention is illustrated by the following figures to which reference is made in the non-limiting examples below.

Figure 1: Intravenous (i.v.) SIVmac239 challenge of rhesus macaques pretreated with an intravaginal iSIV/BCG.

Figure 2: Intrarectal (i.r.) SIVmac239 challenge of rhesus macaques pretreated with an intravaginal iSIV/BCG.

Figure 3: Repeated SIVmac239 challenges (3 times by i.v. and 2 times by i.r.) of rhesus macaques pretreated with an intravaginal iSIV/BCG.

Figure 4: Intravenous SIVmac239 challenge of rhesus macaques pretreated with an intravaginal iSIV/BCG plus an intradermal booster.

Figure 5: Intrarectal SIVmac239 challenge of rhesus macaques pretreated with an intravaginal iSIV/BCG plus an intradermal booster.

Figure 6: Intrarectal SIVmac239 challenge of rhesus macaques pretreated with an oral iSIV/BCG.

Figure 7: *In vitro* antiviral activity of CD8⁺ T cells obtained from rhesus macaques pretreated with an intravaginal iSIV/BCG.

Figure 8: *In vitro* antiviral activity of CD8⁺ T cells obtained from the 4 rhesus macaques pretreated with an oral iSIV/BCG.

Figure 9: SIV-specific suppression of CD4⁺ T-cell activation by autologous CD8⁺ T cells obtained from the 4 rhesus macaques pretreated with an oral iSIV/BCG.

Figure 10a: Anti-SIV IgG antibody titers in plasma samples taken from the rhesus macaques pretreated with iSIV/LP, iSIV or LP.

Figure 10b: SIV-specific T-cell proliferation in PBMC samples taken from the rhesus macaques pretreated with iSIV/LP, iSIV or LP.

Figure 10c: SIV-specific IFN-gamma-secreting T cells upon *in vitro* stimulation in the presence or the absence of CD8 or CD25 T cells.

Figure 10d: SIV-specific suppression of CD4⁺ T-cell activation by autologous CD8⁺ T cells obtained from the 8 rhesus macaques pretreated with an oral iSIV/LP as compared to animals pretreated with an oral LP (n = 4) or iSIV (n = 3).

Figure 10e: SIV-specific CD8⁺ T cells after 60 days following intragastric administration of an iSIV/LP preparation: cytotoxicity of AT-2 SIV-pulsed CD4⁺ T cells in the presence of CD8⁺ T cells or of K562 in the presence of human nature killer cells (hNK) (controls) with or without SEB and anti-CD3/CD28 stimulation.

Figure 11a: *In vitro* antiviral activity (in CD4 cells) of autologous CD8⁺ T cells obtained from the 8 rhesus macaques pretreated with an oral iSIV/LP as compared to animals pretreated with an oral LP (n = 4) or iSIV (n = 3).

Figure 11b: *In vitro* antiviral activity (in CD4 cells) of heterologous or allogenic CD8⁺ T cells obtained from 4 out of the 8 rhesus macaques 80 days after the treatment of an oral iSIV/LP.

Figure 11c-g: Anti-SIV activity of CD8⁺ T cells after 60 days following oral immunization in a delayed (c), insert (d), allogenic (e) culture system, in the presence of anti-MHC-Ia/ABC or anti-MHC-Ib/E antibodies (f), and in the CD8⁺ T cells depleted of TCR $\gamma\delta$ ⁺ or V β 8⁺ subset (g).

Figure 12a: Plasma viral load levels (SIV RNA copies per ml of plasma) following intrarectal and intravenous SIVmac239 challenges in the rhesus macaques pretreated with an oral iSIV/LP as compared to animals pretreated with an oral LP or iSIV.

Figure 12b: Cellular viral load levels (SIV DNA copies per million PBMCs) following intrarectal and intravenous SIVmac239 challenges in the rhesus macaques pretreated with an oral iSIV/LP as compared to animals pretreated with an oral LP or iSIV.

Figure 13: Depletion of peripheral blood and lymph node CD8⁺ T cells of the 8 iSIV/LP-treated macaques by infusion of the anti-CD8 antibody cMT807. a, Peripheral blood CD8⁺ T-cell counts before and after receiving three injections of cMT807; b, % of lymph node CD8⁺ T cells before and after receiving three injections of cMT807; c, Plasma viral load before and after receiving three injections of cMT807; d, PBMC DNA SIV load before after receiving three injections of cMT807; e, Lymph node SIV DNA load before and after receiving three injections of cMT807.

Figure 14: Plasma (a) and PBMC (b) viral loads following a third intrarectal challenge performed intrarectally with SIVB670 in 8 rhesus macaques immunized with an oral preparation made of iSIV and LP and 2 additional naïve monkeys.

Figure 15: *In vitro* and *in vivo* CD8⁺ T cell-mediated antiviral activity following intragastric immunization with iSIV and LP (iSIV/LP immunization No. 2). a, Anti-SIV activity (fold of viral suppression) of CD8⁺ T cells during 60-420 days post-immunization in 8 rhesus macaques that will be challenged intrarectally; b and c, Plasma and cellular viral loads following intrarectal SIVmac239 challenge of those 8 rhesus macaques immunized with an oral iSIV/LP and of 8 control monkeys treated with LP alone (n = 4) or iSIV (n = 4) alone.

Figure 16: SIV DNA and RNA loads in rectal mucosa intraepithelial lymphocytes (IPLs) (a-b), lamina propria cells (LPC) (c-d), and in pelvic lymph nodes (PLN) (e) post intrarectal challenge of SIVmac239 in 8 macaques (iSIV/LP immunization No. 2).

Detailed description of the invention

The present invention is directed to a therapeutic mucosal (preferably oral) or intradermal or intraepithelial vaccine composition for use in a method for treating an infection by a HIV virus in a human in need thereof, comprising:

- an antigen made of inactivated autologous HIV virus; and
- a tolerogenic adjuvant which, when combined to said antigen in said therapeutic vaccine composition, induces and, preferably, maintains immunotolerance to said HIV virus in said human, thereby treating said infection in said human.

Said appropriate adjuvant is thus herein designated as a "tolerogenic adjuvant" or a "tolerogenic carrier" or a "carrier of tolerance" or a "carrier of tolerization", these terms being synonymous.

Said antigen is hereinafter also called "a viral antigen" or "an immunogen" or "a viral immunogen".

Said vaccine composition is also referred to herein as a "therapeutic tolerogenic vaccine composition" or a "therapeutic tolerogenic vaccine" or a "therapeutic tolero-immunogenic composition", these terms being equivalent.

Reference can also be made here to the terms "tolerogenic vaccine composition" or "tolerogenic vaccine" or "tolero-immunogenic composition", these terms being equivalent, to more specifically deal with the vaccine composition that, as illustrated in the Examples below, is capable of inducing a new type of HIV- or SIV-specific immunotolerance upon mucosal or intradermal or intraepithelial administration to mammals (including humans), said immunotolerance being at least characterized by: 1) a strong non-cytotoxic, MHC (for "Major Histocompatibility Complex")-Ib/E-restricted CD8+T cell response suppressing the early activation of HIV or SIV antigen-presenting CD4+T cells; 2) an absence of proliferation of CD4+T cells and a lack of gamma interferon secretion by CD8+T cells upon HIV or SIV antigen stimulation; 3) a lack of production of systemic anti-HIV or anti-SIV IgM and IgG antibodies. In fact, the above-mentioned "therapeutic tolerogenic vaccine composition" can be regarded as a preferred embodiment of the "tolerogenic vaccine composition" illustrated in the Examples below and which has both therapeutic and prophylactic applications.

According to the present invention, a "human in need thereof" means a human in need of a treatment as provided here, that is to say a human to be treated because he/she is suffering from a HIV infection.

Due to the great variability in the HIV genome, which results from mutation, recombination, insertion and/or deletion, HIV has been classified in groups, subgroups,

types, subtypes and genotypes. There are two major HIV groups (HIV-1 and HIV-2) and many subgroups because the HIV genome mutates constantly. The major difference between the groups and subgroups is associated with the viral envelope. HIV-1 is classified into a main subgroup (M), said subgroup M being divided into nine subtypes (clades or subtypes) designed A through J (Hu et al., JAMA 275:210-216, 1996 ; Korber et al., Science 280:1868-1871, 1998), and a 10th outlier subgroup (O). Many other subgroups resulting from *in vivo* recombinations of the previous ones also exist (Papathanasopoulos MA, et al. Virus Genes 2003, 26:151-163).

Preferably, the HIV virus is HIV-1 or HIV-2. Yet preferably, it is HIV-1.

The antigen

In the therapeutic vaccine composition according to the present invention, the "antigen" or "immunogen" from viral origin is "autologous", which means that it derives from the HIV virus infecting the human to be treated. Thus, in practice, to prepare the therapeutic vaccine composition of the invention, the HIV virus is isolated from the human to be treated, more particularly from the CD4⁺ T cells of said human. The thus isolated HIV virus is cultured and inactivated (preferably at least inactivated twice). The thus obtained antigen is then associated with a tolerogenic adjuvant so as to obtain an appropriate therapeutic tolerogenic vaccine composition.

As explained below, it is essential for a HIV virus to be an antigen for use in the context of the present invention that it expresses at least HIV Gag and/or Pol.

Thus, "an antigen made of inactivated autologous HIV virus" according to the present invention is an antigen comprising or consisting of the HIV virus infecting the human to be treated and appropriately inactivated for a safe therapeutic administration to humans.

Alternatively, it can be a recombinant microorganism such as a recombinant virus (HIV or any other appropriate virus type) or a recombinant bacterium or a recombinant fungus comprising (preferably, expressing) Gag and/or Pol from the HIV virus infecting the human to be treated, and appropriately inactivated for a safe therapeutic administration to humans.

Instead of using a virus as the antigen, one may use an antigen selected from recombinant microorganisms (including recombinant virus, bacteria and fungi), virus particles, recombinant virus particles, virus-like particles, polymeric microparticles presenting on their surface viral proteins or peptides, and also viral proteins and viral

peptides, with the proviso that the antigen comprises or expresses at least Gag and/or Pol, preferably at least GAG, from the HIV virus infecting the human to be treated.

All the foregoing alternative embodiments are encompassed by the terms "an antigen made of inactivated autologous HIV virus".

It was suspected for a long time by the scientific community that the activation of CD4⁺ T cells, the principal target of both HIV-1 and SIV, contributed directly to viral replication (Andrieu and Lu, 1995; Korin and Zack, 1999). However, it was only recently that the interplay between CD4⁺ T cell activation and the successive steps of the SIV or HIV infectious process was clarified. In quiescent CD4⁺ T cells, virus penetration was followed within 2 hours post entry by the presentation at the plasma membrane of Gag and Pol protein-derived epitopes of incoming virions while Env and Nef proteins needed *de novo* synthesis (Sacha et al., 2007). However, the subsequent phases of the infectious process, i.e., reverse transcription followed by virus integration, developed very inefficiently in quiescent cells (Vatakis et al., 2009a and 2009b). In contrast, when CD4⁺T cells were activated before or within the 48 hours following the presentation of Gag and Pol epitopes at the plasma membrane, HIV/SIV reverse transcription and DNA integration were extremely active which allowed very efficient virus replication and release (Vatakis et al., 2009a and 2009b).

Hence, the Inventors postulated that specifically blocking *in vivo* the early development of HIV/SIV Gag or Pol-specific CD4⁺ T-cell activation after HIV/SIV exposure will result in the prevention of active viral replication.

Bearing this in mind, in order to induce the suppression of the activation of HIV Gag/Pol antigen-presenting CD4⁺ T cells, and in turn to prevent *in vivo* HIV replication and dissemination in virus-exposed humans, the therapeutic vaccine composition of the present invention is required to comprise an antigen derived from a HIV virus and comprising at least GAG and/or POL.

Alternatively or additionally, said antigen derived from a HIV virus may comprise one or more proteins encoded by GAG such as the capsid protein (p24) and the matrix protein (p17), and/or one or more proteins encoded by POL such as the integrase, the reverse transcriptase and the protease.

In particular, any other viral proteins selected in the group consisting of ENV, VIF, VPR, VPU for HIV-1, VPX for HIV-2, REV, NEF, TAT, and the like, are not essential

components of the antigen comprised in the therapeutic vaccine composition disclosed here. Anyone of these proteins, if present, are only optional components of the antigen to be used in the vaccine composition provided by the Inventors.

An effective amount of the viral antigen to be used in the context of the invention can easily be determined by the skilled person, using the common general knowledge and in the light of the Examples disclosed hereafter, in connection with SIV or HIV virus.

The tolerogenic adjuvant

As used herein, a "tolerogenic adjuvant" is an entity that, when administered by the mucosal or the intradermal or the intraepithelial route together with an appropriate viral antigen as defined above, will induce and will preferably maintain a state of immunotolerance to the antigen, thus enabling to treat a viral infection in humans.

Preferably, the equivalent terms "tolerogenic adjuvant", "tolerogenic carrier", "carrier of tolerance" or "carrier of tolerization" refer to an entity that is used in combination with a HIV virus-derived antigen in order to achieve a specific immunotolerance to the viral antigen, thereby treating an HIV infection in humans.

An "entity" is defined herein as a substance or a combination of substances. A "substance" can be chemical or biological, natural or synthetic; it may be, e.g., a compound, a molecule, a cell, or a bacterium.

Advantageously, the tolerogenic adjuvant is selected from:

- non-pathogenic bacteria, especially probiotics and commensal bacteria;
- attenuated pathogenic bacteria; and
- inactivated (optionally, also previously attenuated) pathogenic bacteria.

The tolerogenic adjuvant may be recombinant or not.

"Non-pathogenic bacteria" to be used as tolerogenic adjuvants in the context of the present invention do not generally induce any pathology in humans. This is the reason why they are Generally Recognized As Safe (GRAS). Of course, such bacteria have to be administrable to humans.

Preferred non-pathogenic bacteria to be used as tolerogenic adjuvants are commensal bacteria. Such bacteria are well-known to the skilled artisan. Non-limiting

examples include *Bacillus* sp. (e.g., *B. coagulans*), *Bifidobacterium animalis*, *Bifidobacterium breve*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Bifidobacterium bifidum*, *Bifidobacterium lactis*, *Escherichia coli*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus johnsonii*, *Lactobacillus plantarum*, *Lactobacillus reuteri*, *Lactobacillus rhamnosus*, *Lactobacillus brevis*, *Lactobacillus gasseri*, *Lactobacillus salivarius*, *Lactococcus lactis*, *Streptococcus thermophilus*, and the like.

A "commensal bacterium" for use as a tolerogenic adjuvant in the context of the present invention is advantageously a lactic acid bacterium or a bifidobacterium which is more particularly selected in the list above, including also combinations thereof. A preferred commensal bacterium is *Lactobacillus* sp., and more preferably *Lactobacillus plantarum*. The Examples reported below show for the first time that *Lactobacillus plantarum* is a tolerogenic adjuvant, leading to viral immunotolerance when administered together with a viral antigen.

Advantageously, a combination of non-pathogenic bacteria, such as two or more commensal bacteria, may be used as the tolerogenic adjuvant.

As used herein, the terms "pathogenic bacteria" refer to bacteria inducing pathologies in humans. Such bacteria are well known from the skilled person and include *inter alia* *Listeria* species (e.g., *Listeria monocytogenes*), *Corynebacterium* species, *Mycobacterium* species, *Rhococcus* species, *Eubacteria* species, *Bortadella* species and *Nocardia* species. Preferably, a pathogenic bacterium is selected among *Mycobacterium* species, and is more preferably *Mycobacterium bovis*.

As used herein, "attenuated pathogenic bacteria" are pathogenic bacteria which are less virulent compared to their wild-type counterpart because of one or several mutations or of one or more attenuation treatments (e.g., chemical treatment and/or successive passages on specific media). Such attenuated pathogenic bacteria are well known from the one of skill in the art. Non-limiting examples of attenuated pathogenic bacteria include attenuated *Salmonella typhimurium* and *Mycobacteria* with a preference for attenuated *Mycobacteria*. As an example of attenuated *Mycobacteria*, one can cite the "Bacille de Calmette Guerin", also known as "BCG", and, more especially, among others, the six widely used BCG strains - the evolutionarily early strain BCG Japanese, the two evolutionarily late strains in DU2 Group III (BCG Danish and Glaxo), and the three evolutionarily late strains in DU2 Group IV (BCG Connaught, Pasteur, and Tice). As another example of attenuated *Mycobacteria*, one can also cite

recombinant BCG such as the strain rBCG30 disclosed in HOFT *et al.* (2008), the recombinant BCG disclosed in WANG *et al.* (2008), and also the recombinant BCG disclosed in International patent applications WO 2005/111205 and WO 02/102409, and disclosed in patents US 7,122,195 and US 6,261,568.

Advantageously, instead of or additionally to being attenuated, pathogenic bacteria may be inactivated to be used as tolerogenic adjuvants in the context of the present invention, but attenuated pathogenic bacteria may also be used after having been inactivated.

"Inactivated pathogenic bacteria" are well known from the one of skill in the art. Methods of preparation of such inactivated pathogenic bacteria form part of the common general knowledge in the art. As an example of such methods, one can cite phage mediated lysis, chemical inactivation such as formalin treatment (see US 7,393,541), thermal inactivation, physical inactivation such as lyophilisation (e.g., Extended Freeze Drying) or U.V or gamma irradiation (see WO 2008/128065) or microwave exposure, and combinations thereof.

Preferably, said tolerogenic adjuvant is an attenuated derivative of pathogenic bacteria like BCG. The Examples reported below show for the first time that BCG is a tolerogenic adjuvant, leading to viral immunotolerance when administered together with a viral antigen.

According to a specific embodiment, the tolerogenic adjuvant is an attenuated derivative of pathogenic bacteria. As an example, said attenuated derivative of pathogenic bacteria corresponds to *Salmonella typhimurium* or *Mycobacteria* (e.g., BCG) which expresses or produces at least one HIV protein (preferably selected or derived from Gag and Pol proteins) as described previously. Alternatively, the tolerogenic adjuvant is an attenuated derivative of pathogenic bacteria which does not express any HIV protein.

An effective amount for a tolerogenic adjuvant can be easily determined by the skilled person and examples of such an effective amount are disclosed hereinafter.

The vaccine composition

It may be possible to administer the tolerogenic adjuvant and the antigen either simultaneously, or separately, or sequentially.

It is an object of the present invention to provide products containing:

- an appropriate adjuvant, that is to say a tolerogenic adjuvant as defined above; and
 - an antigen made of an inactivated autologous HIV virus as defined above,
- as a combined therapeutic tolerogenic vaccine composition for simultaneous, separate or sequential use in mucosally or intradermally or intraepithelially vaccinating a human in need thereof, thereby treating a HIV infection in said human.

The tolerogenic adjuvant and the antigen are two separate and distinct entities that are comprised into the therapeutic tolerogenic vaccine composition of the present invention. This means that said tolerogenic adjuvant and said antigen are present as distinct components in said composition.

More advantageously, the therapeutic tolerogenic vaccine composition of the invention does not comprise any oligonucleotide (e.g., CpG or dsRNA) as adjuvant.

The novel tolerance mechanism achieved by the tolerogenic vaccine composition

The expressions "tolerance", "immunological tolerance", "immunotolerance", "immunotolerance to a virus", "new type of virus-specific tolerance", "immunotolerance to viral antigens", "immunotolerance to viral immunogens", and "Ts" immunotolerance" are synonymous. Thereby, it is meant herein an actively-induced strong non-cytotoxic, MHC-Ib/E-restricted CD8+T cell response suppressing the early activation of HIV or SIV antigen-presenting CD4+T cells associated with an absence of proliferation of CD4+T cells. If the human or animal subject is not yet infected by a HIV or SIV virus, this can further be associated with a lack of significant gamma interferon secretion by CD8+T cells upon HIV or SIV antigen stimulation and/or a lack of significant production of systemic anti-HIV or anti-SIV IgM and IgG antibodies.

These CD8+ T cells are also called CD8+ "regulatory" T cells.

Also, TCR $\gamma\delta$ and V β 8 have been shown not to be involved in CD8+T cell suppression of viral replication, suggesting that TCR $\alpha\beta$ should play a central role in the recognition of MHC-Ib/E-peptide presentation on infected CD4+T cells.

By the terms "lack of significant gamma interferon secretion by CD8+T cells upon HIV or SIV antigen stimulation", it is meant herein that the level of gamma interferon secretion by CD8+T cells which can be observed upon HIV or SIV antigen stimulation

is zero or weak. A “weak” gamma interferon secretion by CD8+T cells upon HIV or SIV antigen stimulation is typically less than about 80 SFCs per 2×10^5 PBMCs.

By the terms “lack of significant production of systemic anti-HIV or anti-SIV IgM and IgG antibodies”, it is meant herein that the level of production of systemic anti-HIV or anti-SIV IgM and IgG antibodies which can be observed is zero or weak. A “weak” production of systemic anti-HIV or anti-SIV antibodies is typically a titer of anti-HIV or anti-SIV of about 300 or less.

A “usual immune response to an antigen derived from a virus” can be observed *inter alia* upon vaccination with a conventional preventive vaccine composition comprising an antigen and a standard or conventional adjuvant (*i.e.*, any form of physical, chemical or biological adjuvant aimed at stimulating and/or facilitating and/or increasing the immune response associated with the antigen, such as those described in the Chapter entitled “Adjuvants” in “Vaccines” by S. Plotkin et al). In a subject not yet infected by a virus, such a “usual immune response to an antigen” derived from this virus involves humoral, cellular, or both humoral and cellular immune responses, and is conventionally characterized by:

- (i) the proliferation of virus-specific CD4 cells upon specific *in vitro* stimulation; and/or
- (ii) the induction of a specific systemic humoral response via the production of systemic antibodies against viral antigenic proteins and/or peptides; and/or
- (iii) the induction of a specific cellular response associated with the production of gamma interferon by CD8 T cells, and/or
- (iv) the absence of non-cytotoxic CD8+T cell response suppressing the activation of viral antigen-presenting CD4+T cells.

By contrast, a tolerogenic vaccine comprising a viral antigen and a tolerogenic adjuvant, as illustrated in the Examples below, generates an “immunotolerance to a virus” and, more particularly, a ““Ts” immunotolerance”, which is characterized by, in a subject not yet infected by a virus:

- (i) the absence of proliferation of virus-specific CD4 cells upon specific *in vitro* stimulation; and

- (ii) the absence of any significant systemic humoral response, that is to say either no specific detectable systemic antibody response can be detected by classical clinical laboratory methods such as ELISA , or if systemic antibodies are detected, they are not protective against virus infection; and
- (iii) the absence of any cytotoxic CD8+ T cell response associated with the production of gamma interferon (e.g., detectable by ELISpot) upon adequate *in vitro* stimulation, or if a cytotoxic CD8 T cell response is detected, it is not protective against virus infection; and
- (iv) as an essential feature, the actively-induced strong non-cytotoxic, MHC-Ib/E-restricted CD8+T cell response suppressing the early activation of HIV Gag and/or Pol antigen-presenting CD4+T cells.

The "therapeutic tolerogenic vaccine composition" of the present invention is a pharmaceutical composition that, upon administration to a human in need thereof, is capable of treating an infection by a HIV virus in said human by inducing or producing, and preferably maintaining, immunotolerance to antigens made of inactivated autologous HIV virus as defined above. The immunotolerance mechanism that is achieved by the therapeutic tolerogenic vaccine composition in a HIV-infected human is mainly characterized by item (iv) above (i.e., the actively-induced strong non-cytotoxic, MHC-Ib/E-restricted CD8+T cell response suppressing the early activation of Gag and/or Pol antigen-presenting CD4+T cells).

Preferably, said therapeutic tolerogenic vaccine composition maintains a decreased or even undetectable plasma viral load after the withdrawal of antiviral therapy. Alternatively, it decreases and advantageously maintains the viral load preferably to undetectability in an HIV-infected human not under antiviral therapy.

A tolerogenic vaccine composition as illustrated in the Examples below comprises a tolerogenic adjuvant and a viral antigen. Actually, a tolerogenic adjuvant (also identified as a "tolerogenic carrier", a "carrier of tolerance or a "carrier of tolerization") combined to a viral antigen induces a state of virus-specific immunotolerance in a human, instead of eliciting a usual immune response as defined above. Thus, an antigen administered by the mucosal or the intradermal or the intraepithelial route, alone or associated with a standard adjuvant is generally capable of eliciting a usual immune response, excepting when it is associated to a tolerogenic adjuvant as in a tolerogenic vaccine composition. Under these specific circumstances,

the association tolerogenic adjuvant / antigen in the tolerogenic vaccine composition induces an immunotolerance as defined above. This means that immunotolerance can only be achieved upon administering, by the mucosal or the intradermal or the intraepithelial route, an appropriate combination of a tolerogenic adjuvant and a viral antigen. If one is administered to a human in the absence of the other, the human will not be "vaccinated".

The term "vaccination" refers to the action(s) (especially, administering the therapeutic tolerogenic vaccine composition of the present invention) that is(are) taken for treating an infection by a HIV virus in a human in need thereof. Actually, the tolerogenic vaccine composition of the invention is useful for inducing and, preferably, maintaining immunotolerance to antigens made of inactivated autologous HIV virus in a human that is to say, in other words, for vaccinating (or "tolerizing") and, in turn, treating said human. Thus, vaccinating a human using the tolerogenic vaccine of the present invention is regarded as a "tolerogenic vaccination" (or a "tolerization" or a "tolerisation"). Since said tolerogenic vaccination is, in the context of the present invention, for therapeutic purposes, it is a "therapeutic tolerogenic vaccination" or a "therapeutic tolerization" or a "therapeutic tolerisation"

If, after the mucosal or the intradermal or the intraepithelial administration of the therapeutic tolerogenic vaccine composition of the invention (i.e., after treatment of a human in need thereof by therapeutic tolerogenic vaccination), immunotolerance is successfully induced in said human, said human is considered as being "vaccinated" (or "tolerized" or "tolerant") and, in turn, "treated".

The response of a "vaccinated" human to the treatment may be monitored by evaluating *ex vivo* the viral RNA load of said "vaccinated" human to an *in vitro* viral infectious challenge. Therapeutic vaccination (and, thus, treatment) can thus be predicted as successful if the viral RNA load of a "vaccinated" human to an *in vitro* viral infectious challenge is reduced by at least about 50%, more preferably by at least about 70%, yet more preferably by at least about 75% or 80% or 85% or 90% or 95%, or even more, relative to the plasma viral RNA load of said human prior to vaccination.

As yet mentioned, the immunotolerance (also called "Ts" immunotolerance) induced or achieved upon administering the therapeutic tolerogenic vaccine composition provided by the Inventors is preferably at least characterized by a strong non-cytotoxic, MHC-Ib/E-restricted CD8+T cell response suppressing the early activation of HIV or SIV antigen-presenting CD4+T cells.

Advantageously, "Ts" immunotolerance can be determined in said human by *in vitro* detecting the presence and activity of virus-specific non-cytotoxic (in particular, non-gamma interferon-producing) MHC-Ib/E-restricted suppressive CD8 cells. Such detection may be performed by standard *in vitro* techniques, such as those described in the Examples below.

The administration route of the vaccine composition

For example, when the tolerogenic adjuvant is a bacterium, it may be administered orally (e.g., as an oral drug or a food supplement), and the antigen is administered mucosally (preferentially orally) or intradermally or intraepithelially.

Of course, appropriate pharmaceutical vehicles may be used in order to ensure a suitable delivery of each to the expected site (e.g., a mucosal surface). The time and dose for administering each of the tolerogenic adjuvant and the antigen will be easily adapted by the skilled artisan.

Since the therapeutic vaccine composition according to the present invention is for mucosal or intradermal or intraepithelial administration, it is formulated for such an administration. In particular, the therapeutic vaccine composition may further comprise one or more appropriate pharmaceutical vehicles (or supports) for mucosal or intradermal or intraepithelial delivery of said antigen and of said appropriate adjuvant.

Preferably, a "mucosal delivery" is herein selected from nasal, oral, sub-lingual, tracheal, pharyngeal, bronchial, esophageal, gastric, duodenal, intestinal, rectal, preputial and vaginal deliveries. A "mucosal delivery" is a delivery to a mucosal surface, such as nasal, oral, sub-lingual, tracheal, bronchial, pharyngeal, esophageal, gastric, and mucosae of the duodenum, small and large intestines, including the rectum, as well as preputial and vaginal mucosae. In the present context, the mucosal surface also includes the external surface of the eye, i.e., the mucosa of and that surrounding the eye. Yet preferably, the mucosal surface refers to vaginal and digestive mucosa, and more preferably to digestive mucosa.

Thus, the therapeutic tolerogenic vaccine composition may also comprise one or more pharmaceutical vehicles depending on the route of administration. Those of ordinary skill in the pharmaceutical art are familiar with, or can readily ascertain, vehicles for drug delivery to a mucosal surface or for an intradermal or intraepithelial delivery. Useful references in this regard are Chien (Novel Drug delivery system, Chapters 3 through 6 and 9, Marcel Dekker, 1992), *Ullmann's Encyclopedia of Industrial Chemistry*, 6th Ed. (various editors, 1989-1998, Marcel Dekker); and

Pharmaceutical Dosage Forms and Drug Delivery Systems (ANSEL *et al.*, 1994, WILLIAMS & WILKINS).

Exemplary methods and routes for drug delivery useful in the invention are briefly described below.

Administration to the bronchial, bronchiolar, tracheal, nasal, oral, sub-lingual, preputial or pharyngeal mucosa can be obtained by formulating the therapeutic tolerogenic composition as inhalable, spray and the like (e.g., nasal spray, aerosol spray or pump spray and the like), solution, gel etc. Nebulizer devices suitable for delivery of pharmaceutical compositions to the nasal mucosa, trachea and bronchiole are well-known in the art and will therefore not be described in detail here. The therapeutic tolerogenic composition may then comprise a vehicle selected in the group comprising solutions, emulsions, microemulsions, oil-in-water emulsions, anhydrous lipids and oil-in-water emulsions, other types of emulsions.

Administration to the vaginal mucosa can be obtained by formulating the therapeutic tolerogenic composition as solution, enema, foam, suppository, vaginal tablet or topical gel. Preferred vehicles for vaginal delivery include hydrophilic and hydrophobic vehicles such as those commonly used in formulating emulsion or gel preparations (e.g., oil/water emulsion gel).

Administration to the digestive tract mucosa can be obtained by formulating the therapeutic tolerogenic composition as capsule, microcapsule. Preferred vehicles for digestive delivery correspond to capsules and microcapsules (e.g., capsules and microcapsules of pectin and/or alginate) generally given *per os* such as those commonly used in formulating preparations for digestive delivery (e.g., the microcapsules disclosed in International patent application WO 2007/140613). Alternatively, digestive delivery may be obtained by consuming or administering appropriate liquids and/or foodstuffs, such as beverages, yoghourts, and the like.

Intradermal or intraepithelial administration is well-known to the skilled artisan. Intradermal administration (e.g., injection) can for instance be done with needle-devices such as those disclosed in patent US 6,933,319 and in International patent application WO 2004/101025, or with appropriate needle-free devices.

The therapeutic tolerogenic vaccine composition may further comprise at least one absorption agent. "Absorption agents" are well known from the one of skill in the art. As examples, one can cite surfactants such as polyoxyethylene derivatives of fatty

acid partial esters of sorbitol anhydrides (e.g., Tween® 80, Polyoxyl 40 Stearate, Polyoxyethylene 50 Stearate, polyoxyethylene-9-lauryl ether and Octoxynol), bile salts such as sodium glycocholate, mixed micelles, enamines, nitric oxide donors (eg., S-nitroso-N-acetyl-DL-penicillamine, NOR1, NOR4-which are preferably co-administered with an NO scavenger such as carboxy-PITO or doclofenac sodium), sodium salicylate, glycerol esters of acetoacetic acid (eg., glyceryl-1,3-diacetoacetate or 1,2-isopropylideneglycerine-3-acetoacetate), cyclodextrin or beta-cyclodextrin derivatives (eg., 2-hydroxypropyl-beta-cyclodextrin and heptakis(2,6-di-O-methyl-beta-cyclodextrin)), medium-chain fatty acid such as mono- and diglycerides (eg., sodium caprate-extracts of coconut oil, Capmul), or triglycerides (eg., amyloextrin, Estaram 299, Miglyol 810), polymers such as carboxymethylcellulose, carbopol, polycarbophil, tragacanth and sodium alginate, and other absorption agents adapted for mucosal or intradermal or intraepithelial delivery. For a reference concerning general principles regarding absorption agents, which have been used with success in mucosal or intradermal or intraepithelial delivery of drugs, see Chien, Novel Drug Delivery Systems, Ch. 4 (Marcel Dekker, 1992).

The therapeutic tolerogenic vaccine composition may further comprise one or more additives (e.g., diluents, excipients, stabilizers, preservatives, and the like). See, generally, Ullmann's Encyclopedia of Industrial Chemistry, 6th Ed. (various editors, 1989-1998, Marcel Dekker); and Pharmaceutical Dosage Forms and Drug Delivery Systems (ANSEL et al., 1994, WILLIAMS & WILKINS).

Therapy

It is an object of the present invention to provide a therapeutic tolerogenic vaccine composition as described above, for use as a medicament.

The present invention also relates to a method for treating an infection by a HIV virus in a human in need thereof, comprising at least the step of mucosally or intradermally or intraepithelially administering an effective amount of a therapeutic vaccine composition as defined above to said human.

According to the present invention, a therapeutic tolerogenic vaccination may comprise one or several consecutive administrations of the therapeutic tolerogenic vaccine composition as described previously. Preferably, the therapeutic tolerogenic vaccination may comprise at least two or more consecutive administrations (i.e., vaccinations), and more preferably more than two consecutive administrations of said composition.

Advantageously, the interval between consecutive therapeutic tolerogenic vaccinations is comprised between 1 minute and 3 months, preferably between 15 minutes and 2 months.

Yet advantageously, the therapeutic tolerogenic vaccinations of the invention may also include recall tolerogenic vaccinations one or several years after the first mucosal or intradermal or intraepithelial tolerogenic vaccination.

The new therapeutic tolerogenic vaccinations following the first mucosal or intradermal or intraepithelial therapeutic tolerogenic vaccination may be selected from mucosal, intradermal and intraepithelial therapeutic tolerogenic vaccinations.

In other words, the therapeutic tolerogenic vaccine composition may be administered once only during the life of the human to be treated. Alternatively, it may be administered twice or more times during the life of the human to be treated, on the same day or on different days separated by a period ranging for example from about 1 day to about 1 year, or more. In particular, it may be administered every day or periodically, for periods ranging for example from about 1 day to about 1 year, or more. If necessary, the therapeutic tolerogenic vaccine composition may be administered all along the life of the human to be treated.

According to the present invention, an "effective amount" of the therapeutic tolerogenic vaccine composition is one which is sufficient to achieve the desired biological effect, which is here a curative effect (in other words, a therapeutic tolerogenic vaccinating effect) through induction of an immunotolerance, preferably a "Ts" immunotolerance. It is understood that the effective dosage will be dependent upon the age, sex, health, and weight of the human subject to be treated, the kind of concurrent treatment, if any, the frequency of treatment, and the nature of the expected effect. The ranges of effective doses provided below are not intended to limit the invention and represent preferred dose ranges. However, the preferred dosage can be adapted to the human, as it is understood and determinable by the one of skill in the art, without undue experimentation. See, e.g., Ebadi, Pharmacology, Little, Brown and Co., Boston, Mass. (1985).

For instance with respect to HIV, a typical dosage for a human adult will be from about 10^6 – 10^{12} HIV virus particles per dose, with 10^8 – 10^{10} preferred. Of course, whatever dosage is used, it should be a safe and effective amount as determined by known methods, as also described herein.

Moreover, the one of skill in the art can also determine in the light of his/her general knowledge the effective amount of tolerogenic adjuvant to be administered to a human in order to achieve the desired biological effect.

As an example, said effective amount for attenuated derivative of pathogenic bacteria (e.g., BCG) is comprised in the range of 10^4 to 10^{12} , preferably 10^5 to 10^{10} CFU (colony forming unit) per dose, and more preferably 10^6 to 10^8 CFU. As another example, said effective amount for attenuated derivative of pathogenic bacteria or inactivated pathogenic bacteria (e.g., BCG) is comprised in the range of 0.001 mg to 1 g per dose, preferably 0.01 to 100 mg, and more preferably 0.1 to 10 mg.

As another example, said effective amount for non-pathogenic bacteria (e.g., *Lactobacillus* sp.) is comprised in the range of about 10^6 - 10^{14} CFU per dose, and more preferably about 10^{10} - 10^{12} CFU.

As yet mentioned, for therapeutic purposes, the viral antigen comprised into the vaccine composition is autologous, that is to say it derives from the HIV virus infecting the human to be treated.

Advantageously, the HIV virus is isolated from the human to be treated, then it is cultured (preferably on cells obtained from the patient to be treated) and inactivated (preferably at least inactivated twice), to be finally associated with a tolerogenic adjuvant so as to obtain a therapeutic tolerogenic vaccine composition as described above.

Yet advantageously, the therapeutic tolerogenic vaccine composition comprising inactivated autologous HIV virus is administered to the human subject during a conventional antiviral treatment which will have previously led to an undetectable viral load. The conventional antiviral treatment may then be stopped after one or more therapeutic tolerogenic vaccinations using the therapeutic tolerogenic vaccine composition, provided appropriate *ex vivo* viral replication suppression of non-autologous/allogenic acutely infected CD4 cells is achieved by autologous virus-specific non-cytotoxic MHC-Ib/E-restricted CD8 cells. Advantageously, the treatment using the therapeutic vaccine composition of the present invention may be prolonged for periods of time to be determined in order to maintain the *ex vivo* and *in vivo* desired antiviral effects.

Other applications including prevention of infections

The tolerogenic vaccine compositions disclosed in the Examples below are suitable for treating an HIV-infected human, or for preventing a future HIV infection in a human.

The present invention further provides an *in vitro* method for determining whether a human is protected against a HIV virus, comprising:

- a) isolating peripheral blood CD8 T cells from a blood sample of said vaccinated human;
- b) cultivating under appropriate conditions:
 - (i) said isolated CD8 T cells with allogenic CD4+T cells which were *in vitro* acutely infected by a viral strain equivalent to said HIV virus; and
 - (ii) said *in vitro* acutely infected allogenic CD4+T cells;
- c) recovering the culture supernatants;
- d) measuring the viral load in said supernatants; and
- e) determining whether said human is protected against said HIV virus, or not.

By "a viral strain equivalent to a HIV virus to be tested", it is meant that said viral strain originates from a wild virus and has essential characteristics similar to those of the HIV virus to be tested (for example, one can cite the viral strain HTLVIIIB originating from an individual HIV-1: HTLVIIIB can be considered as "a viral strain equivalent to" HIV-1). Preferably, said viral strain will originate from a wild virus which is the HIV virus to be tested. Said viral strain thus represents an appropriate model for studies involving a HIV virus, especially a wild HIV virus. The viral strain is of course well-adapted for such studies, especially in terms of safety.

All the steps above can be performed using standard techniques that are well-known from the person skilled in the art. In particular, the appropriate culture conditions for step b) are part of the general knowledge in the field of the invention (such as the conventional methods described in the Examples below).

The viral load can be measured in step d) by conventional methods such as those described in the Examples below.

The viral load in the supernatant recovered from the culture of said *in vitro* acutely infected allogenic CD4+T cells according to sub-step b)(ii) will be used as a reference for the determination in step e). One will advantageously calculate the "percent suppression (%)" or "suppressive ratio" or "antiviral effect" by, e.g., comparing the geometric mean of viral concentration in the supernatants from duplicate (or triplicate or quadruplicate or more) wells containing only cells from the *in vitro* acutely infected allogenic CD4+T cells with the geometric mean of viral concentration in the supernatants from duplicate (or triplicate or quadruplicate or more) wells containing CD8 T cells, and cells from the *in vitro* acutely infected allogenic CD4+T cells.

Then, said determination in step e) is preferably performed as follows:

- If the suppressive ratio is higher than about 100, one can conclude that said human is protected. Typically, this will be the case if a HIV-non infected human has been administered an efficient preventive antiviral treatment or if a HIV-infected human has been administered an efficient therapeutic treatment, said efficient preventive or therapeutic treatment comprising preferably a pharmaceutical composition according to the present invention, and it will thus not be necessary to further administer any preventive or therapeutic treatment to the human, as long as it remains protected.
- If the suppressive ratio is lower than about 100, one can conclude that said human is not protected against said virus. Then, the human, either a HIV-non infected human or a HIV-infected human, will advantageously be administered a preventive or therapeutic treatment comprising a pharmaceutical composition according to the present invention, respectively, and the *in vitro* method above will be performed once or more at appropriate time intervals to make sure the human become protected.

Also, the present invention provides a kit for *in vitro* determining whether a human is protected against a HIV virus, comprising allogenic CD4+T cells that can be infected by a viral strain, said viral strain being, as defined above, equivalent to said HIV virus to be tested. The kit may also include an adequate viral strain in appropriate concentration to infect the above-mentioned allogenic CD4+T cells and/or appropriate reagents and/or controls and/or media (such as media for cell suspension, cell culture, cell storage, etc.). The kit of the present invention may be specific to a particular type of HIV virus, or it may be adapted to various types of virus, said types of virus being close (in particular, phylogenetically close).

The present invention can easily be adapted in order to be used for preventing and/or treating any chronic infectious diseases. Non-limiting examples of such diseases are: hepatitis B and C, human papilloma virus (HPV), EBV and other herpes viruses, tuberculosis, leprosis, leishmaniosis, etc.

Globally, each time where one or several pathogenic antigens associated with the above-mentioned infections or diseases are involved in the specific activation of CD4+T cells which present epitopes derived from above-mentioned pathogenic proteins or peptides, the specific suppression/prevention of activation of CD4+T cell can be raised by non-cytotoxic CD8+T cells generated by mucosal or intraepithelial or intradermal tolerogenic vaccines associating the above-mentioned antigen(s) and a tolerogenic adjuvant such as those described herein.

It is herein shown that viral infections and associated diseases can be prevented and/or treated in mammals/humans using the tolerogenic vaccine composition of the present invention. Based on this teaching, it is possible to provide other tolerogenic vaccine compositions comprising (i) tolerogenic adjuvants or tolerogenic carriers such as those disclosed herein; and (ii) any antigens from viral, bacterial, fungal, protozoal or parasitic origin. Such tolerogenic vaccine compositions are formulated for appropriate delivery (preferably, mucosal or intradermal or intraepithelial) of said tolerogenic adjuvants and of said antigens. They are useful for preventing and/or treating chronic infections in mammals caused by the virus, bacteria, fungi, protozoa or parasites which the antigens are derived from. An example is a bacterial tolerogenic vaccine composition comprising (i) anyone of the tolerogenic adjuvants described herein; and (ii) an antigen derived from *Mycobacterium tuberculosis*. This bacterial tolerogenic vaccine composition is formulated for appropriate delivery (preferably, mucosal or intradermal or intraepithelial) of said antigen and of said tolerogenic adjuvant. Of particular interest for preventing and/or treating tuberculosis in humans is such a bacterial tolerogenic vaccine composition wherein the mycobacterial antigen is derived from the Koch's bacillus.

All patents, patent applications and publications referred to above are hereby incorporated by reference.

EXAMPLES

PART A - GENERAL MATERIALS & METHODS

A-I Animals. Colony-bred Chinese rhesus macaques (*Macaca mulatta*) were housed in accordance with the regulations of the National Institutes of Health 'Guide for the Care and Use of Laboratory Animals'. All animals were in good health, 2-4 years old, weighed 4-6 kg and were seronegative for SIV, SRV, simian T cells lymphotropic virus 1, hepatitis B virus, and B virus. X ray and skin test (PPD) were performed at entry for all animals to exclude potential carriers of tuberculosis.

A-II MHC class I typing. Rhesus macaque classical MHC class I alleles were genotyped in peripheral blood mononuclear cells (PBMC) samples using sequence-specific primers (SSP) PCR assays for representative *Mamu-A* and *Mamu-B* sequences as previously described (Muhl et al., 2002; Loffredo et al., 2007).

A-III Antigenic viral preparations.

III-1. The SIV production was performed on CEM174 cells inoculated with SIVmac239 (gift of P.A. Marx). The culture supernatants were collected at peak viral production.

III-2. AT-2-inactivated SIVmac239: SIVmac239 was inactivated by 250 μ M aldrithiol (AT-2) (Sigma) for 2 hours and was washed three times by ultracentrifugation. The AT2-inactivated virus was used in a final dose of 10^9 viral particles for each administration (i.e., vaccination).

III-3. Heat-inactivated SIVmac239: SIVmac239 was inactivated at 56°C for 30 minutes. The heat-inactivated virus was used in a final dose of 10^9 viral particles for each administration.

III-4. AT-2-Heat-inactivated SIVmac239: SIVmac239 was inactivated by 250 μ M aldrithiol (AT-2) (Sigma) for 2 hours and was washed three times by ultracentrifugation. Then, the virus was subjected to a temperature of 56°C for 30 minutes. The inactivated virus is used in a final dose of 10^9 viral particles for each administration.

III-5. The inactivated virus preparations were inoculated to CEM174 cells to verify the 100% inhibition of viral infectivity.

A-IV Assay for antibody responses to SIV. Anti-SIV IgG, IgM, and IgA antibodies in plasma were titrated by an immunofluorescence antibody (IFA) assay (Mederle et al., 2003). Briefly, serial twofold dilutions of test plasma were incubated with SIV-infected CEM174 cell-attached slides at 37°C for 30 minutes. After washing with Hanks, FITC-conjugated goat anti-macaque IgG (Sigma), IgM (ADI, San Antonio, Texas), or IgA

(ADI) were added for additional 30 minutes (at 37° C). Antibody titers were determined as reciprocal of the highest dilution to reach a positive immunofluorescence staining. The sensitivity of IFA assay was a titer of 20 for IgG and a titer of 5 for IgM and IgA. When a plasma sample was negative for the IFA (below the assay sensitivity), a value of 1 was assigned for facilitating data analysis.

Mucosal secretions were collected by washing of the rectum with PBS using a catheter for gastric instillation as described previously (Tsai et al., 1993). Briefly, trypsin inhibitor (10 µg/ml) and EDTA (5 x 10⁻⁴ M) (Sigma) were added to the samples which were then centrifuged for 10 minutes at 10000 x g at 4°C. Supernatants were collected and supplemented with phenylmethylsulfonyl fluoride (10⁻³ M) and sodium azide (0.01%) (Sigma). Samples were stored at -80°C until use. Anti-SIV IgA titers in rectum were detected by the above IFA assay.

A-V Flow cytometry. Flow-cytometry analysis was carried out with FACScalibur (BD Biosciences, San Jose, California) using fluorescence-labeled monoclonal antibodies against the following: CD3-PE-Cy7 (clone SP34-2), CD4-PE (clone MT477), CD8-PerCP (clone RPA-T8), and secondary rabbit anti-mouse-APC (BD Biosciences). The Ki-67-PE (BD Biosciences) and FITC-conjugated anti-P27 monoclonal antibody (Fitzgerald, Concord, MA) or biotin-conjugated anti-P27 monoclonal antibody (Fitzgerald) coupled with APC-SAv (BD Biosciences) were used for intracellular staining after permeabilization.

PE-conjugated monoclonal antibodies against TCRγδ (clone B1), Vβ8, and CD antigens (CD7, CD16, CD28, CD62L, CD95, CD122, CD137, CD150, CD183, CD184, CD195, CD196, CD197, CD226, CD272, and CD305) were purchased from BD Biosciences; PE-conjugated monoclonal antibodies against CD antigens (CD11a, CD25, CD27, CD39, CD101, CD129, CD215, CD277, and CD357) were purchased from BioLegend (San Diego, CA, USA); and PE-conjugated monoclonal antibodies against CD antigens (CD127, CD247, and CD279) were purchased from eBioscience (San Diego, CA, USA).

A-VI Cell proliferation. PBMCs were obtained as described previously (Lu et al. 2003). The proliferation of SIV-specific CD4⁺ or CD8⁺ T cells was evaluated by carboxy-fluorescein diacetate, succinimidyl ester (CFSE) labeling assay (Molecular Probes, Eugene, Oregon) according the manufacturer's instruction. PBMC were stained with 3 µM CFSE for 15 minutes at 37°C. After washing, the CFSE-labeled cells were stimulated for 5 days with 10 µg/ml recombinant SIV core protein P27 (ImmunoDiagnostics, Wobun, MA), 2 µg/ml SIV gag 15-mer peptides (GLS, Shanghai, China), 10⁹/ml AT-2-inactivated SIV or medium alone. After labeling with anti-CD3 and

anti-CD4 or anti-CD8 antibodies, PBMC were fixed in 1% paraformaldehyde for flow cytometry.

A-VII Cell activation. Fresh PBMCs, depleted (or not) with CD8 or CD25 by magnetic beads were single-round infected with AT-2-treated SIVmac239 for 2 hours at a viral concentration of 10^{10} /ml. Infected cells were stimulated overnight with staphylococcal enterotoxin B (2.5 µg/ml) and anti-CD3 (2.5 µg/ml)/anti-CD28 (2.5 µg/ml) antibodies. Intracellular staining of SIV P27 and Ki-67 was performed 48 hours after stimulation in order to determine the percentage of activation (Ki-67+) within infected (P27+) CD4+ cells.

A-VIII ELISPOT assay. The rhesus macaque IFN-γ and IL-10 ELISPOT assays were carried out in uncultured PBMC in the presence or the absence of P27 or AT-2-inactivated SIV using a commercial kit (Cell Sciences, Canton, MA). A TGF-β1 ELISPOT kit was purchased from R&D Systems (Minneapolis, MN). The data were read with an automated ELISPOT reader (AID, GmbH, Straßberg, Germany). The number of SIV-specific spot forming cells (SFCs) was calculated by subtracting the nonspecific SPCs in the presence of medium alone.

A-IX Antiviral assay. Autologous CD4+ T cells from each animal purified by magnetic positive-labeling (MicroBeads, Miltenyi Biotec) were acutely infected with SIVmac239 (10^{-3} MOI) in the presence or the absence of magnetically purified CD8+ T cells at a CD4/CD8 ratio of 1:2 and then stimulated with SEB (Sigma) for 16 hours. After washing, the cells were cultured in quadruplicates in a final volume of 200 µl per well of RPMI 1640 medium (Invitrogen, Shanghai, China) containing 100 IU of human rIL2 in 96-well plates for 5 days at 37°C in the presence of 5% CO₂. The cell cultures were replaced once with half of fresh medium at day 3. The culture supernatants collected at day 5 were used for the measurement of viral load by a real-time RT-PCR (see below). Percent suppression (%) was calculated by comparing the geometric mean of viral concentration in the culture supernatants from duplicate wells containing only CD4+ infected cells with the geometric mean of viral concentration in the supernatants from quadruplicate wells containing the mixed CD8+ and CD4+ cells. CD4+ T cells were also co-cultured with allogenic CD8+ T cells in order to determine the correlation between viral suppression and HLA restriction.

A-X Viral load measures. SIV RAN in plasma or cell-associated SIV DNA was quantified by a real-time RT-PCR or PCR using primers (sense, SEQ ID No. 1: 5'-GAGGAAAAGAAATTTGGAGCAGAA-3'; antisense, SEQ ID No. 2: 5'-GCTTGATGGTCTCCCACACAA-3') and probe (SEQ ID No. 3: 5'-FAM-

AAAGTTGCACCCCCTATGACATTAATCAGATGTTA-TAMRA-3') specifically optimized for SIVmac239 and for SIVmac251.

A-XI SIV-specific suppressive T-cell assay. Fresh PBMCs, depleted (or not) with either CD8 or CD25 by magnetic bead-conjugated anti-CD8 or anti-CD25 antibodies according to the protocol provided by the manufacturer (Miltenyi Biotec) were infected with SIVmac239 for 2 hours at 0.5 multiplicity of infection (MOI). Infected cells were treated overnight with staphylococcal enterotoxin B (SEB) (2.5 µg/ml) (Sigma) and anti-CD3 (2.5 µg/ml)/anti-CD28 (2.5 µg/ml) antibodies (BD Biosciences). Simultaneous intracellular staining of SIV P27 and Ki-67 were performed 48 hours after in vitro stimulation in order to determine the percentage (%) of T-cell activation (Ki-67+) within infected (P27+) cell populations.

A-XII Viral challenges.

XII-1. The SIV production was performed on macaques PBMC inoculated with SIVmac239 (gift of P.A. Marx). The culture supernatants were collected at peak viral production.

XII-2. Intrarectal challenge (IRC): Following vaccination, the animals were inoculated (repeatedly) intrarectally with 5000 MID 100 i.e. 5×10^5 TCID₅₀ of pathogenic SIVmac239. This infectious dose generally results in a systemic infection of 100% Chinese rhesus macaques with a peak plasma viral load (10^6 - 10^7 copies/ml) between day 10 and day 14. All SIV-challenged animals were evaluated clinically and biologically every 2-week for 1 month and every 1-month thereafter.

XII-3. Intravenous challenge (IVC): Following vaccination, the animals were inoculated (repeatedly) intravenously with 5 MID 100 i.e. 500 TCID₅₀ (titrated in CEM174 cell line) of pathogenic SIVmac239 (gift of Dr. P.A. Marx from Aaron Diamond AIDS Research Center, New York, USA). This infectious dose generally results in a systemic infection of 100% Chinese rhesus macaques with a peak plasma viral load (10^6 - 10^7 copies/ml) between day 10 and day 14. All SIV-challenged animals were evaluated clinically and biologically every 2-week for 1 month and every 1-month thereafter.

A-XIII Statistical analysis. Impaired data between different groups of animals or paired data before and after immunization were compared by the Mann-Whitney or the Wilcoxon test, respectively.

PART B - SPECIFIC MATERIALS & METHODS

B-I- USE OF BCG AS A TOLEROGENIC VEHICLE

B-I-I Preparation of BCG

I-1. Live BCG: Live BCG prepared in Copenhagen at the Statens Serum Institut (strain SSI 1331) was purchased from Laboratories Sanofi-Pasteur Merck, Sharp and Dome (SPMSD) and was used at a final concentration of 5×10^6 cfu for intestinal or intravaginal administration or at a final concentration of 5×10^5 cfu for each intradermal boost administration.

I-2. Extended freeze drying (EFD) inactivated BCG: The live SSI 133 BCG strain was killed by 5 days extended freeze-drying (EFD) under a vacuum of less than 20 μ m Hg and is used at a final dose corresponding to 5×10^6 cfu for each intestinal or intravaginal administration or 5×10^5 cfu for each intradermal administration.

I-3. Heat inactivated BCG: The live SSI 133 BCG strain was autoclaved for 15 minutes at 115°C in borate buffer and is used at a final dose corresponding to 5×10^6 cfu for each intestinal or intravaginal administration or 5×10^5 for each intradermal administration.

B-I- II Pharmaceutical compositions

The composition was prepared freshly with the use of RPMI 1640 (Invitrogen, Shanghai, China) containing one of the SIV antigens and the tolerogenic vehicle.

B-I- III Animal immunization

At the time of immunization, animals were anesthetized with tiletamine hydrochloride and zolazepan (0.7 mg/kg) injected intramuscularly.

III-1. Intravaginal immunization (IVI): Female animals were immunized under anesthesia by intravaginal injection for 4 hours of one milliliter of pharmaceutical composition or of one tolerogenic vehicle disclosed previously as a control. A booster immunization with the pharmaceutical composition or with the tolerogenic vehicle was given with the same dose at the same site at 8 weeks. All animals were evaluated clinically and biologically every two weeks after the first immunization.

III-2. Oral (intra-gastric) immunization (IGI): Male or female animals under anesthesia were administered intragastrally with 15 ml of 0.1M sodium bicarbonate 15

minutes before ingestion of pharmaceutical composition or of one tolerogenic vehicle disclosed above as a control. Additional 15 ml of the sodium bicarbonate solution was given immediately after administration. The same tolerogenic vaccination than the initial one was repeated two times at 1-month interval to each animal. All animals were evaluated clinically and biologically every two weeks after the first immunization.

III-3. Intradermal boost immunization (IDI): Female intravaginally immunized animals (see above IVI section) were given at 90 days after the first immunization under anesthesia an intradermal booster with 0.1 ml of pharmaceutical composition containing 10^9 copies of AT-2-inactivated SIV and 5×10^5 cfu of live BCG. All animals were evaluated clinically and biologically every two weeks after the first immunization.

B-I-IV Antiviral assay. The threshold corresponding to sterile immunity after intrarectal challenge is at least 20.

B-II- USE OF *Lactobacillus plantarum* AS A TOLEROGENIC VEHICLE

B-II-I Bacterial preparation (tolerogenic vehicle preparation). *Lactobacillus plantarum* (LP) (ATCC8014) was cultured at 37°C in MRS medium with a rotation rate of 200 rpm. To obtain LP at the logarithmic (midlog) phase of bacterial culture, bacteria were cultured until reaching an optical density of 1.0 at 600 nm with a final LP concentration of around 10^{10} cfu/ml (obtained in about 3.5 hours).

B-II-II Animal immunization by oral (intra-gastric) delivery. Animals were fasted overnight (without breakfast). At the time of oral administration, animals were anesthetized with tiletamine hydrochloride and zolazepan (0.7 mg/kg) injected intramuscularly.

Immunisation No. 1: Eight animals were administered intragastrically 30 ml of a made of a viral-bacterial preparation containing 4×10^7 copies/ml of DI-SIV and 3×10^9 cfu/ml of living LP in maltodextrin (20%) solution. After this first immunization, monkeys were receiving intragastrically 25 ml of the same viral-bacterial preparation (i.e., pharmaceutical composition) each 30 minutes for 3 hours. This oral delivery protocol was performed 5 times over 5 consecutive working days. As controls 4 animals were administered living LP alone and other 3 received only twice inactivated SIV in parallel.

Immunisation No. 2: Twelve animals (iSIV/LP#9-20) were intragastrically administered 30 ml of a preparation of 4×10^7 copies/ml of iSIV (AT-2/heat-inactivated SIVmac239) and 3×10^9 cfu/ml of living LP in maltodextrin (20%) solution. Then, animals were

receiving 25 ml of the same preparation every 30 minutes for 3 hours (6 times) on 5 consecutive days. Six animals (LP#5-10) were intragastrically administered 30 ml of 3×10^9 cfu/ml of living LP in maltodextrin (20%) solution. Then, animals were receiving 25 ml of the same preparation every 30 minutes for 3 hours (6 times) on 5 consecutive days. Finally, another 6 animals (iSIV#5-10) were intragastrically administered 30 ml of a preparation of 4×10^7 copies/ml of iSIV alone. Then, animals were receiving 25 ml of the same preparation every 30 minutes for 3 hours (6 times) on 5 consecutive days.

B-II-III Depletion of CD8+ T cells *in vivo*. Macaques were first anesthetized and then given an intravenous injection of a chimeric anti-CD8 monoclonal antibody (cMT-807, Centocor Research & Development, Inc., Malvern, Pennsylvania, USA) at 5 mg/kg on days 0, 4, and 7) as described earlier (Schmitz et al., 1999). Peripheral blood samples (5 ml) were taken from each animal at day 0 and at various time points after antibody injection.

B-II-IV Antiviral assay. The threshold corresponding to sterile immunity after intrarectal challenge is at least 100.

B-II-V CD8+ T cell SIV suppression assay. Autologous CD4+ T cells from each animal purified by magnetic positive-labeling (MicroBeads, Miltenyi Biotec) were acutely infected with SIVmac239 (10^{-3} multiplicity of infection) in the presence or the absence of magnetically purified CD8+ T cells at a CD4/CD8 ratio of 1:3 and then stimulated with SEB and anti-CD3/anti-CD28 antibodies for 16 hours. After washing, the cells were cultured in quadruplicates in 96-well plates. Cultures were maintained in a final volume of 200 μ l per well of RPMI 1640 medium containing 100 IU of human rIL2 (Roche Diagnostics GmbH, Mannheim, Germany) for 5 days. Culture supernatants collected at day 5 were used for the measurement of viral load by a real-time RT-PCR (see below). Fold suppression was calculated as follows: the geometric means of viral concentration in the culture supernatants from the infected CD4+ target cells only/ the geometric means of viral concentration in the supernatants from the mixed CD8+ and CD4+ T cells).

In some experiments, CD8+ and CD4+ T cells were cultured without cell-to-cell contact by using a Multiwell Insert System (BD Biosciences) (CD8 in the insert well and CD4 in the bottom well); CD4+ T cells were cocultured with allogenic CD8+ T cells in order to determine the correlation between viral suppression and MHC restriction; and CD8+ and CD4+ T cells were also co-cultured in the presence of anti-MHC-ABC (BioLegend)

or anti-MHC-E (Cell Science) antibodies to define the modes of MHC restriction. To define the subsets of CD8⁺T cells associated with antiviral activity, CD8⁺T cells were purified from PBMCs immediately after their depletion with PE-conjugate anti-TCR $\gamma\delta$, anti-V β 8, or other anti-CD antigen antibodies using anti-PE microbeads through a LD column (Miltenyi Biotec).

B-II-VI SIV-specific CD8⁺ T cell's cytotoxicity assay. Both purified CD8⁺ T cells (effector cells) and purified CD4⁺ T cells pulsed with 10^{10} AT-2-treated SIVmac239 (target cells) were labeled with 40 nM 3,3'-dihexyloxacarbocyanine (DiOC₆) (Marchetti et al., 1996) (Molecular Probes) for 10 min at 37°C. Target cells were labeled with PerCP-Cy5-conjugated anti-CD4 (BD Bioscience) for 20 min on ice. After washing 3 times, effector cells were mixed with target cells in a U-bottomed 96-well plate at different E/T ratios (3:1, 1:1, 0.3:1) in triplicate. K562 cells (target) with APC-conjugated anti-CD32 (BD Bioscience) and purified CD56⁺ (NK) cells (effector) from 4 healthy donors were included as an assay control. After 4 hrs incubation at 37°C in the presence of SEB and anti-CD3/anti-CD28, cells were harvested and analyzed by flow cytometry. Percent cytotoxicity was calculated as follows: $100 \times (\% \text{ of total apoptotic target cells} - \% \text{ of spontaneous apoptotic target cells}) / (100 - \% \text{ of spontaneous apoptotic target cells})$.

B-II-VIII Viral challenges.

First study: Four months after the oral administration of the vaccine or the controls in the first batch of experiment (immunization No. 1), the 8 immunized animals and their 7 controls were inoculated intra-rectally with 2500 MID₁₀₀ (100,000 TCID₅₀) of pathogenic SIVmac239. Two months later, 4 vaccinated and already protected monkeys were rechallenged by intrarectal route (100,000 TCID₅₀) while the 4 other protected monkeys were intravenously rechallenged with 5 MID₁₀₀ (200 TCID₅₀) of SIVmac239. As controls, 2 monkeys received an intrarectal challenge and other 2 an intravenous challenge. These infectious doses generally result in a systemic infection of 100% Chinese rhesus macaques with a peak plasma viral load ($10^7 - 10^9$ vp/ml) between day 10 and day 14.

Second study (immunization No. 2): On day 420 post-immunization in the second set of study, 16 animals (8 monkeys immunized with iSIV and LP) and 8 controls (4 iSIV and 4 LP) were intrarectally challenged with 100,000 TCID₅₀ of SIVmac239.

PART C - RESULTS

C-I BCG IS A TOLEROGENIC VEHICLE

C-I-I Protection against the intravenous SIVmac239 challenge following intravaginal administration:

Six animals (Compositions_1, 2, 3, 4, 5, and 6) were administered intravaginally one milliliter of a tolerogenic composition comprising AT2-inactivated virus as an antigen and live BCG. A booster administration was given with the same tolerogenic composition at the same site at 8 weeks.

Simultaneously, 5 other animals (controls_1, 2, 3, 4, and 5) were given intravaginally one milliliter of a composition comprising only live BCG. A booster administration was also given with the same composition at the same site at 8 weeks.

Four months after the initial administration, all 11 animals (Compositions_1-6 and controls_1-5) were challenged by an intravenous viral inoculation.

The viral loads were determined regularly in the plasma of the treated animals.

Figure 1 shows the virus loads (plasma SIV RNA copies/ml) as a function of time (days) in animals which have received the composition (Compositions_1-6) and in control animals (controls_1-5) following a single intravenous viral challenge.

The results show that, after intravenous viral challenge, the 5 control animals (Controls_1-5) showed a typical primary infection with a peak plasma viral load (10^6 - 10^7 copies/ml) between days 10-14 post-challenge as expected. The plasma viral load of this group of control animals remained still high ($>10^5$ vp/ml) over the 60 days post viral-challenge and thereafter.

In contrast, 4/6 animals which had received intravaginally the tolerogenic composition made of an AT-2-inactivated SIVmac239 plus BCG showed a very low plasma viral load peak (<1000 vp/ml; between days 10-14), which became undetectable (<10 vp/ml) rapidly (one month after viral challenge). The 2 animals with a high plasma viral load peak ($> 10^6$ copies/ml) had a lower set-point viral load level (<1000 copies/ml) than the control group ($> 10^5$ copies/ml) at day 60.

C-I-II- Protection against the intrarectal SIVmac239 challenge following intravaginal administration of the composition:

Seven animals (Compositions_7, 8, 9, 10, 11, 12, and 13) were administered intravaginally one milliliter of a tolerogenic composition comprising AT2-inactivated virus as an antigen and live BCG as a tolerogenic vehicle. A booster administration was given with the same composition at the same site at 8 weeks.

Simultaneously, five other animals (controls_6, 7, 8, 9, and 10) were given intravaginally one milliliter of a composition comprising only live BCG. A booster administration was also given with the same composition at the same site at 8 weeks.

Four months after the initial administration, all 12 animals (Compositions_7-13 and controls_6-10) were challenged with SIVmac239 through an intrarectal viral inoculation.

The viral loads were determined regularly in the plasma of the treated and control animals.

Figure 2 shows the virus loads (plasma SIV RNA copies/ml) as a function of time (days) in animals which received the composition (Compositions_7-13) and in control animals (controls_6-10) following intrarectal viral challenges.

The results show that, following intrarectal viral challenges, the 5 animals which received the intravaginal administration of live BCG alone (Controls_6-10) showed a typical primary infection with a peak plasma viral load (10^6 - 10^7 vp/ml) between days 10-14 post-challenge as expected. The plasma set-point viral load of this group of control animals remained still high ($>10^5$ copies/ml) over the 60 days post viral-challenge.

In contrast, 4/7 animals which received intravaginally AT-2-inactivated SIVmac239 plus live BCG showed surprisingly undetectable viral load level (<10 copies/ml) over a period of 60 days post-challenge. The 3 other animals showed a typical primary infection with a peak plasma viral load between days 10-14 post-challenge. However, their set-point viral load (10^3 - 10^5 copies/ml) was significantly lower than the control animals' level ($>10^5$).

C-I-III- Protection against repeatedly intravenous or intrarectal SIVmac239 challenges following intravaginal administration of the pharmaceutical composition:

Two and eight months later, the 3 animals with an undetectable viral load following intravenous challenge (Compositions_1, _2, and _3) were subjected to a second and a third intravenous challenge with the same dose of viral inocula.

After the second and third intravenous viral challenges of this group of monkeys, a similar low peak plasma viral load was observed at day 10. However, by 30 days after viral challenge, viral loads became again undetectable (Figure 3).

Sixteen and twenty three months after the initial administration of the composition, the 3 animals which already had a total of 3 intravenous challenges (Compositions_1, _2, and _3) were further challenged by intrarectal inoculation.

As expected, these 3 animals (which initially received intravaginally AT-2-inactivated SIVmac239 plus BCG) showed again no detectable (<10 copies/ml) plasma viral load peak after 2 successive intrarectal viral challenges (Figure 3).

These results have established that efficiency on inhibiting viral replication is stable since this inhibition is still observed more than 20 months after the initial administration of the composition

C-I-IV- Protection against the intravenous or intrarectal SIVmac239 challenge following intravaginal administration of the pharmaceutical composition plus an intradermal booster:

As expected, after following intravenous (controls 17 and 18, figure 4) or intrarectal (controls 19 and 20, Figure 5) viral challenges, the 4 animals which had received intravaginal administration of live BCG alone showed a typical primary infection with a peak plasma viral load (10^6 - 10^7 vp/ml) between days 10-14 post-challenge as expected. The plasma set-point viral load of this group of control animals remained still high ($>10^5$ copies/ml) by 60 days post viral challenge.

In contrast, the 3/4 (75%) animals (Compositions 14, 15, and 17) which received intravaginally the composition made of AT-2-inactivated SIVmac239 and live BCG plus an intradermal booster with the same composition showed undetectable plasma viral load (<10 copies/ml) over a period of 60 days post-intravenous challenge (see Figure 4). The remaining one animal (composition 16) showed a primary infection with a peak plasma viral load ($>10^5$ copies/ml) between days 10-14 post-challenge (see Figure 4). However, its set-point viral load reached relatively low level (10^4 copies/ml) at day 60.

Moreover, the 4/4 (100%) animals (compositions 18-21) which received intravaginally the composition made of AT-2-inactivated SIVmac239 plus live BCG plus an intradermal booster of the same composition showed undetectable plasma viral load (<10 copies/ml) over a period of 60 days post-intrarectal challenge (see Figure 5).

C-I-V- Protection against the intrarectal SIVmac239 challenge following oral administration of the pharmaceutical composition:

Four animals (Compositions_22, _23, _24, and _25) were administered intragastrically one milliliter of a composition comprising AT2-inactivated virus and live BCG.

Simultaneously, four other animals (controls 21-24) were intragastrically given one milliliter of live BCG alone.

The same administration given initially to each animal was repeated three times at day 15, 30 and 60 following the first administration step.

The results show that after intrarectal viral challenge (performed at day 90), the 4 animals which received live BCG alone (controls 21-24) showed a typical primary infection with a peak plasma viral load (10^6 - 10^7 copies/ml) between days 10-14 post-challenge whereas the 4 animals (Compositions_22-25) which received the AT-2-inactivated SIV plus live BCG composition showed surprisingly an undetectable plasma viral load (<10 copies/ml; between days 10-14) (Figure 6).

C-I-VI- Immune correlates and protection against SIVmac239 challenge following the administration of the composition made of AT2-inactivated virus plus live BCG:

No systemic antibody directed against SIV was detected in the blood of the treated animals. However, some specific systemic humoral response has been detected when intradermal boost composition administration has been used. Consequently, the observed protection against SIV infection for the treated animals does not result from a systemic humoral response.

Moreover, no conventional SIV-specific gamma interferon-producing cytotoxic T lymphocytes were detectable by ELIspot (data not shown). For the purpose of evaluating whether a SIV-specific non-conventional cellular response existed, blood samples were taken for each treated or control animal, and CD4+ and CD8+ cells were purified from each sample according to conventional methods. The previously obtained CD4+ cells were cultured and then infected with SIV mac239 according to conventional methods. The SIV-infected CD4+ cells were then cultured in the presence or in the absence of the previously obtained autologous CD8+ cells for 5 days. The supernatant SIV concentration was assayed by a quantitative real-PCR.

Figure 7 shows the fold of suppression of viral replication in SIV-infected CD4+ obtained in the presence or in the absence of autologous CD8+ cells obtained in the course of the experiments presented in Figure 2. The tested CD8 were obtained from animals which received, by the intravaginal route, the composition of AT2-inactivated virus plus BCG (Compositions_7-13) or from control animals (controls 6-10).

The results show that the CD8 T cells from animals protected against virus infection (composition_7, composition _8, composition _10, and composition _11) provide a level of viral suppression in SIV-infected CD4 cells greater than 20 fold, whereas the CD8 T cells from animals non-protected against virus infection (composition_9, composition_12, and composition_13) provide a level of viral suppression inferior or equal to 10 fold (Figure 7). Moreover, a more than 20 fold viral suppression has been also observed in the 4 animals protected against intravenous viral challenges presented in Figure 1 (data not shown).

Figure 8 shows the levels of viral suppression in SIV-infected CD4+ obtained in the presence or in the absence of autologous CD8+ cells obtained in the course of the experiments presented in Figure 6. The tested CD8 were obtained from the 4 animals which received the composition (compositions_22-25) by oral administration of AT2-inactivated virus plus BCG or from the 4 control animals (controls 21-24).

Figure 9 shows the levels of T-cell activation (Ki-67+) in SIV (P27+)-infected CD4 cell population obtained in the presence or in the absence of autologous CD8+ cells obtained in the course of the experiments presented in Figure 6. The tested CD8 were obtained from the 4 animals which received the composition (compositions_22-25) by oral administration of AT2-inactivated virus plus BCG or from the 4 control animals (controls 21-24). A SIV-specific suppression of CD4+ T-cell activation by autologous CD8+ T cells was observed in the 4 animals which received the composition.

The results confirm that the prevention of systemic or mucosal SIV infection obtained by intravaginal or oral administration of AT2-inactivated plus BCG induced a state of immunotolerance characterized by a non-cytotoxic CD8+ T-cell response associated with an SIV-infected CD4 cell anergy. In view of these results, BCG can thus be identified as a tolerogenic adjuvant.

Taken together, these findings have demonstrated that a steady state of immunotolerance to SIV antigens is for the first time achieved by intravaginal or oral (or intragastric) administration of a composition made of inactivated SIV virus plus live BCG. At the same time, it was shown for the first time that an intravaginal or oral

administration of a pharmaceutical composition comprising AT2-inactivated SIV virus plus live BCG according to the invention is effective (>50%) to prevent chronic viral infection following intrarectal or intravenous challenge.

C-II- USE OF *Lactobacillus plantarum* AS A TOLEROGENIC VEHICLE

C-II-I- Induction of SIV-specific immunotolerance by oral co-administration of double inactivated SIV and *Lactobacillus plantarum* (iSIV/LP)

On the one hand, SIV-specific antibodies (IgG, IgM, and IgA) were not detected in animals treated with oral iSIV/LP (Fig. 10a). On the other hand, no significant SIV P27-specific peripheral blood CD4⁺ T cell proliferation was observed in iSIV/LP-treated animals while iSIV-treated animals did show significant P27-specific peripheral blood CD4⁺ T cell proliferation.

C-II-II- Anti-activation and antiviral activities of non-cytotoxic CD8⁺ cells

SIV P27-specific peripheral blood CD8⁺ T cell proliferation was observed both in iSIV/LP-treated and iSIV-treated animals (Fig. 10b). However, no interferon-gamma secreting T cells (upon to *in vitro* stimulation) were detected in iSIV/LP-treated animals and the depletion of either CD8⁺ or CD25⁺ cells did not alter the unresponsiveness of P27-specific effector T cells (Fig. 10c). Moreover, a strong suppression of activation (Ki-67⁺) of infected (P27⁺) CD4⁺ T cells by non-cytotoxic CD8⁺ T cells was also observed in acutely *in vitro* infected PBMCs taken from iSIV/LP-treated animals and the depletion of CD25⁺ cells did not alter the potent suppression operated by CD25⁺ CD8⁺ T cells on the activation of infected CD4⁺ T cells (Fig. 10d).

Of note the fact that no cell lysis was detected by a high-sensitive cytotoxicity assay (Marchetti et al., 1996) after co-incubating CD8⁺ T cells and CD4⁺ T cells pulsed with non-replicative SIVmac239 in the presence or the absence of SEB and anti-CD3/anti-CD28 antibodies (Fig. 10e).

Finally, the peripheral blood CD8⁺ T cells taken from animals treated since ≥ 2 months by iSIV/LP showed a strong inhibiting activity against viral replication in acutely *in vitro* infected autologous CD4⁺ T cells (Fig. 11a). Furthermore, such a strong antiviral activity of CD8⁺ T cells was also observed equally in acutely *in vitro* infected heterologous CD4⁺ T cells (Fig. 11b), suggesting that a non-classical HLA1 restricted mechanism is involved in the suppressive/inhibiting activity of CD8⁺ T cells.

Purified peripheral blood CD8⁺ T cells taken from macaques immunized with LP/iSIV $\geq$ 2 months earlier had a strong antiviral activity on autologous acutely SIVmac239-infected CD4⁺ T cells stimulated overnight with SEB and anti-CD3/anti-CD28 antibodies and then co-cultured for 5 days. Once SIV-specific CD4⁺ T cells activation is established (48 hours post-stimulation), adding CD8⁺ T cells can no longer inhibit viral replication (Fig. 11c). This observation argues against the potential lysis of target (productively infected) CD4⁺ T cells by CD8⁺ T cells in prolonged culture, as suggested by a previous study in human autoimmune type 1 diabetes (Jiang et al., 2010). This CD8⁺ T cell-mediated antiviral activity needed cell-to-cell contact (Fig. 11d) and also was classical MHC1a-unrestricted as shown by the strong inhibition of viral replication operated by CD8⁺ T cells on acutely infected CD4⁺ T cells from other immunized animals or from control animals (Fig. 11e). Finally, the CD8-mediated antiviral activity was blocked by an anti-MHC-Ib/E antibody but not by the anti-MHC-Ia/ABC antibody, indicating a non-classical MHC-Ib/E-restricted CD8⁺ T cell activity (Fig 11f).

It is established that a CD8⁺ T cell TCR expression is necessary to recognize MHC-Ib/E-peptide complexes carried by target CD4⁺ T cells (Sarantopoulos et al., 2004; Van Kaer, 2010). Using an *in vitro* depletion by antibody-conjugated magnetic microbeads, TCR $\gamma\delta$ and V β 8 were shown not to be involved in CD8⁺ T cell suppression of viral replication (Fig. 11g). TCR $\alpha\beta$ thus appears to play a central role in the recognition of MHC-Ib/E-peptide presentation on infected CD4⁺ T cells. Moreover, by depleting CD8⁺ T cells with available anti-human antibodies cross-reacting with membrane CD (for "Cluster Differentiation") antigens of non-human primates (CD7, CD11a, CD16, CD25 (IL-2RA), CD27, CD28, CD39, CD62L, CD95, CD101, CD122 (IL-2RB), CD127 (IL-7R), CD129 (IL-9R), CD137, CD150, CD183 (CXCR3), CD184 (CXCR4), CD195 (CCR5), CD196 (CCR6), CD197 (CCR7), CD215 (IL-15Ra), CD218 (IL-18Ra), CD223 (LAG3), CD226, CD247, CD272, CD277, CD279 (PD-1), CD305 (LAIR1), and CD357), no CD antigen associated with MHC-Ib/E-restricted CD8⁺ T cells activity could be identified (Table 1). Table 1 below shows the antiviral activity (fold suppression, geometric mean $\pm$ SE) of CD8⁺ T cells taken from 8 iSIV/LP-immunized animals before and after depletions of CD antigen-defined subsets* in the first immunisation study (immunisation No. 1).

Table 1

CD antigens value	Undepleted CD8 ⁺ T cells	Depleted CD8 ⁺ T cells	P
CD7	1387 $\pm$ 301	964 $\pm$ 326	0.313

CD11a		941 ± 377	0.568
CD16	529 ± 152	533 ± 99	0.772
CD25		691 ± 258	0.490
CD27	704 ± 242	761 ± 122	0.867
CD28		1021 ± 177	0.407
CD39	970 ± 361	1256 ± 354	0.710
CD62L		1013 ± 302	0.832
CD95	813 ± 238	775 ± 239	0.954
CD101		980 ± 197	0.613
CD122	997 ± 411	784 ± 265	0.412
CD127		715 ± 339	0.545
CD129	872 ± 325	855 ± 252	0.813
CD137		868 ± 306	0.852
CD150	889 ± 223	924 ± 231	0.959
CD183		633 ± 198	0.354
CD184	1452 ± 253	1265 ± 447	0.841
CD195		1083 ± 295	0.374
CD196	789 ± 245	652 ± 280	0.882
CD197		878 ± 247	0.789
CD215	1221 ± 213	1214 ± 445	0.621
CD218		739 ± 371	0.477
CD223	623 ± 293	1208 ± 248	0.197
CD226		1234 ± 192	0.237
CD247	914 ± 288	940 ± 279	0.991
CD272		1056 ± 231	0.846
CD277	1247 ± 216	957 ± 282	0.523
CD279		1197 ± 151	0.616
CD305	798 ± 245	1157 ± 241	0.233
CD357		820 ± 127	0.807

*In each batch of experiment, antiviral activity (fold suppression, geometric mean ± SE) of CD8+ T cells taken from 8 iSIV/LP-immunized animals depleted (or not) with 2 anti-CD antigen antibodies was performed.

C-II-III Protection of animals from intra-rectal challenges by oral immunotolerance

Three months after the administration of iSIV/LP or control preparations, the 8 iSIV/LP-immunized and the 7 control animals were intrarectally challenged with a single high dose (100,000 TCID₅₀) of SIVmac239. Eight out of 8 iSIV/LP-treated animals were protected from intra-rectal challenge of pathogenic SIVmac239 while the 4 iSIV-treated and the 4 LP-treated animals were infected by the same intra-rectal viral challenge (Fig. 12a and b, left part of the Figures).

C-II-IV Protection of animals from intravenous challenge by oral immunotolerance

Two months after this first challenge, 4 out of the 8 monkeys received a second challenge via the intravenous route (200 TCID₅₀). All of them showed a slight peak of replication (≤ 200 SIV DNA copies/million PBMC and 200 SIV RNA copies/ml of plasma) at day 10 post-challenge; however by day 30, PBMC SIV DNA had decreased to ≤ 10 copies/million cells and plasma SIV RNA was undetectable (≤ 10 copies/ml), indicating the lack of *in vivo* active replication of the virus (Fig. 12a and b, right part of the Figures). In contrast, 2 naïve animals which received the same intravenous SIVmac239 challenge (200 TCID₅₀) were successfully infected. The 4 remaining monkeys were intrarectally re-challenged (100.000 TCID₅₀) and all of them remained fully protected (Fig. 12a and b, right part of the Figures).

C-II-V Confirmation *in vivo* of the role of CD8⁺ T cells

Five months after this second challenge, in order to confirm *in vivo* the role of CD8⁺ T cells, 3 intravenous injections of a mouse-human chimeric monoclonal anti-CD8 antibody (cMT-807, Centocor) were given over a period of one week (days 300, 304 and 307 post-immunization) to the 8 already-challenged monkeys to temporarily deplete their CD8⁺ T cells from peripheral blood and lymphoid organs (Fig. 13a & b). No viral RNA or DNA emergence was detected in the 4 macaques re-challenged by intrarectal route, demonstrating again their full sterile protection; in contrast, a strong viral replication accompanied the depletion of CD8⁺ T cells from lymphoid organs of the 4 intravenously challenged animals as shown by their plasma viral loads that peaked at 10^6 RNA copies/ml and their PBMC and lymph node proviral loads that reached 10^4 DNA copies/ 10^6 cells by day 15 (the nadir of CD8⁺ T cells depletion); by days 60-90, when the 4 monkeys had recovered baseline CD8⁺ T cells concentrations, plasma SIV RNA and PBMC and lymph nodes SIV DNA recovered also baseline levels (Fig. 13 c, d & e). This confirmed the unique role of iSIV/LP-induced CD8⁺ T cells in the control of *in vivo* viral replication in intravenously SIV-challenged animals in which replication-competent virus remained latent in presumably in quiescent memory CD4⁺ T cells.

Eight months after the second challenge, the 4 intrarectally rechallenged monkeys as well as the 4 intravenously rechallenged ones received a third challenge, this time via the intrarectal route with SIVB670 (100,000 TCID₅₀), a distinct infectious SIV strain. The 8 animals remained fully protected over the next 12 months as shown by their undetectable SIVB670 DNA and RNA levels whereas 2 naïve animals were successfully infected by the same SIVB670 challenge, demonstrating that LP/iSIVmac239-generated MHC-Ib/E-restricted CD8⁺ T cells were cross-protective

through preventing the activation of CD4+ T cells infected by other SIV strains (Figures 14a & b).

To determine the duration of efficacy for preventing SIV diseases in the iSIV/LP-treated animals, a second immunization with iSIV/LP was conducted in 8 new macaques of Chinese origin and the *in vitro* antiviral activity of their CD8+ T cells was checked overtime without SIV challenge. Such an *in vitro* antiviral activity was detected as from 60 days post-immunization as compared to the control animals either treated with LP (n = 4) or iSIV (n = 4) alone.

Ex-vivo anti-SIV activity levels were maintained until day 420 in 7 out of 8 monkeys while the antiviral activity of one monkey progressively decreased from day 360 to reach baseline levels of control monkeys by day 420 (Fig. 15a). On day 420 post-immunization, the 16 animals were intrarectally challenged with 100,000 TCID₅₀ of SIVmac239. Seven out of the 8 iSIV/LP-immunized animals acquired a sterile immunity without any SIV RNA and DNA emergence in plasma and PBMC (Fig. 15b & c), as well as in rectal mucosa lymphocytes (where they were measured from day 1 post challenge) and pelvic lymph nodes (Fig. 16a to 16e) while one immunized monkey was fully infected. Importantly, the evolution of the *ex-vivo* antiviral activity of the 8 vaccinated monkeys allowed to predict from day 360 post immunization (i.e., 60 days before their challenge) the 7 protected monkeys and the unprotected one (Fig. 15a to c).

C-II-VI Conclusions

It is disclosed herein, in the macaque model, that the administration of inactivated SIVmac239 (iSIV) and commensal *Lactobacillus plantarum* (LP) (referred to as a tolerogenic adjuvant) generates MHC-Ib/E-restricted CD8+ T regulatory cells that induced the suppression of activation of SIV antigen-presenting CD4+ T cells and thereby the suppression of SIV replication and the protection of macaques from SIV challenges.

A mixture made of inactivated iSIV and LP was administered intragastrically to a total of 16 animals and 15 controls. Four to 14 months later, all animals were challenged intrarectally with pathogenic SIVmac239.

Full protection against SIV infection was observed in 15 out of 16 iSIV/LP-administered animals; in contrast, infection was established in all control animals and one vaccinated monkey. The unprotected monkey can be predicted by an *ex vivo* antiviral assay 60 days before the intrarectal challenge. Eight protected animals remained protected after

a second SIVmac239 challenge given intravenously in 4 monkeys and intrarectally in the other 4.

The 8 iSIV/LP-delivered animals had complete lack of SIV-specific peripheral blood CD4+ T cell proliferation and did not raise any systemic SIV-specific antibodies (IgG, IgM, or IgA).

Moreover, their SIV-specific peripheral blood CD8+ T cell had several particularities:

- 1) they proliferated well but without interferon- γ secretion upon to *in vitro* stimulation;
- 2) they strongly suppressed the activation of acutely infected autologous CD4+T cell;
- 3) both functions remained unchanged after depletion of CD25+ cells;
- 4) they inhibited also SIV replication in acutely infected allogenic CD4+ T cells; and
- 5) their suppressive/inhibiting action was MHC-Ib/E-restricted.

These results show that intra-gastric co-administration of iSIV and LP allows macaques to develop virus-specific non-cytotoxic MHC-Ib/E-restricted CD8+ regulatory T cells which generate an SIV-specific immunotolerance and that very surprisingly such a virus-specific immunotolerance is associated with vaccine protection of animals against the establishment of SIV infection.

It is shown hereinabove that the pharmaceutical composition according to the present invention prevents HIV and SIV infections in humans/mammals. This preventive action is obtained in macaques by inducing a "Ts" immunotolerance in the tolerogenically-vaccinated subjects (i.e., the mammals having been administered the pharmaceutical composition). Said "Ts" immunotolerance is herein demonstrated to involve virus-specific non-cytotoxic MHC-Ib/E-restricted suppressive CD8 regulatory T cells, the presence and the activity of which being shown to:

- inhibit SIV replication in acutely-infected CD4+T cells of macaques having been administered the pharmaceutical composition of the present invention (*in vitro*); and/or
- prevent SIV replication in tolerogenically-vaccinated macaques that are challenged with infectious SIV (*in vivo*).

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CLAIMS

1. A therapeutic mucosal or intradermal or intraepithelial vaccine composition for use in a method for treating an infection by a HIV virus in a human in need thereof, comprising:
 - an antigen made of inactivated autologous HIV virus; and
 - a tolerogenic adjuvant.
2. The composition according to claim 1, wherein said tolerogenic adjuvant and said antigen are present as distinct components in said composition.
3. The composition according to claim 1 or 2, wherein said tolerogenic adjuvant is selected from:
 - non-pathogenic bacteria;
 - attenuated pathogenic bacteria; and
 - inactivated (optionally, also attenuated) pathogenic bacteria.
4. The composition according to claim 3, wherein said attenuated pathogenic bacteria is BCG.
5. The composition according to claim 3, wherein said non-pathogenic bacterium is one or more *Lactobacillus* sp.
6. The composition according to claim 5, wherein said non-pathogenic bacterium is *Lactobacillus plantarum*.
7. The composition according to anyone of claims 1 to 6, wherein it is a mucosal composition, preferably selected from nasal, oral, sub-lingual, tracheal, pharyngeal, bronchial, esophageal, gastric, duodenal, intestinal, rectal, preputial, and vaginal compositions.

8. The composition according to anyone of claims 1 to 7, wherein said HIV virus is HIV-1 or HIV-2.
9. The composition according to anyone of claims 1 to 8, wherein said composition induces and, preferably, maintains immunotolerance to said HIV virus in said human, thereby treating said infection in said human.
10. The composition according to claim 9, wherein said immunotolerance is at least characterized by a strong non-cytotoxic, MHC-Ib/E-restricted CD8+T cell response suppressing the early activation of HIV Gag and/or Pol antigen-presenting CD4+T cells.
11. The composition according to claim 9 or 10, wherein said immunotolerance is determined in said human by *in vitro* detecting the presence and activity of virus-specific non-cytotoxic MHC--Ib/E-restricted suppressive CD8 cells.

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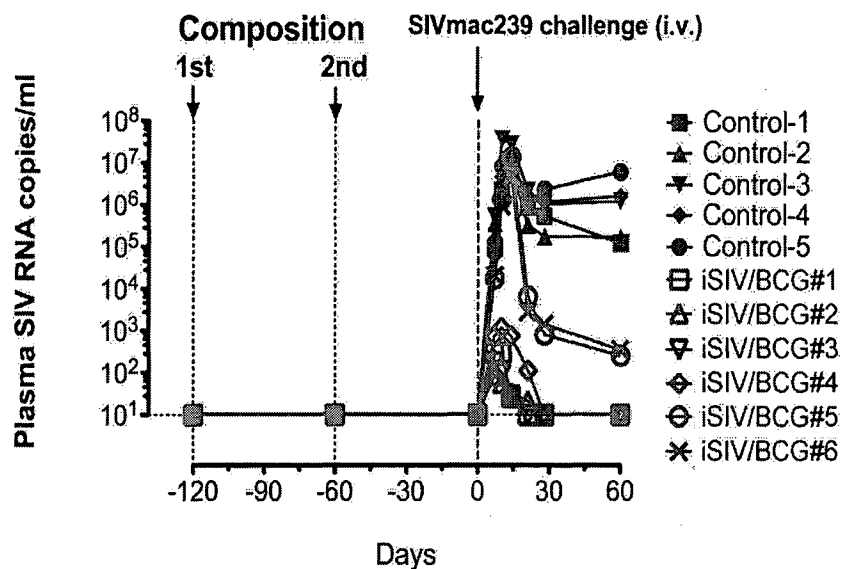


Figure 1

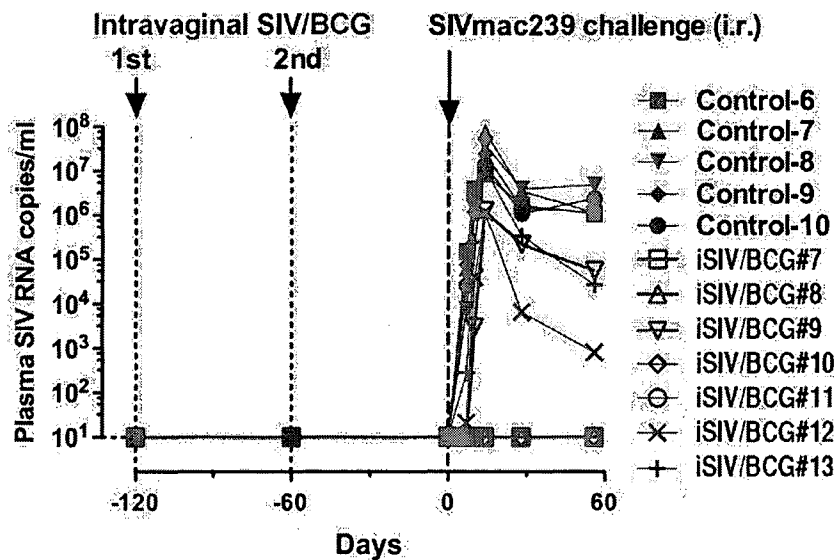


Figure 2

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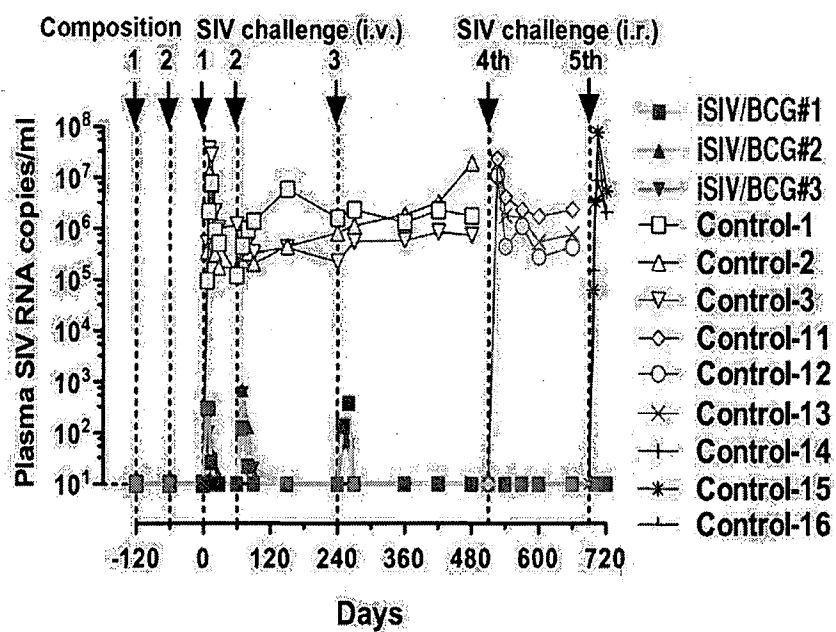


Figure 3

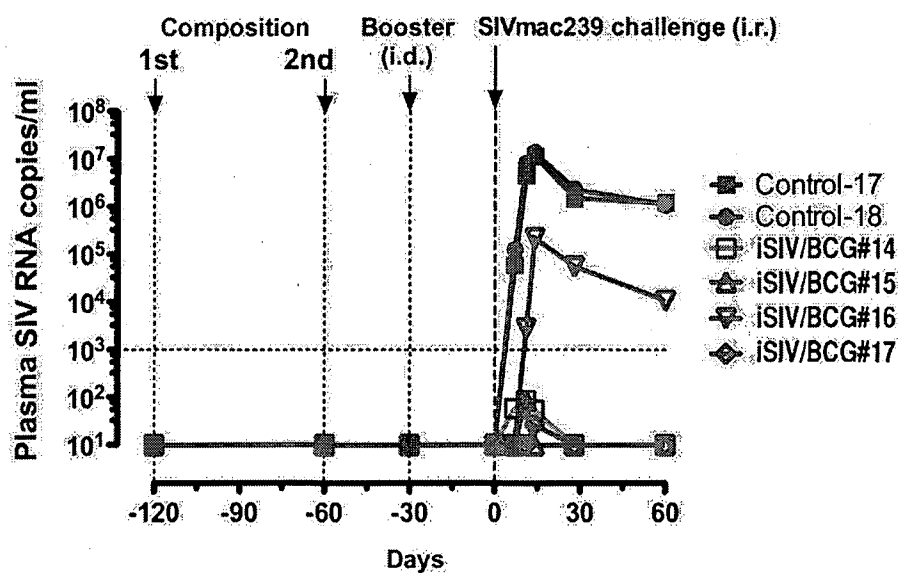


Figure 4

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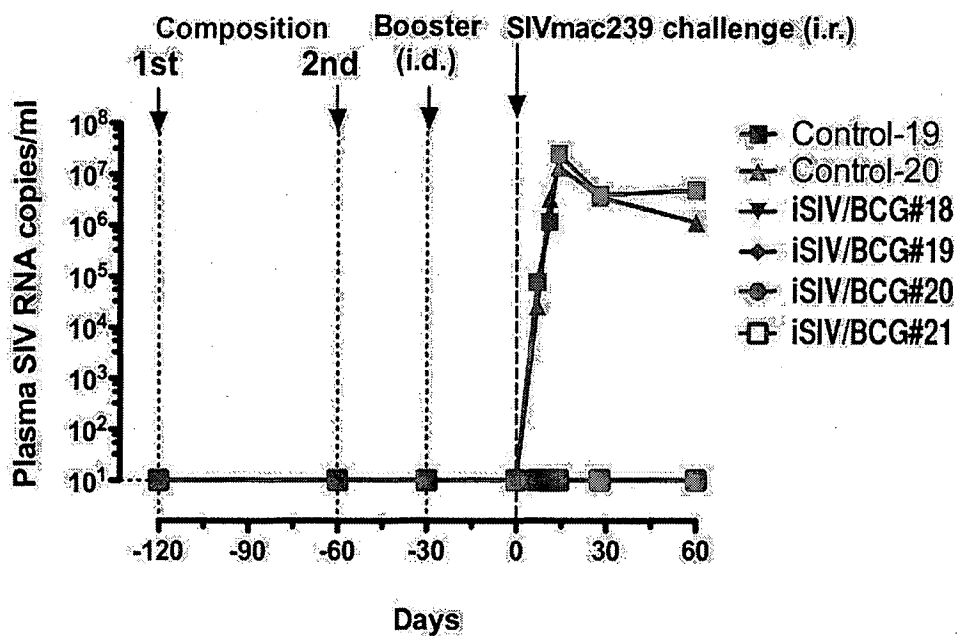


Figure 5

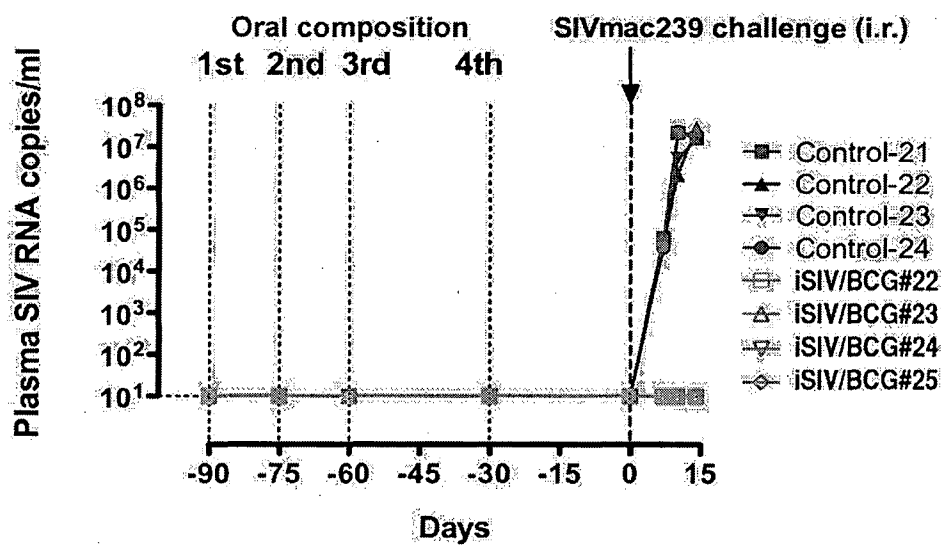


Figure 6

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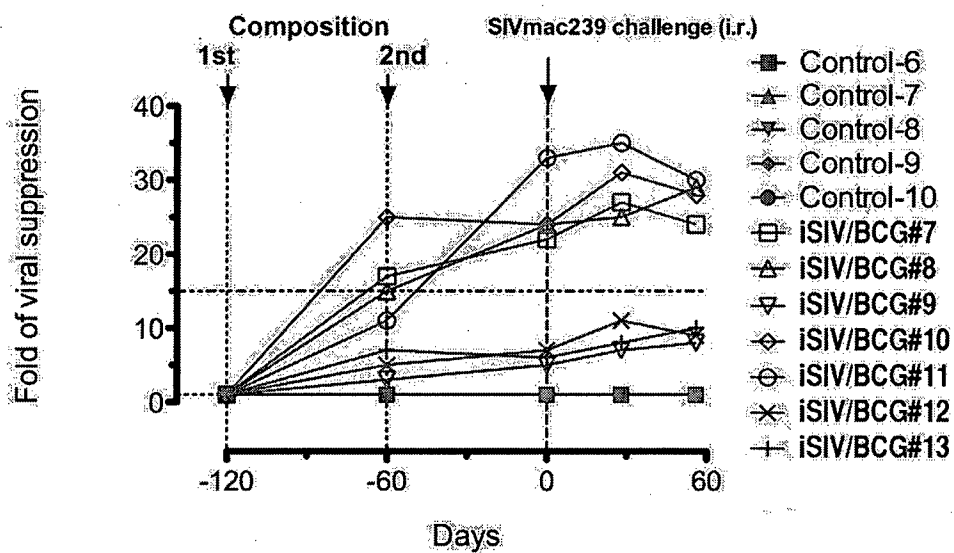


Figure 7

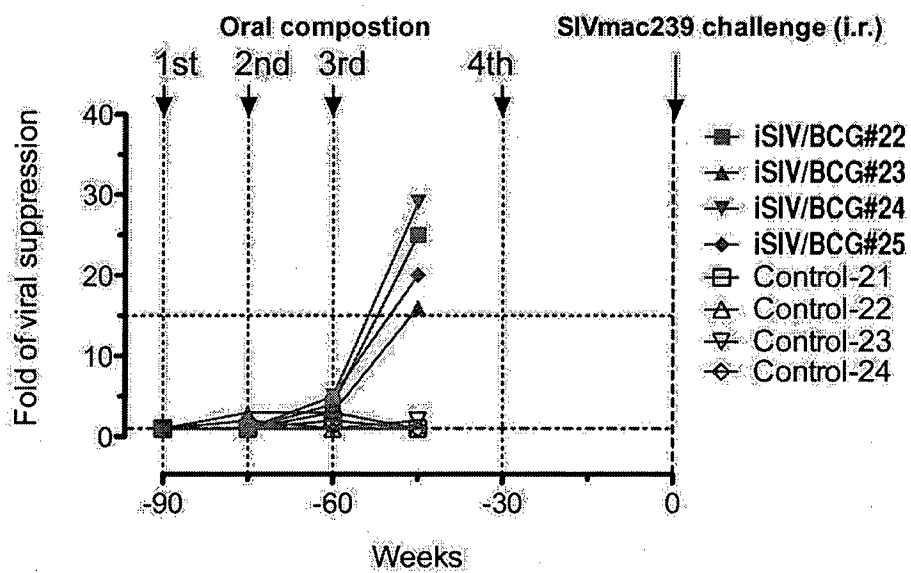


Figure 8

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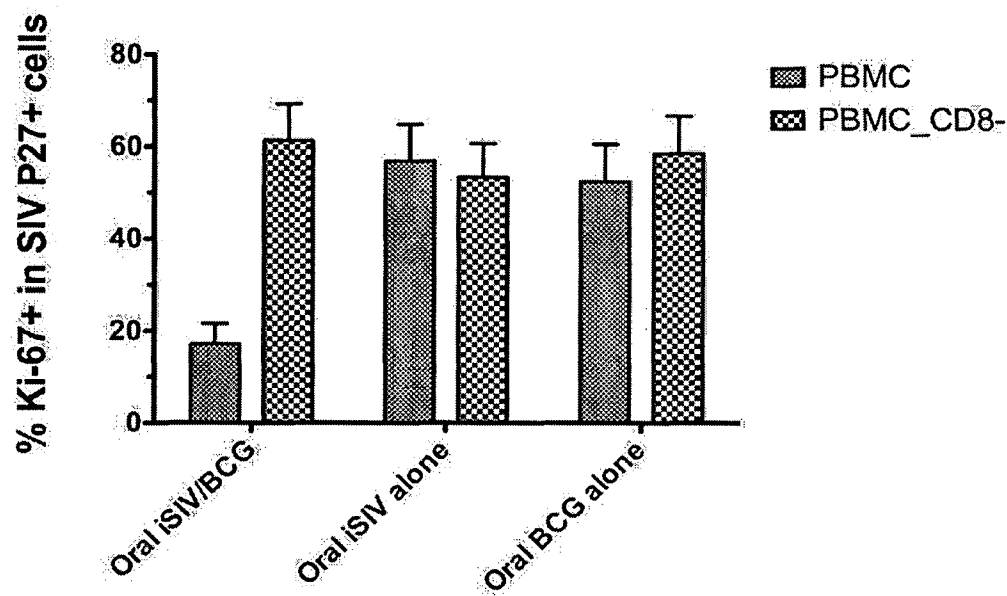


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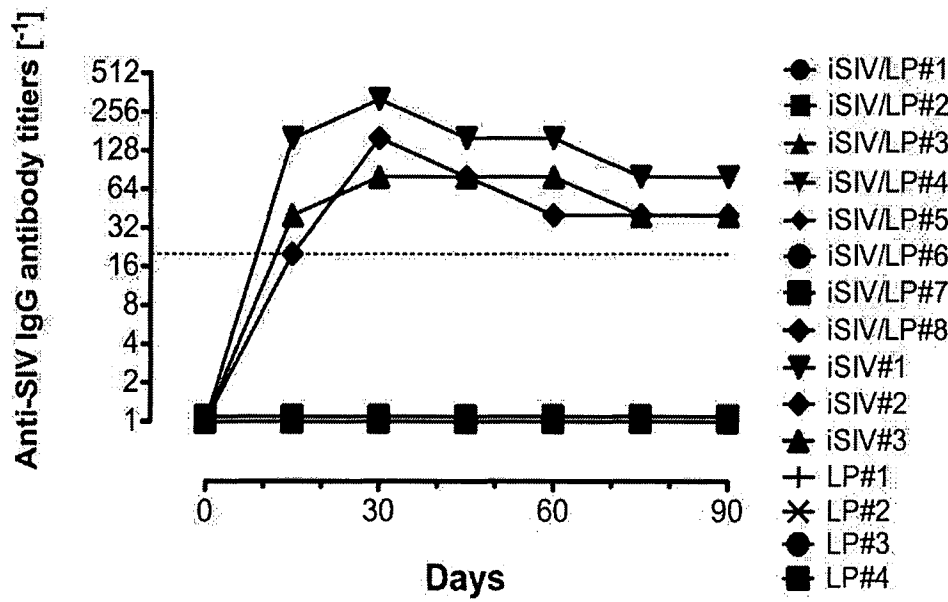


Figure 10a

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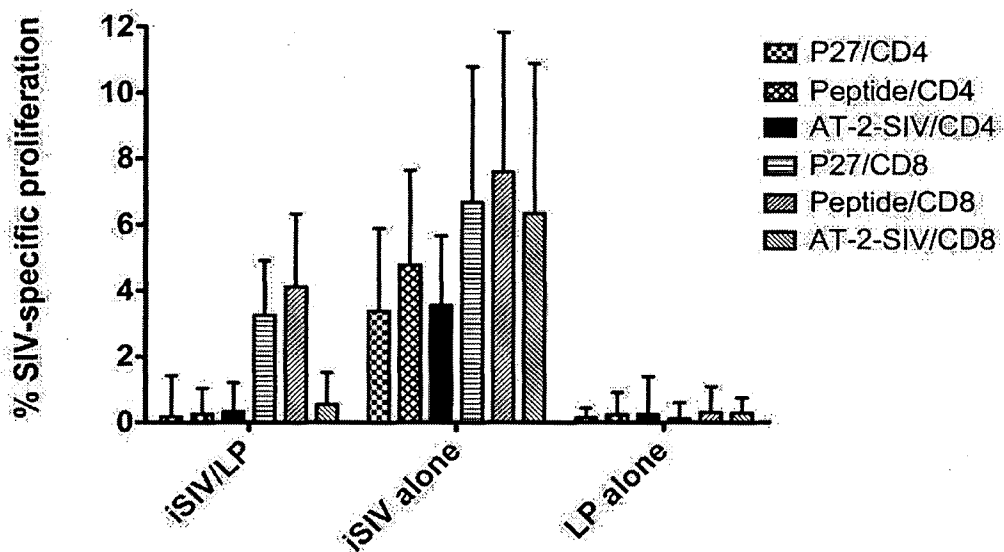


Figure 10b

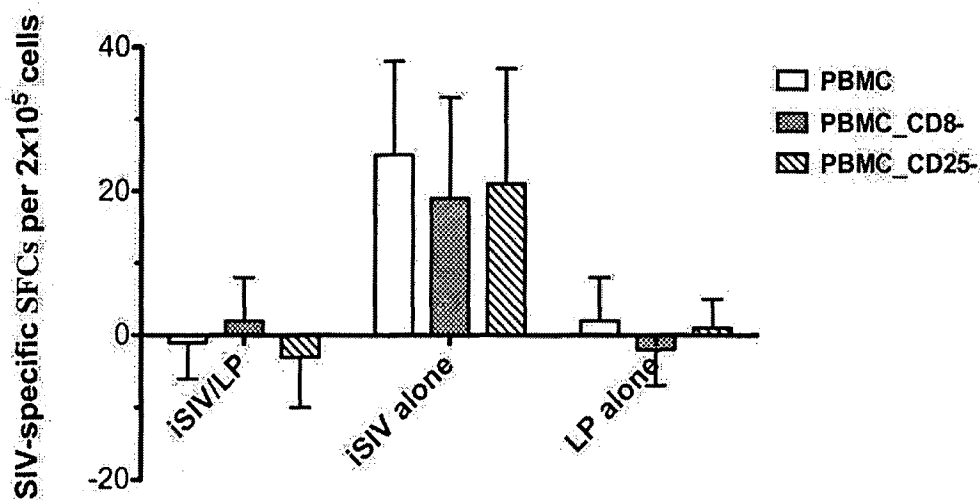


Figure 10c

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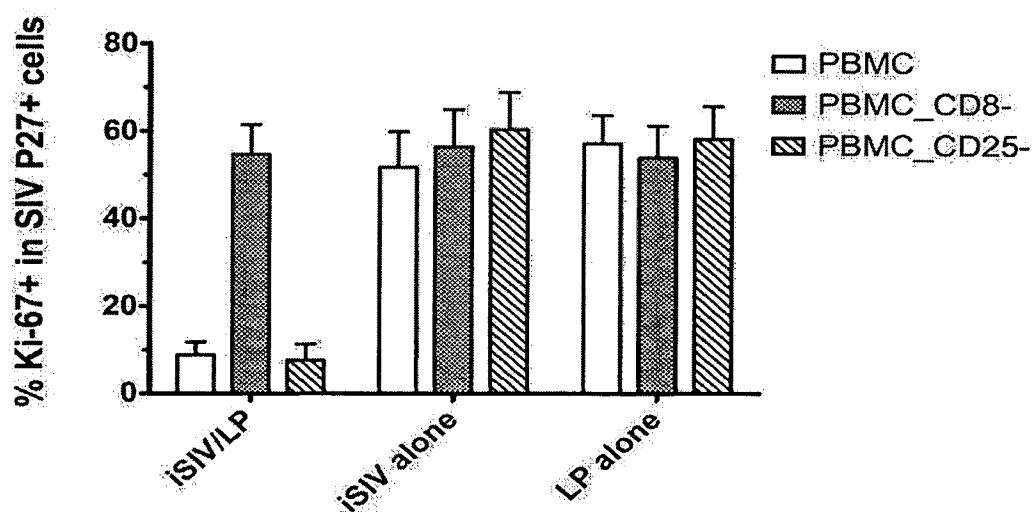


Figure 10d

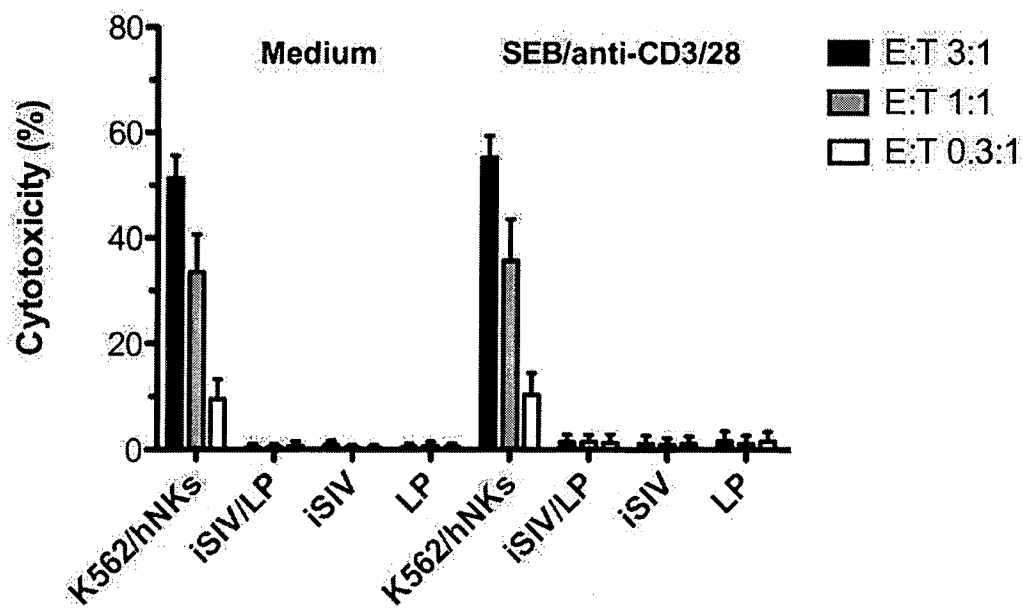


Figure 10e

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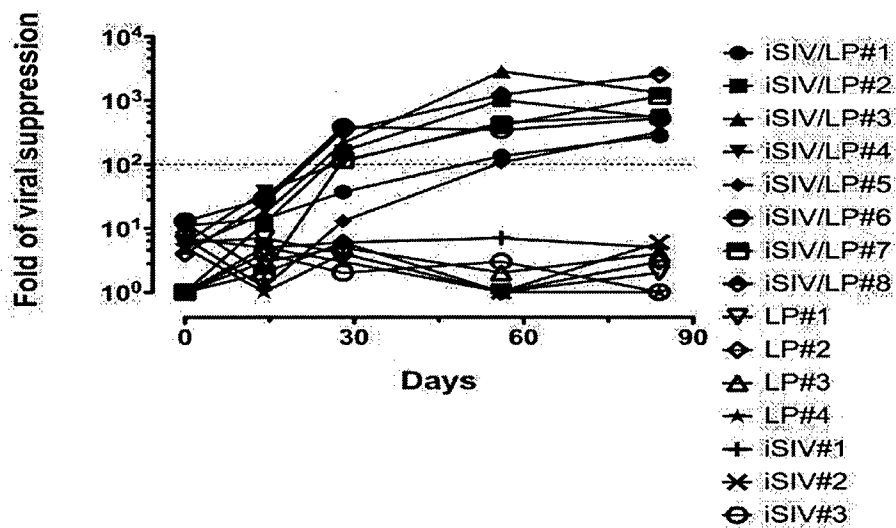


Figure 11a

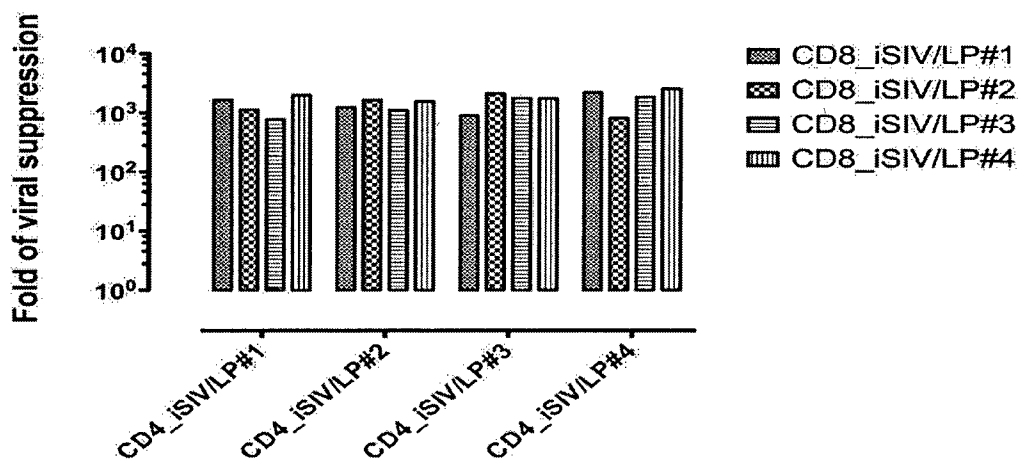


Figure 11b

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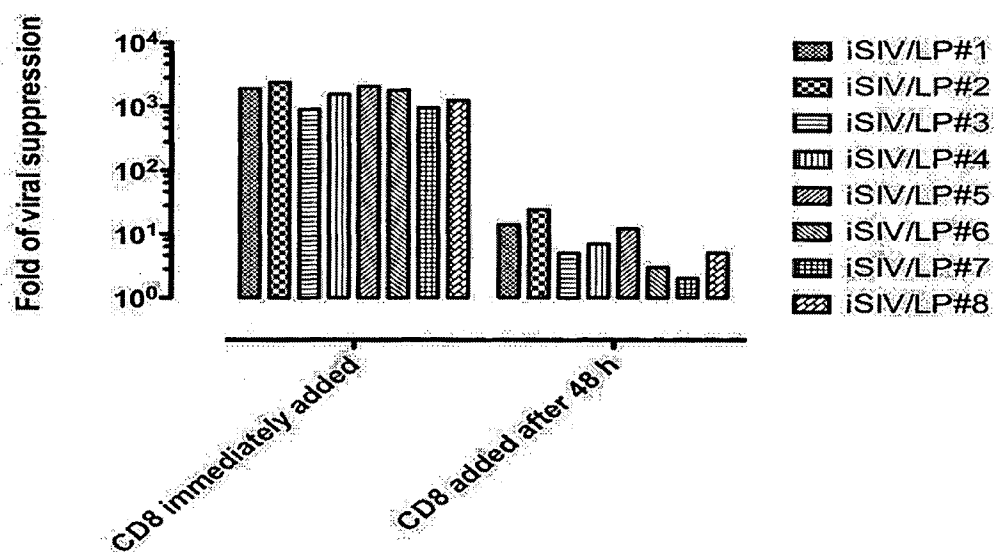


Figure 11c

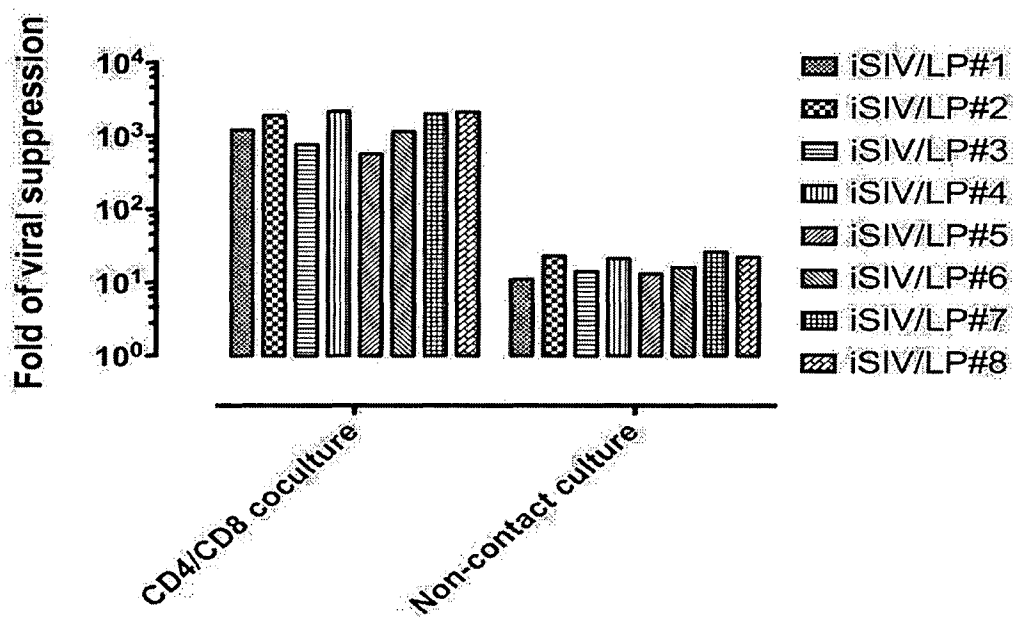


Figure 11d

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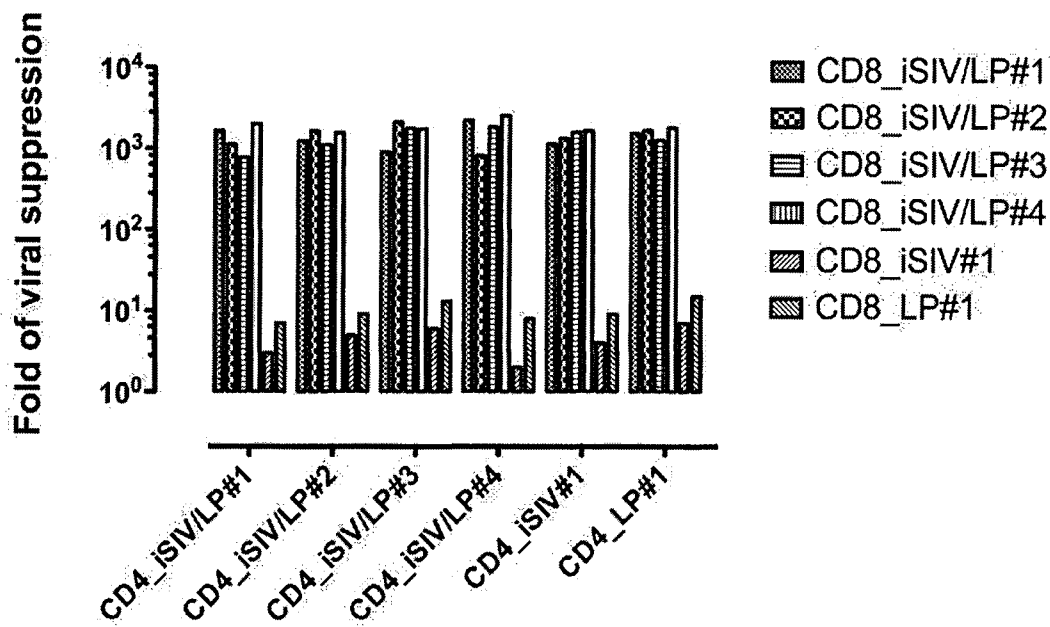


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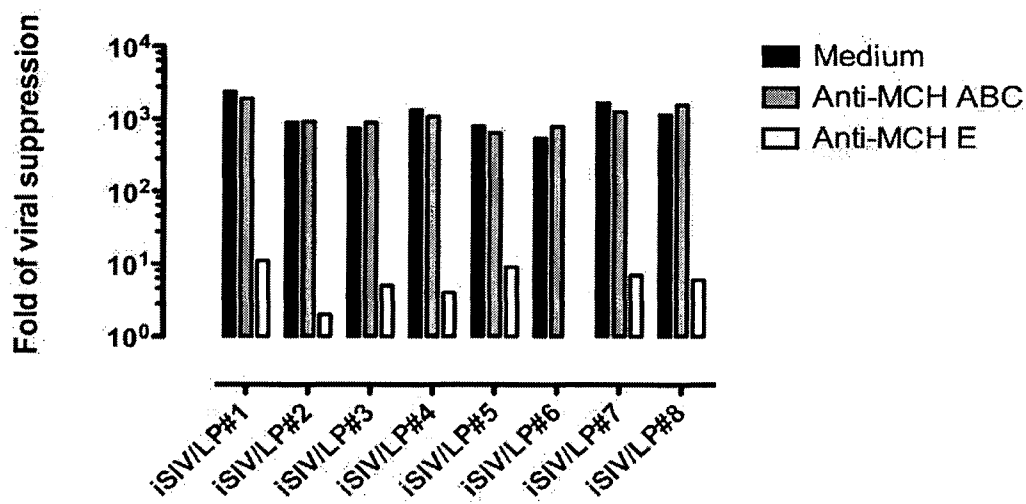


Figure 11f

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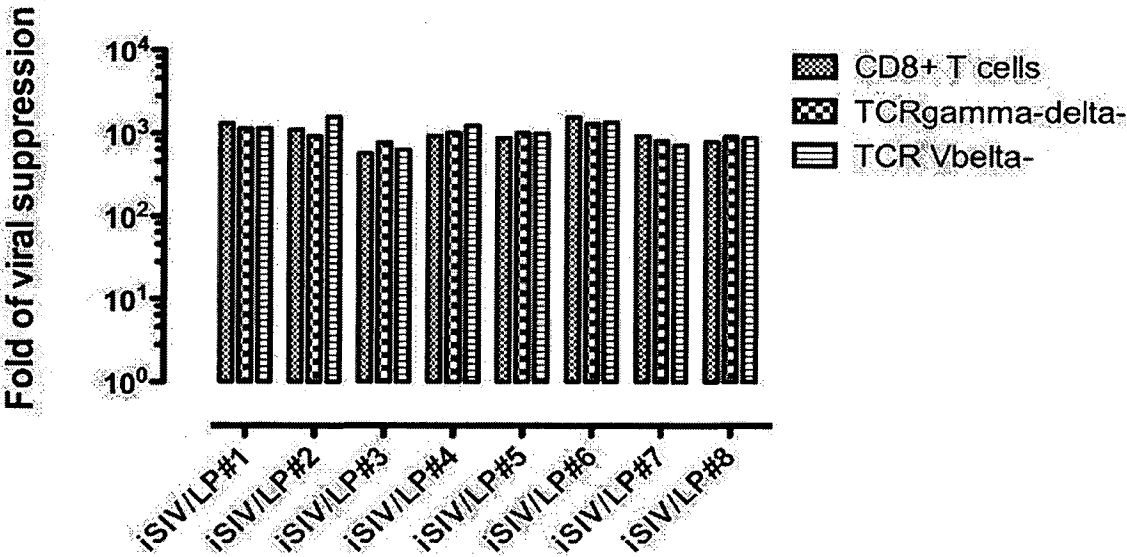


Figure 11g

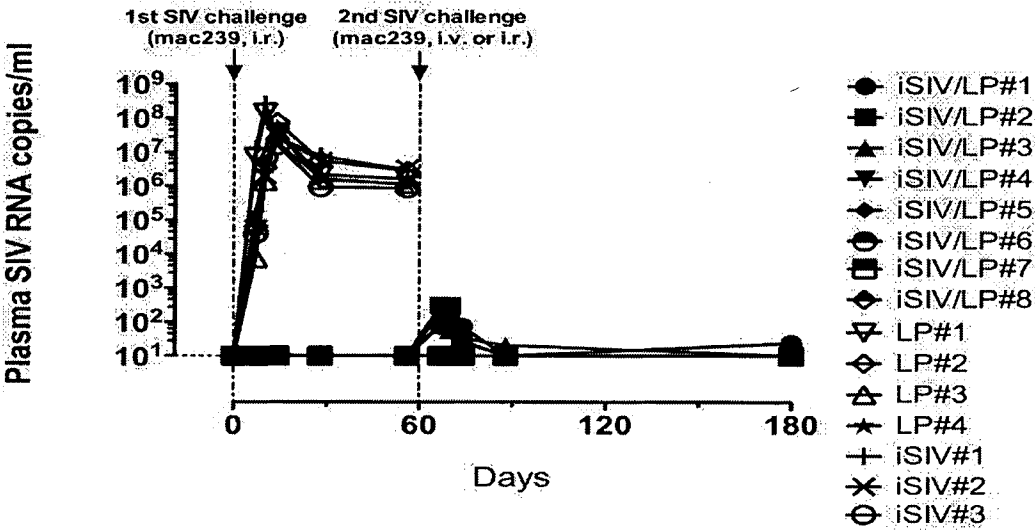


Figure 12a

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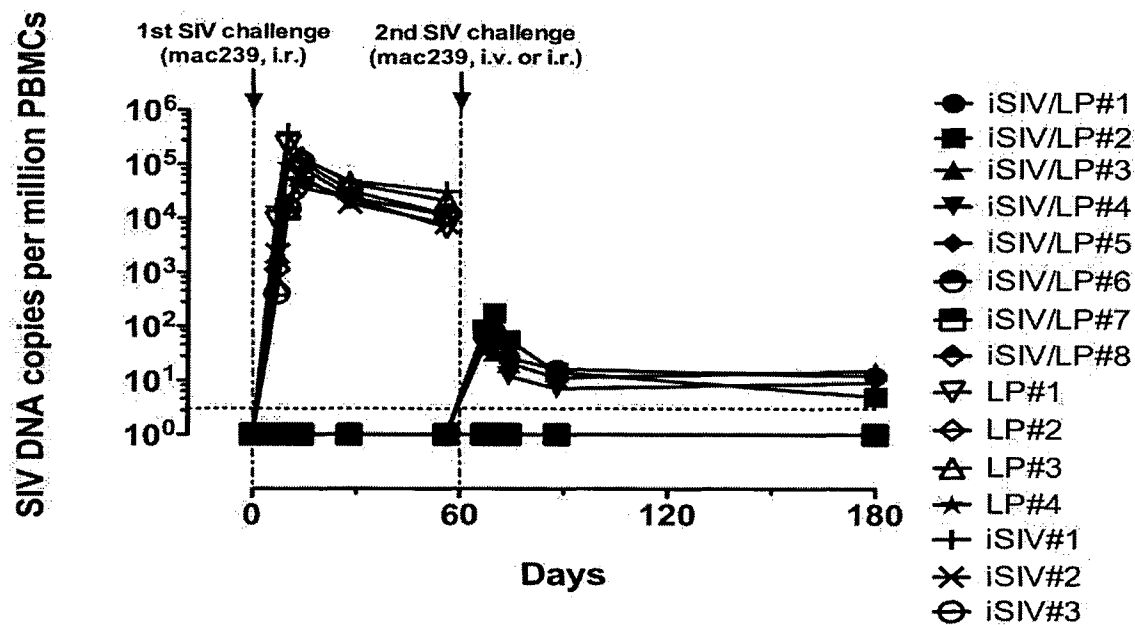


Figure 12b

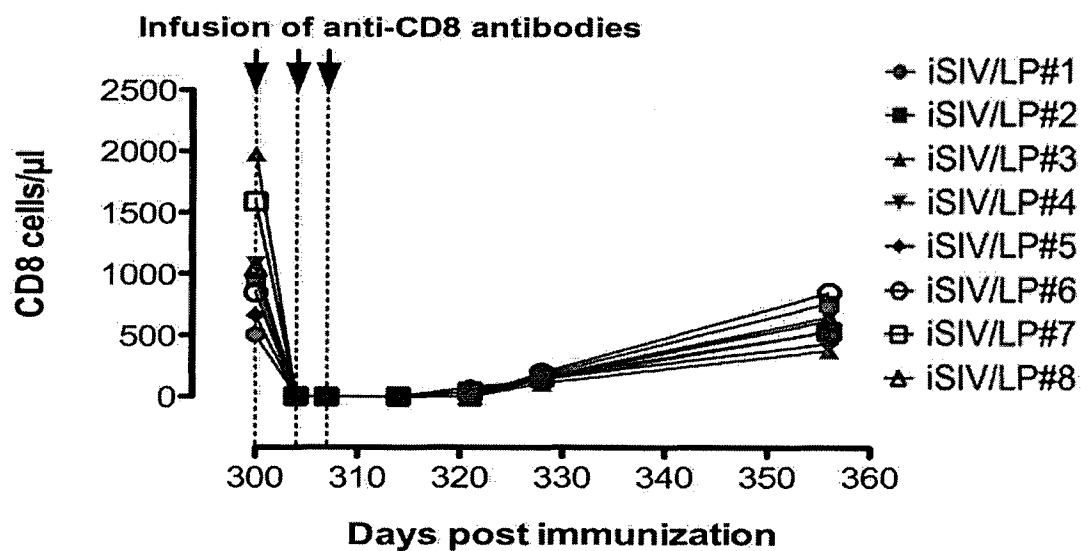


Figure 13a

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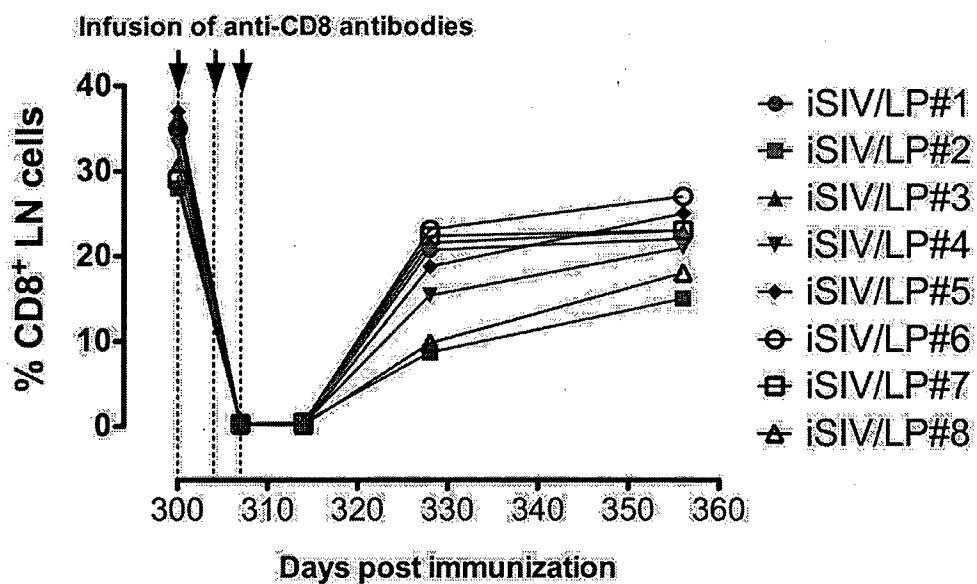


Figure 13b

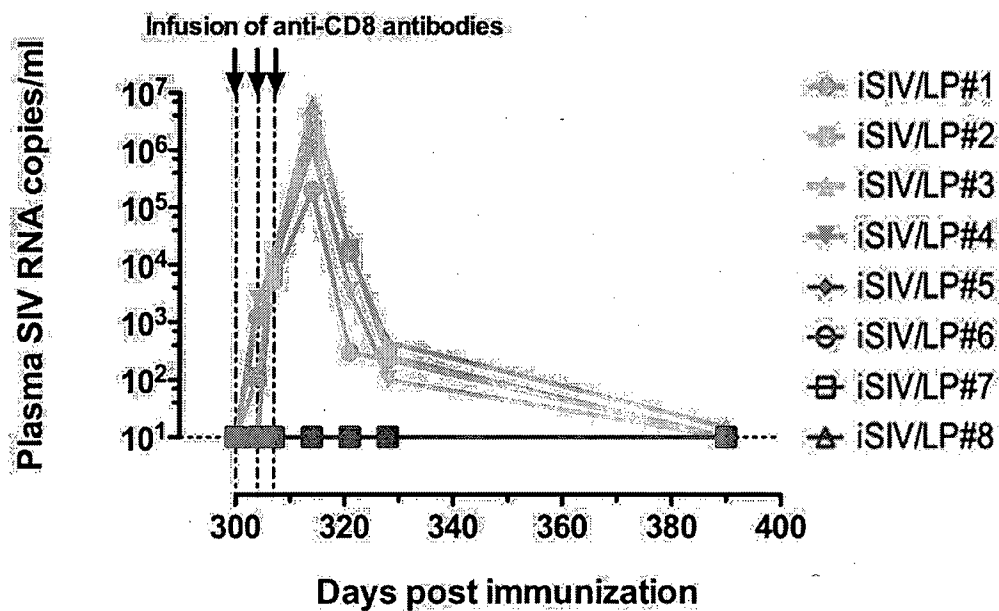


Figure 13c

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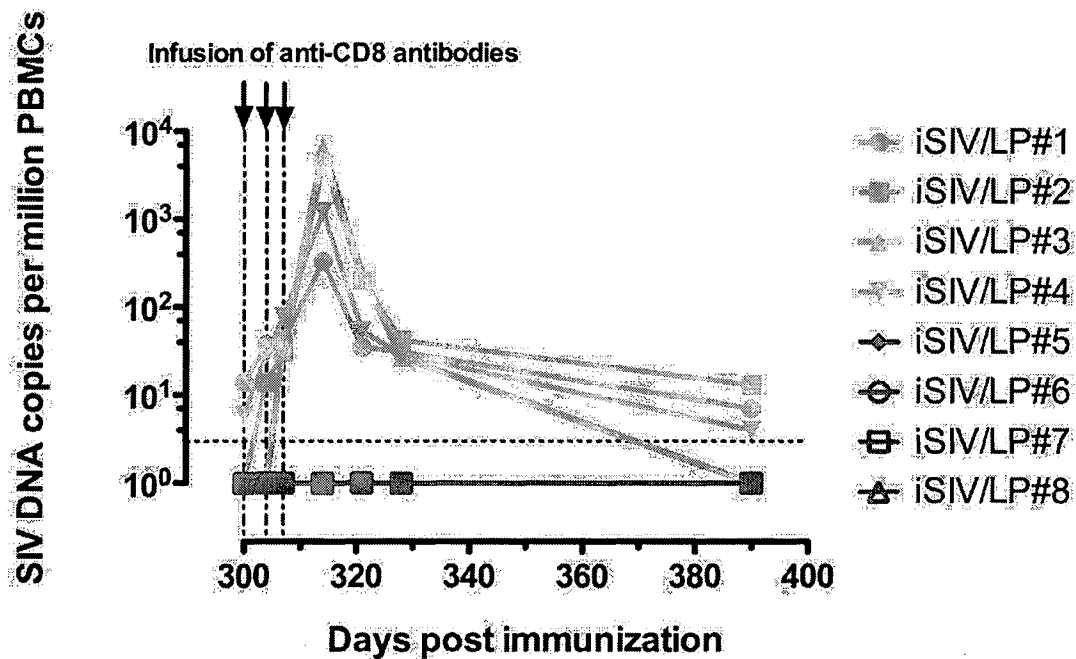


Figure 13d

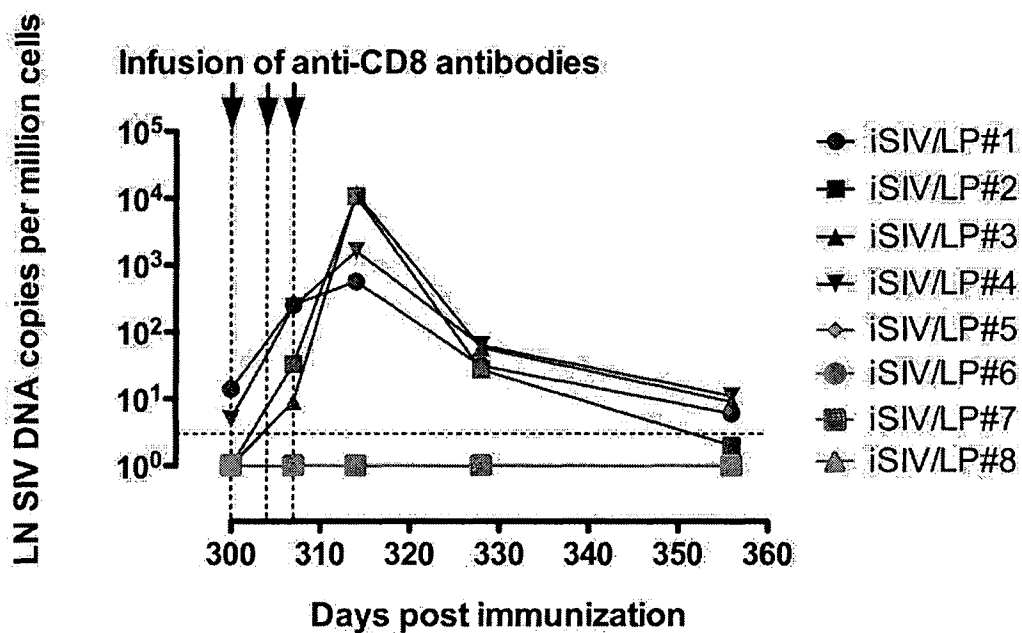


Figure 13e

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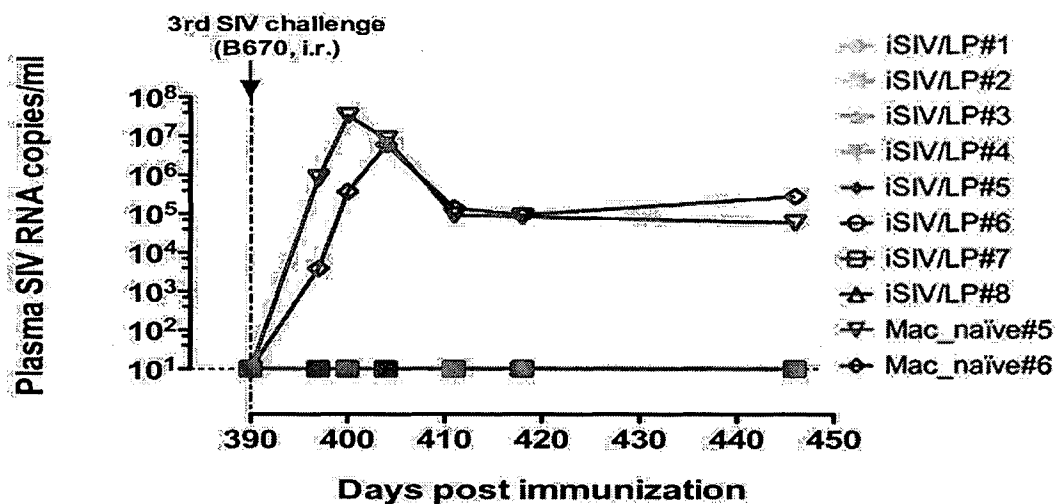


Figure 14a

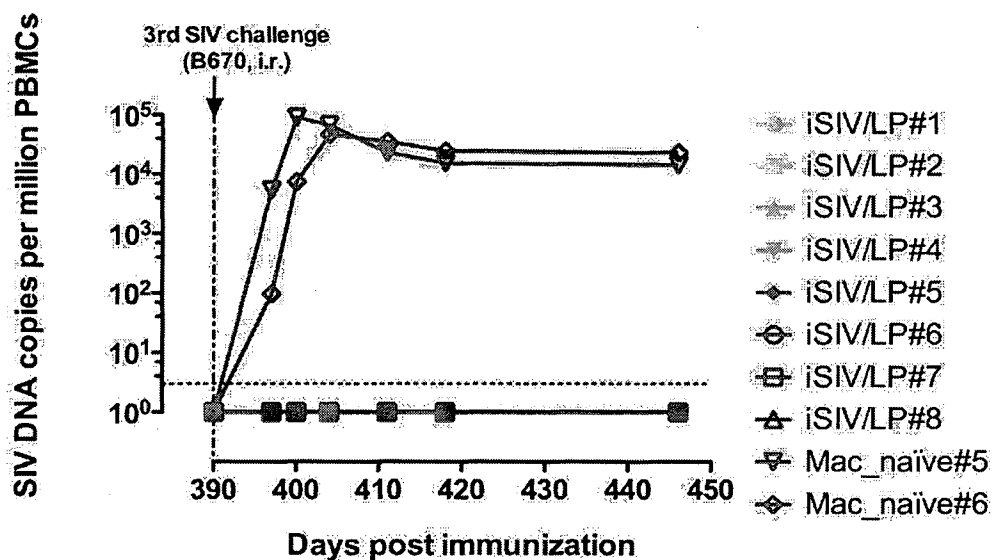


Figure 14b

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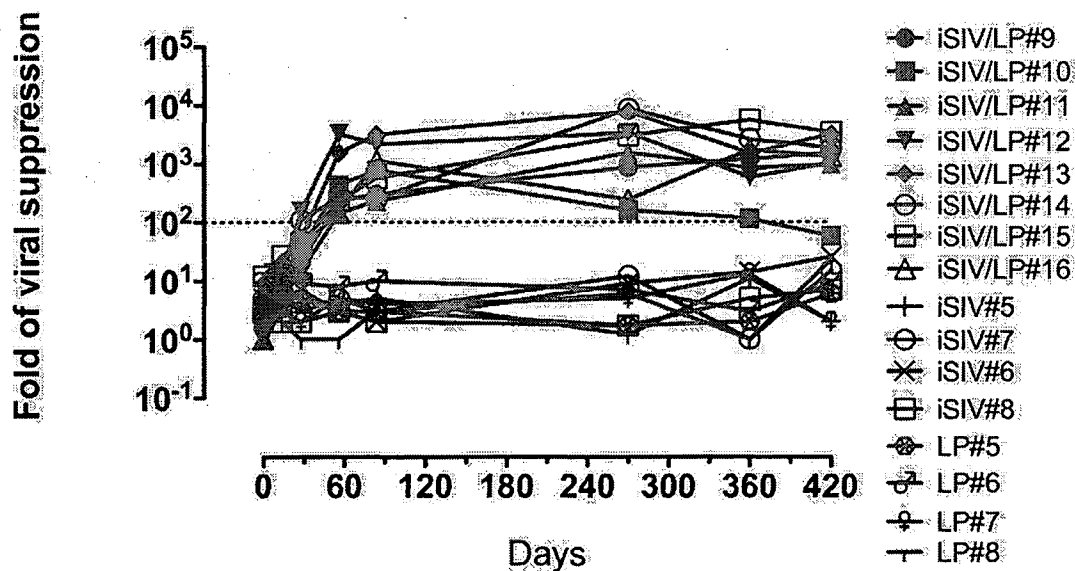


Figure 15a

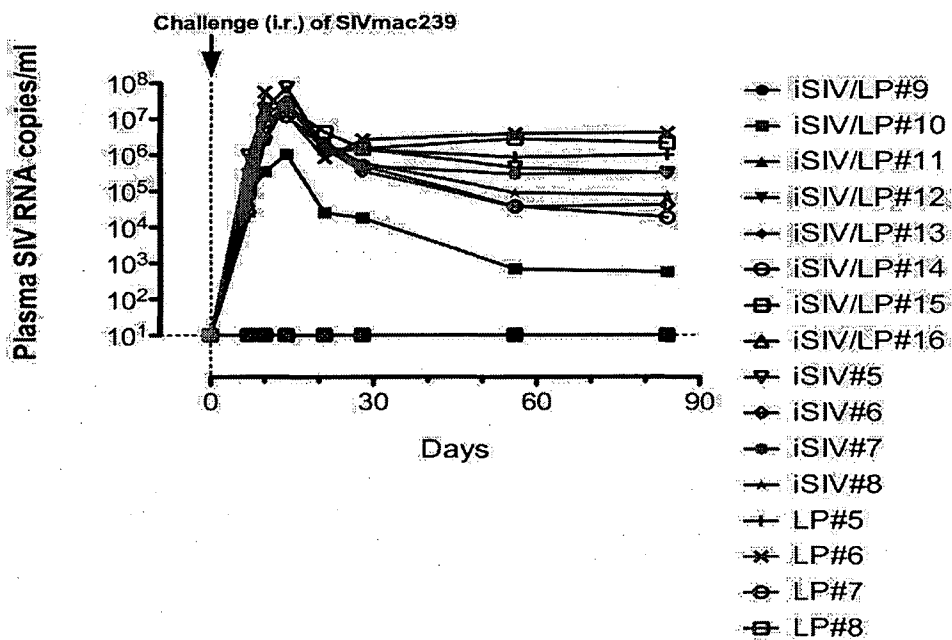


Figure 15b

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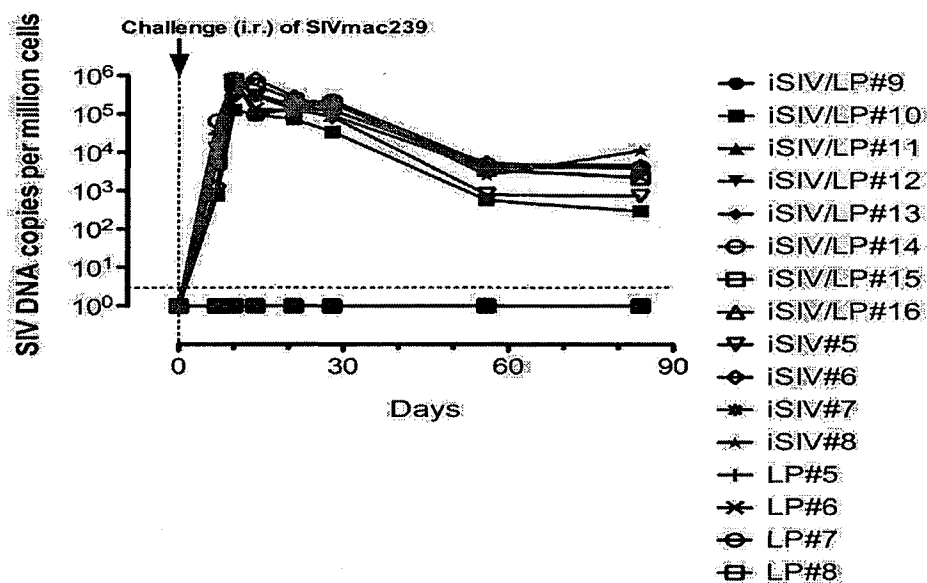


Figure 15c

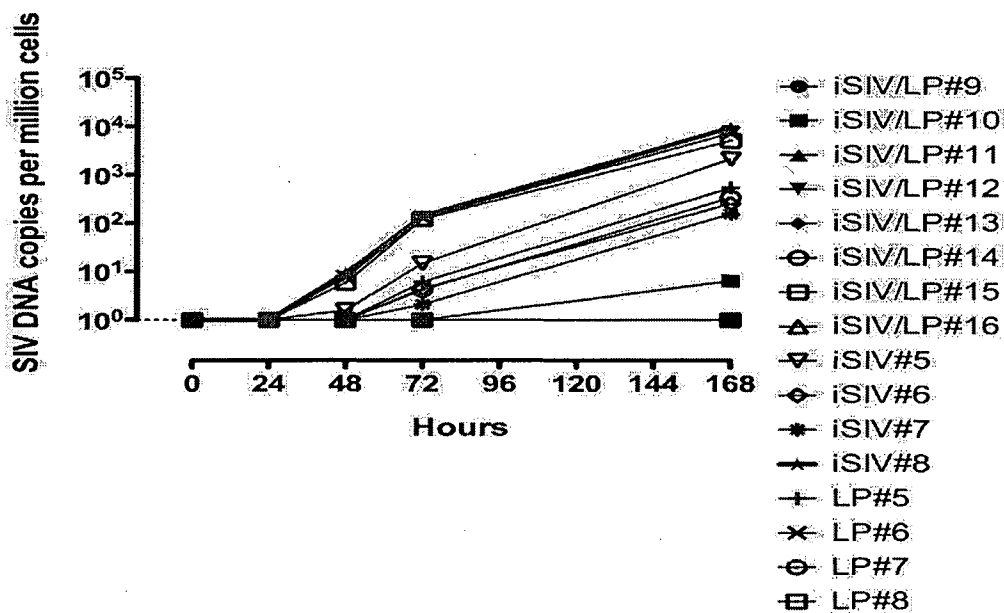


Figure 16a

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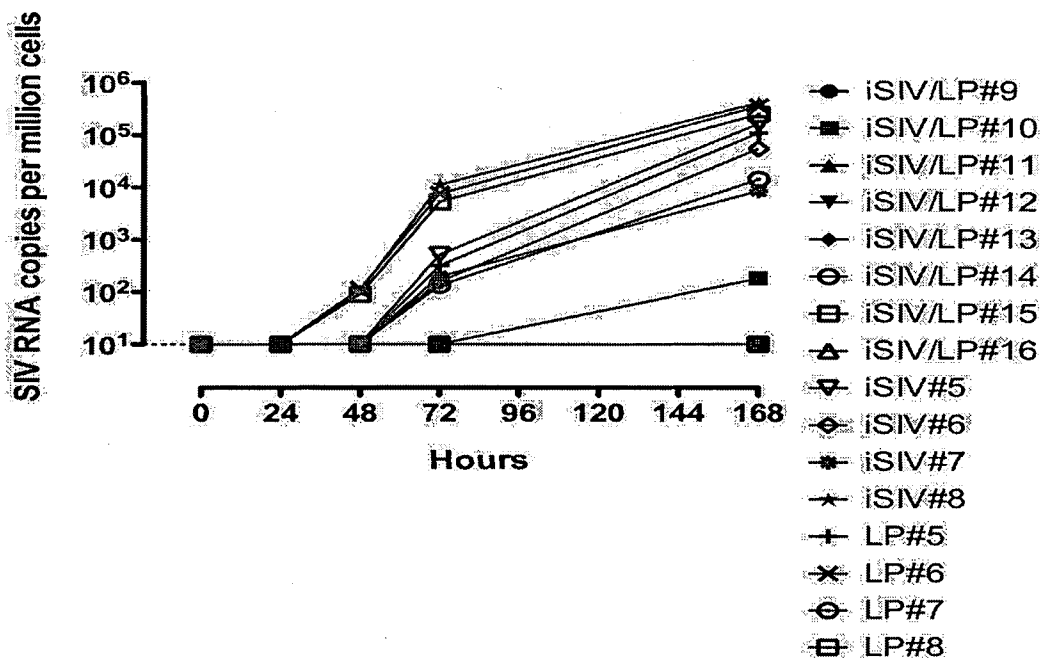


Figure 16b

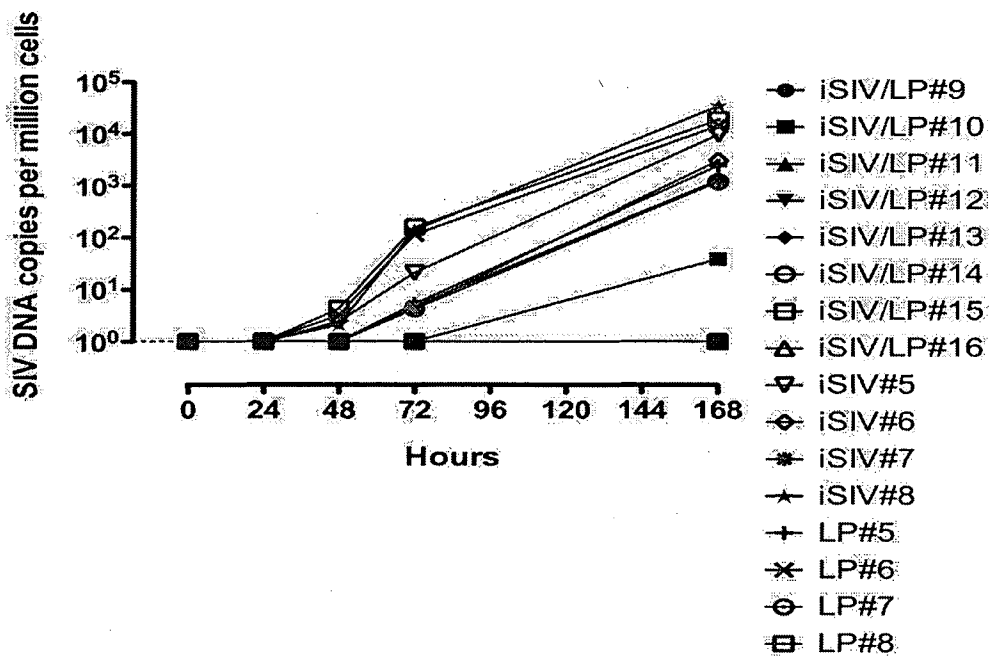


Figure 16c

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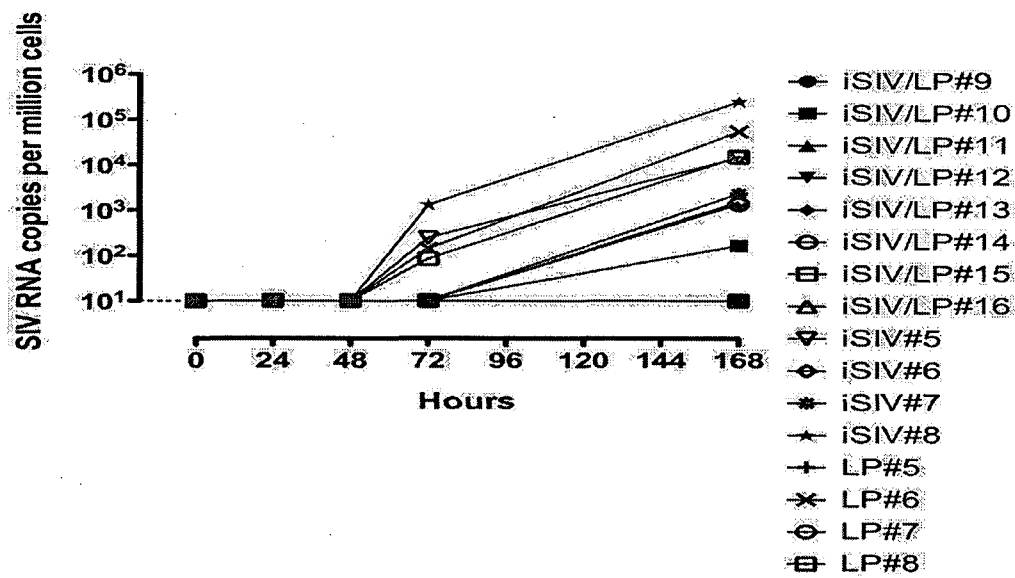


Figure 16d

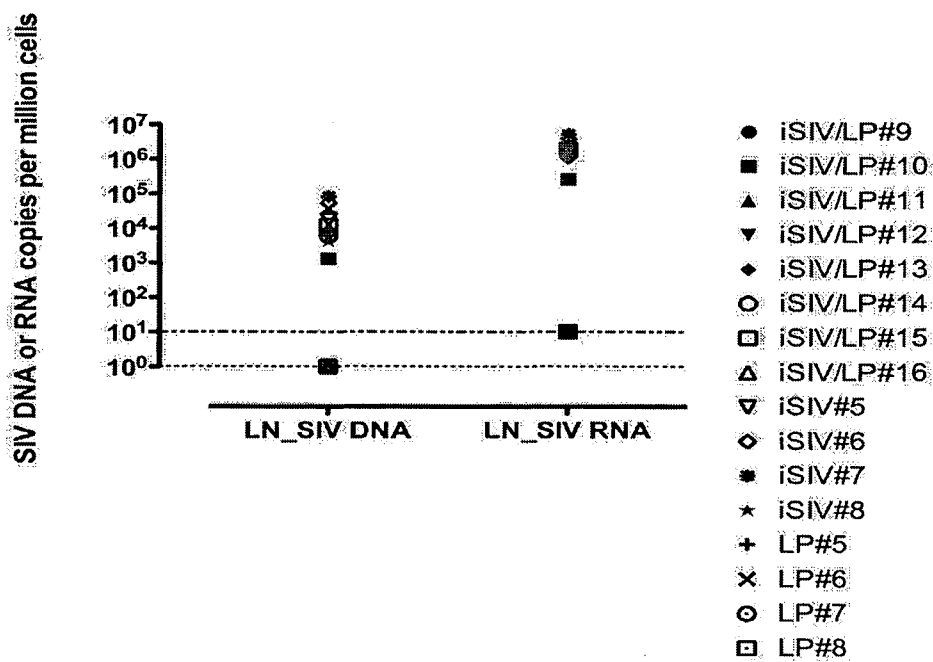


Figure 16e

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2012/000866

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K39/09 A61K39/21 A61K39/295 A61P31/18
ADD.

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

B. FIELDS SEARCHED

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

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Y. WANG ET AL: "Enhanced Antibody Responses Elicited by a CpG Adjuvant Do Not Improve the Protective Effect of an Aldrithiol-2-Inactivated Simian Immunodeficiency Virus Therapeutic AIDS Vaccine", CLINICAL AND VACCINE IMMUNOLOGY, vol. 16, no. 4, 1 April 2009 (2009-04-01), pages 499-505, XP55031411, ISSN: 1556-6811, DOI: 10.1128/CVI.00471-08 page 503; figures 1-4; table 1 ----- -/--	1-11

☒ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search

2 July 2012

Date of mailing of the international search report

13/07/2012

Name and mailing address of the ISA/

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NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Cilensek, Zoran

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2012/000866

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AHLERS JEFFREY D ET AL: "New paradigms for generating effective CD8+ T cell responses against HIV-1/AIDS", DISCOVERY MEDICINE, US, vol. 9, no. 49, 14 June 2010 (2010-06-14), pages 528-547, XP009154181, ISSN: 1944-7930 the whole document	1-11
A	----- XIN KE-QIN ET AL: "Immunogenicity and protective efficacy of orally administered recombinant Lactococcus lactis expressing surface-bound HIV Env", BLOOD, AMERICAN SOCIETY OF HEMATOLOGY, US, vol. 102, no. 1, 1 July 2003 (2003-07-01), pages 223-228, XP009130271, ISSN: 0006-4971, DOI: 10.1182/BLOOD-2003-01-0110 figures 1-5	1-11
A	----- JAE-SUNG YU ET AL: "Generation of Mucosal Anti-Human Immunodeficiency Virus Type 1 T-Cell Responses by Recombinant Mycobacterium smegmatis", CLINICAL AND VACCINE IMMUNOLOGY, AMERICAN SOCIETY FOR MICROBIOLOGY, WASHINGTON, DC, US, vol. 13, no. 11, 1 November 2006 (2006-11-01), pages 1204-1211, XP007912590, ISSN: 1556-6811, DOI: 10.1128/CVI.00195-06 figures 1-5	1-11
A	----- Y. SUI ET AL: "Innate and adaptive immune correlates of vaccine and adjuvant-induced control of mucosal transmission of SIV in macaques", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, vol. 107, no. 21, 25 May 2010 (2010-05-25), pages 9843-9848, XP55031386, ISSN: 0027-8424, DOI: 10.1073/pnas.0911932107 the whole document	1-11
A	----- JIRI MESTECKY ET AL: "Perspectives on Mucosal Vaccines: Is Mucosal Tolerance a Barrier?", JOURNAL OF IMMUNOLOGY, AMERICAN ASSOCIATION OF IMMUNOLOGISTS, US, vol. 179, 1 January 2007 (2007-01-01), pages 5633-5638, XP007912594, ISSN: 0022-1767 the whole document ----- -/--	1-11

INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2012/000866

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KAWAHARA M ET AL: "Intradermal and oral immunization with recombinant Mycobacterium bovis BCG expressing the simian immunodeficiency virus Gag protein induces long-lasting, antigen-specific immune responses in guinea pigs", CLINICAL IMMUNOLOGY, ACADEMIC PRESS, US, vol. 119, no. 1, 1 April 2006 (2006-04-01), pages 67-78, XP024911786, ISSN: 1521-6616, DOI: 10.1016/J.CLIM.2005.11.005 [retrieved on 2006-04-01] the whole document -----	1-11
A	MANSOUR MOHAMADZADEH ET AL: "Lactobacilli activate human dendritic cells that skew T cells toward T helper 1 polarization", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, NATIONAL ACADEMY OF SCIENCES, US, vol. 102, no. 8, 22 February 2005 (2005-02-22), pages 2880-2885, XP007912593, ISSN: 0027-8424 the whole document -----	1-11