### Preparations containing virus-like particles as immunopotentiators administered through the mucosa

**Abstract:** Preparations containing virus-like particles as immunopotentiators administered through the mucosa.

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**References cited:**
- EP-A- 0 534 615
- EP-A- 0 835 663
- WO-A-94/12617

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Description

Technical branch

[0001] The present invention is related to the branch of medicine, particularly with the use of new vaccine immunoenhancing strategies. In this case, the adjuvant is a virus-like particle (VLP), which at the same time constitutes an antigen of interest in the formulation. The adjuvant mechanism is based on the positive effect of one antigen on others or on the synergic interaction between the antigens of the formulation.

Previous technique

[0002] The technical objective pursued with the present invention is, precisely, the development of formulations capable of enhancing the immune response to antigens administered through mucosal routes, minimizing the number of components in the formulation. The enhancing activity is supported by the interaction between particles at the mucosal level, generating systemic as well as mucosal immunity. Furthermore, the development of combined vaccines to the mucosal route taking as a central antigen the HBsAg, increased the immune response to one or more of coadministered antigens. The obvious advantage is the elimination of all other element or compounds different from the antigen of interest and the use of a different route. We consider that this is the basis or nucleus to develop combined vaccines for a mucosal use.

[0003] HBcAg is an extremely immunogenic antigen during the Hepatitis B Virus (HBV) infection or after immunization. In many HBV chronic patients, this is the only antigen capable of inducing an immune response. It can even induce an immune response in mice in nanogram quantities. Recently, a few structural studies have demonstrated some important characteristics explaining its potent immunogenicity. HBcAg specifically binds membrane immunoglobulin receptors in a large number of as well as mucosal immunity. Furthermore, the development of combined vaccines to the mucosal route taking as a central antigen the HBsAg, increased the immune response to one or more of coadministered antigens. The obvious advantage is the elimination of all other element or compounds different from the antigen of interest and the use of a different route. We consider that this is the basis or nucleus to develop combined vaccines for a mucosal use.

[0004] Serologic and biochemical studies indicate that the resolution of HBV acute infection occurs in the context of an efficient cell-mediated immune response, while the chronic infection is characterized by a poor and undetectable cell-mediated immune response and a "relatively efficient" humoral response.

[0005] The humoral immunity and the cell-mediated immunity are regulated by different groups of helper T cells. Factors influencing the induction in mice of a Th1 or Th2 response to the HBV antigens (HBcAg/HBeAg) revealed that this balance was influenced (1) by the antigen structure (HBcAg is a particulated structure and HBeAg is not; (2) the major histocompatibility complex (MHC) of the host and the T cell antigens which are recognized; (3) the cross regulation between Th 1 and Th2 cells; (4) the T cell tolerance, which is more complete for Th1 than for Th2 cells; (5) the activity of secreted HBeAg that preferentially delete Th1 cells (6) the treatment with cytokines, used to modulate in vivo the response toward Th1 or Th2 cells. This balance Th1/Th2 is relevant to the acute or chronic course of the HBV infection. Th2 cells preferentially evade the induction of tolerance compared with Th1. As HBeAg acts as a tolerogen during HBV vertical transmission, deleting Th1 cells, the predominance of Th2 specific cells for HBeAg could influence in the initiation and maintenance of a chronic carrier state. In this case the cytokine therapy endowed to modulate the response towards Th1, could be beneficial in the treatment of HBV chronic infection (Milich, D.R. 1997 J. Viral. Hepat.; 4 Suppl 2: 48-59).

[0006] The effect of HBeAg circulation on HBcAg Th 1 specific T cells was examined by transferring HBeAg/ HBcAg specific T cells to double (HBeAg and HBcAg) transgenic mice. The presence of serum HBeAg eliminated the Th1 mediated response against HBcAg and changed the balance to the Th2 phenotype. This result suggest that, in the context of the hepatitis B infection, circulating HBeAg has the potential to preferentially eliminate inflammatory specific Th1 cells needed for viral clearance, promoting the persistency of HBV (Milich-DR et al. 1998 J-Immunol. Feb 15; 160(4): 2013-21).

[0007] It is known that antibodies against HBcAg are present since the beginning of the infection and reach high concentrations in sera of HBV chronically infected patients, but these antibodies are not protective. Antibodies passively transmitted to newborn children by chronic carrier mothers, do not protect children of infection. (Beasley et al. 1977. American Journal of Epidemiology 105:914-918). However, it has been demonstrated that immunizing chimp with HBcAg partially or completely protected them from HBV infection (Iwarson, S. et al. 1985 Gastroenterology 88: 763-767; Murray, K. et al. 1987 Journal of Medical Virology 23: 101-107). In Iwarson’s study, three chimp were completely protected. After challenge with HBV, antibody levels against HBcAg and HBeAg increased but only one chimp seroconverted against HBsAg. In Murray’s study, 2 out of 4 immunized chimp showed a low level of viral replication after challenge, HBsAg was detectable in sera for 2 or 3 weeks, and after that they developed an anti-HBsAg antibody response. It was hypothesized that the incomplete protection could be due to the low immune response in vaccinated animals without adjuvant.

[0008] After immunizing with woodchucks hepatitis core antigen (WHcAg) in Freund Complete Adjuvant (ACF), it was possible to protect woodchucks from challenge with the virus (WHV) without signs of infection de-
detectable antibodies against the surface protein (WHsAg). Although the hypothesis that T helper anti-nucleocapsid immune response could enhance undetectable antibodies against the surface antigen can not be discarded, the cytotoxic activity was considered as the main responsible of protection (Roos, S. et al. 1989 J. Gen. Virol. 70, 2087-2095). In a second study using woodchucks the role of HBcAg and WHcAg in protection was determined as well as the possible mechanism. Animals were immunized with WHcAg and HBcAg and afterwards challenged using a high dose of WHV. In this experiment it was found that WHcAg is a protecting antigen there is a cross protection because 4 out of 6 woodchucks immunized with HBcAg were protected from the challenge. Both antigens generated a high antibody titer with a cross reactivity lower than 1%, confirming previous reports of protection using internal hepatitis B virus antigens. Since dominant B epitopes of both antigens do not appear to be conserved, this result also demonstrated that antibodies directed against core antigens are not important for protection. Woodchucks immunized with WHcAg/HBcAg reacted with a rapid response of serum antibodies against surface proteins after challenge with WHV, indicating an increased helper T cell response as a potential mechanism of protection after immunization with an internal antigen of HBV/WHV. (Schodel-F et al. Vaccine. 1993; 11(6): 624-8)  

[0009] Transfection of established cell lines from BALB/C mice hepatocytes with dimeric HBV DNA (ML lines) resulted in the expression of HBV antigens in these lines. The adoptive transfer of spleen cells of BALB/c mice immunized with ML-1.1 cells expressing HBsAg as well as HBcAg, caused a regression of tumours cells expressing the corresponding antigens in athymic mice. Furthermore, the transfer of spleen cells of BALB/c mice immunized with HBsAg or HBcAg also caused tumoral regression. These results demonstrated that surface and nucleocapsid antigens could induce immunity capable of rejecting the hepatocellular carcinoma in vivo (Chen, S.H. et al. 1993 Cancer-Res. Oct 1; 53 (19): 4648-51).  


[0011] HBcAg has been demonstrated to be a very good carrier. HBcAg represents a highly immunogenic antigen in human and animal models. HBcAg activates directly B cells and generates strong T cell responses, furthermore, the efficient processing and presentation of HBcAg by the antigen presenting cells makes it the ideal carrier molecule. Hence a large number of epitopes has been chemically linked or genetically fused to the HBcAg molecule to successfully increase their immunogenicity. Expression vectors have been designed in bacteria to enable the insertion of heterologous B cell epitopes in different positions inside the particles of HBcAg and the efficient purification of hybrid particles.  

[0012] Positional studies of B cell epitopes demonstrated that internal insertions by the amino acid 80 continue to be immunodominant, permitting an increase in the production of antibodies as compared to other fusion proteins.  

[0013] Immunogenicity studies have been performed with experimental challenge in different systems. For example, a peptide from Plasmodium berghei Circumsporozoite was inserted in this site and the purified hybrid particle HBcAg/CS was highly immunogenic and protected 100% of challenged mice against malaria. Aimed to the development of oral vaccines, attenuated avirulent Salmonella strains have been used to introduce genes coding for hybrid particles of HBcAg (Milich, D.R. et al. 1995 Ann. N.Y. Acad. Sci. May 31; 754: 187-201).  

[0014] In conclusion, apart from the relationship between HBcAg and protection, total or partially evidenced in chimps or indirectly referred by the experiments with WHcAg, this protein has a number of properties that makes it unique. HBcAg behaves as a T dependent as well as a T independent antigen (Milich, D.R. et al. 1986 Science 234, 1398-1401), it is very immunogenic, even without the help of adjuvants and its inoculation preferentially sensitises Th 1 cells (Milich, D.R. et al. 1997, J. Virol. 71, 2192-2201). HBcAg is a very efficient carrier protein (Schodel, F. et al. 1992 J. Virol. 66: 106-114; Milich-DR et al. 1995 Ann-N-Y-Acad-Sci. May 31; 754: 187-20) and Th HBcAg specific cells mediate the antibody response against HBcAg as well as anti-HBsAg (Milich, D.R. et al. 1987 Nature (London) 329: 547-549). These immunologic characteristics are unique for the particulated HBcAg and do not apply to the non-particulated form of the antigen, the HBsAg (Milich, D.R. et al. 1997 Proc. Natl. Acad. Sci USA Dec 23; 94(26): 14648-53).  

[0015] EP-A 0 835 663 discloses multivalent vaccine compositions comprising HBsAg and other antigens. The vaccine compositions contain aluminium salts as adjuvants to which the antigens are adsorbed. Administration of the vaccine compositions is by injection.  

[0016] WO 94/12617 discloses hepatitis B virus vaccine formulations which contain several live recombinant vaccinia viruses. Together, these live recombinant viruses express a plurality of hepatitis B virus epitopes comprising core antigen epitopes, surface antigen epitopes or combinations thereof. Various routes of administration are mentioned.  

[0017] EP-A 0 271 302 discloses an immunogenic polypeptide conjugate comprising HBcAg operatively linked through an amino acid residue side chain to a polypeptide immunogen. Said polypeptide immunogen can be, for example, HBsAg. The HBcAg part of the conjugate functions as a carrier for the polypeptide immunogen. Vaccine compositions containing the conjugate may comprise other components, including for example adjuvants. Parenteral, rectal and oral routes of administration are mentioned.  

disclose peptides for inducing cytotoxic T lymphocyte responses to hepatitis B virus and vaccine formulations containing the same. The peptides contain at least one CTL epitope and are relatively short, such as from 8 to 17 amino acids or 6 to 19 amino acids. Vaccine compositions containing the peptides may comprise other components, including for example adjuvants. Various routes of administration are mentioned.

**Detailed description of the invention**

[0019] In the present invention it is reported for the first time a vaccine formulation having as main compounds: HBsAg and HBcAg in adequate proportions. Other compounds may be introduced as stabilizers and preservatives.

[0020] The novelty of HBsAg/HBcAg formulation is linked to the anti HBsAg enhancing effect generated when HBsAg is mixed with HBcAg. Both antigens are compounds of HBV and hence, the role of the adjuvant is taken by other viral antigen attractive per se as a vaccine antigen, becoming a vaccine formulation with a wider anti-hepatitis B immune response spectrum. Other formulations of nucleocapsid antigens combined with surface antigens, for example the formulation HBsAg/Virus like particle of Human Papilloma Virus and extended to other viral antigens, results in an increase in titers against both antigens. After mixing HBsAg with other antigens, an increase in the immunogenicity over other coinculated antigens could be shown, evidencing a synergic effect produced by the combination X + HBsAg through the nasal route. In general, these results enables the generation of HBsAg mucosal combined vaccines, and enables the use of the positive interactions between HBsAg and HBcAg, we can obtain a superior product as compared to the single HBsAg commercially available vaccine because:

- It is possible to obtain a wider spectrum of immune response generated by HBcAg regarded as an important antigen per se in anti-VHB protection. Furthermore, the IgG seric levels anti-HBsAg reached by mucosal inoculation is as intense as the one obtained with the systemic inoculation in alum.
- The route of inoculation offers special advantages such as: systemic and mucosal immunity at the same time, the elimination of strong quality controls such as sterility and pyrogens as well as the high prices of injected vaccines, the related toxicity.
- The toxic effect generated by alum-based vaccines and the toxic effects of adjuvant injection can be avoided because the antigen number 2 is at the same time the adjuvant.
- It is possible to use the initial HBsAg + HBcAg formulation as a nucleus of combined vaccines.
- It is possible to immunize non-responders to the surface antigen and immunodepressed patients using this preparation, due to the inoculation route and the introduction of the nucleocapsid antigen regarded as a protective antigen per se.
- The characteristics of this formulation make it an ideal formulation for therapeutic use.

[0023] In the second place, nucleocapsid antigens, favour the increase of coincolated antigens immunogenicity. We found a great simplicity of resulting formulations and, at the same time, the increased valence of these potential vaccines with a minimal number of antigens due to the possibility of avoiding the use of adjuvants, which are per se non-interesting antigens for protection. In this way very reduced combinations can be obtained if desired, for single or combined vaccines.

[0024] In the third place, it is possible to generate combined vaccines having as a nucleus the HBsAg whose immunoenhancing effect on other coincolated antigens is demonstrated in the example 4. The advantages of these formulations are based on the effective association of HBsAg, as a central antigen of the anti HBV vaccine, with other antigens, with a demonstrated synergic effect in the generated response for both antigens. This fact, not only has the attraction of previously described variants but it also makes the HBsAg, -protecting antigen for a widely distributed world disease- the central antigen of combined formulations.

[0025] In general terms, compared to other mucosal vaccines, it is possible to detach the following advantages:

- The ‘adjuvation’ process -mixing antigens- does not require the adsorption of antigens, and the quantity of the HBcAg antigen is at similar levels of HBsAg. The filtration as a sterilizing process can be used due to the small size of the particles, while other strategies and adjuvants over 0.2μm can not be sterilized in this way.
- The simplicity of the production process for HBcAg makes it a very cheap antigen as compared to other adjuvants.

**EXAMPLES OF PERFORMANCE**

**Example 1**

With the aim of evaluating the immunogenicity of HBcAg through the nasal route, 3 groups of 8 female BALB/c mice were inoculated with a dose of 10μg of HBcAg in all cases. The first group was inoculated with HB-
Example 2

With the aim of demonstrating the immunoenhancing activity of HBcAg on HBsAg when both are mixed and inoculated through the nasal route, 4 groups of 8 female BALB/c mice were assayed. A two inoculations schedule was carried out. The inoculations were on days 0 and 14. The extraction was on day 21. The group 1 was inoculated with 10 μg of HBsAg in PBS, group 2 with μg of HBsAg in acemannan (CIGB, La Habana) 3mg/mL (dry weight), group 3 with 10 μg of HBsAg and 10 μg of HBcAg. Group 4 was used as a systemic control, inoculating subcutaneously 10 μg of HBsAg in alum (Fig. 2).

The Student t test was performed to analyse statistically the results, p<0.05 was considered a significant difference.

Example 3

With the aim of studying the enhancing effect of HBcAg at different doses in the murine model, 6 groups of 6 female BALB/c mice were selected. The schedule had three inoculations (days 0, 14 and 28) and two extractions (days 26 and 42). The assayed groups corresponded with: (1) HBsAg 5 μg in PBS; (2, 3 y 4) HBsAg 5 μg with 5, 10 y 20 μg of HBcAg respectively, (5) HBsAg 5 μg in acemannan 3mg/mL (dry weight) and (6) HBsAg 5 μg in alum 0.5mg/mL. All groups except 6 were inoculated nasally. Group 6 was inoculated intramuscularly.

Example 4

Different antigens were employed with the aim of studying the interaction of virus-like particles of Human Papilloma Virus 16 (VLP del VPH 16), HBsAg and HBcAg. Were immunized 8 groups of 6 female BALB/c mice with a schedule based in inoculations on days 0, 14 and the extraction 7 days after second inoculation.

Comparing antibody titers against HBsAg, the response of acemannan formulation (group 6) has the same intensity to the HBcAg/HBsAg formulation (group 7) respectively. This is the third time that we demonstrate the enhancing effect of HBcAg.

From this experiment we also concluded that neither acemannan nor HBcAg enhanced antibody responses against VLP of Human Papilloma Virus (HPV), represented as groups 4, 5 and 8 in the third graphic. Statistical analysis using Student’s t test (p<0.05 was considered a significant difference) did not show any difference between these groups.

Analysing the response against HBcAg in the group 5, where HBcAg and VLP of HPV were inoculated, low levels of antibody titers against HBcAg could be demonstrated as compared to group 7, where HBcAg was introduced along with HBsAg. Perhaps, the presence of these two particles antagonizes at the mucosal level. However, in group 2, high anti HBcAg and anti VLP of HPV could be achieved with the addition of HBsAg, being significantly higher the increase in these responses as compared to group 5 and do not differ from anti HBcAg response of group 7 (along with HBsAg). Hence we could realize a positive interaction between HBsAg and core antigens and a negative interaction between VLPs and HBcAg. The enhancing effect at mucosal level can occur...
in both senses, enabling the design of combined vaccines having as a nucleus HBsAg or the HBsAg/HBcAg combination.

**0040** HBsAg effect on group 2 not only enhanced the response against HBcAg, but it also enhanced the antibody response against the VLP of HPV. The same effect can be appreciated comparing the response against VLP between groups 1, 2 and 3 with group 8 where VLP were inoculated in PBS. Groups 1, 2 and 3 had statistically similar antibody levels, all of them higher than the group 8 level.

**0041** Group 1 (acemannan + HBsAg + VLPs HPV) and group 3 (HBsAg and VLP), did not differ in anti-HB-sAg antibody titers. There was no statistical difference between group 3 and groups 6 and 7 (HBsAg/Acemannan and HBsAg/HBcAg respectively). This result evidenced the enhancing effect of VLP of HPV on HBsAg immunogenicity.

**0042** These results support the use of combined formulations through nasal routes with HBsAg as a central immunoenhancing antigen. For example, the simple mixture of HBsAg and HPV VLP is very attractive and makes real the possibility of introducing more antigens, enhanced by the interaction with HBsAg.

**0043** The creation of complex formulations is possible without reduction of antibody response against each component, for example: VLP of HPV, HBcAg and HB-sAg can be mixed without affecting IgG response against each component.

Example 5

**0044** With the aim of demonstrating the immunoenhancing activity of Hepatitis C Virus Nucleocapsid (HCV NC) on HBsAg when both are mixed and inoculated through the nasal route, 3 groups of 8 female BALB/c mice were assayed. A two inoculations schedule was carried out. The inoculations were on days 0, 14 and 28. The extraction was on day 42. The group 1 was inoculated with 10μg of HCV NC, group 2 was inoculated with 5μg of HBsAg in PBS and group 3 with 5μg of HBsAg and 10μg of HCV NC in PBS (CIGB, La Habana) (Fig. 5).

**0045** The Student t test was performed to analyse statistically the results, p<0.05 was considered a significant difference.

**0046** From this experiment we concluded that it is possible to enhance the IgG response with the mucosal (IN) coadministration of HBsAg and HCV NC. The IgG serum response was significantly higher compared to the group immunized with HBsAg in PBS.

**DESCRIPTION OF FIGURES**

**0047**

**Figure 1.** Three doses schedule (days 0, 14 and 28). Extraction was performed on day 42. Groups 1 and 2 were inoculated with 50μL through the nasal route.

Group 3 was inoculated subcutaneously with 100μL.

**Figure 2.** Two doses schedule (days 0 and 14). Extraction was performed on day 21. Groups 1, 2 and 3 were inoculated with 50μL through the nasal route. Group 4 was inoculated subcutaneously with 100μL.

**Figure 3.** Three doses schedule (days 0, 14 and 28). Extraction was performed on day 26. Groups 1, 2, 3, 4 y 5 were inoculated through the nasal route. Group 6 was inoculated intramuscularly with 100μL.

**Figure 4.** Two doses schedule (days 0 and 14). Extraction was performed on day 26. All groups were inoculated nasally with 50μL. The composition of experimental groups is shown in table added to the figure.

**Figure 5.** Two doses schedule (days 0, 14 and 28). Extraction was performed on day 42. Groups 1, 2 and 3 were inoculated with 50μL through the nasal route.

**Claims**

1. A vaccine formulation for nasal administration comprising a mixture of (a) Hepatitis B surface Antigen (HBsAg) and (b) at least one viral nucleocapsid antigen, and optionally comprising preservatives and/or stabilizers, wherein said HBsAg and said viral nucleocapsid antigen have the form of Virus-Like Particles and wherein the vaccine formulation is free of adjuvants other than (a) and (b).

2. A vaccine formulation according to claim 1, wherein component (b) comprises non-living Virus-Like Particle of the nucleocapsid antigen of Hepatitis B Virus.

3. A vaccine formulation according to claim 1, wherein component (b) comprises non-living Virus-Like Particle of the nucleocapsid antigen of Human Papilloma Virus.

4. A vaccine formulation according to claim 1, wherein component (b) comprises non-living Virus-Like Particle of the nucleocapsid antigen of Hepatitis C Virus.

5. Use of (a) Hepatitis B surface Antigen and (b) at least one viral nucleocapsid antigen for manufacturing a vaccine formulation for nasal administration for treating or preventing viral infection, wherein said HBsAg and said viral nucleocapsid antigen have the form of Virus-Like Particles and both act as immunogen and as adjuvant.

6. Use according to claim 5, wherein component (b) comprises non-living Virus-Like Particle of the nucleocapsid antigen of Hepatitis B Virus.

7. Use according to claim 5, wherein component (b) comprises non-living Virus-Like Particle of the nu-
cleocapsid antigen of Human Papilloma Virus.

8. Use according to claim 5, wherein component (b) comprises non-living Virus-Like Particle of the nucleocapsid antigen of Hepatitis C Virus.

Patentansprüche

1. Impfstoffformulierung für die nasale Verabreichung, umfassend ein Gemisch von (a) einem Hepatitis B-Oberflächenantigen (HBsAg) und (b) zumindest einem Virus-Nucleocapsid-Antigen und gegebenenfalls umfassend Konservierungsmittel und/oder Stabilisatoren, wobei das HBsAg und das Virus-Nucleocapsid-Antigen die Form von virusartigen Partikeln haben und wobei die Impfstoffformulierung keine anderen Adjuvanten als (a) und (b) enthält.

2. Impfstoffformulierung nach Anspruch 1, wobei die Komponente (b) ein nichtlebendes, virusartiges Partikel des Nucleocapsid-Antgens des Hepatitisvirus B umfaßt.

3. Impfstoffformulierung nach Anspruch 1, wobei die Komponente (b) ein nichtlebendes, virusartiges Partikel des Nucleocapsid-Antgens des Human-Papillomavirus umfaßt.

4. Impfstoffformulierung nach Anspruch 1, wobei die Komponente (b) ein nichtlebendes, virusartiges Partikel des Nucleocapsid-Antgens des Hepatitisvirus C umfaßt.

5. Verwendung (a) des Hepatitis B-Oberflächenantigen- und (b) von zumindest einem Virus-Nucleocapsid-Antigen für die Herstellung einer Impfstoffformulierung für die nasale Verabreichung zur Behandlung oder Prophylaxe einer Virusinfektion, wobei das HBsAg und das Virus-Nucleocapsid-Antigen die Form von virusartigen Partikeln haben und beide als Immunogen und als Adjuvans wirken.

6. Verwendung nach Anspruch 5, wobei die Komponente (b) ein nichtlebendes, virusartiges Partikel des Nucleocapsid-Antgens des Hepatitisvirus B umfaßt.

7. Verwendung nach Anspruch 5, wobei die Komponente (b) ein nichtlebendes, virusartiges Partikel des Nucleocapsid-Antgens des Human-Papillomavirus umfaßt.

8. Verwendung nach Anspruch 5, wobei die Komponente (b) ein nichtlebendes, virusartiges Partikel des Nucleocapsid-Antgens des Hepatitisvirus C umfaßt.

Revidications

1. Formulation de vaccin pour l’administration nasale, comprenant un mélange (a) d’Antigène de surface de l’Hépatite B (HBsAg) et (b) d’au moins un antigène de nucléocapside viral, et comprenant éventuellement des conservateurs et/ou des stabilisants, où ledit HBsAg et ledit antigène de nucléocapside viral ont la forme de Particules de Type Virus et où la formulation de vaccin est dépourvue d’adjuvants autres que (a) et (b).

2. Formulation de vaccin selon la revendication 1, dans laquelle le composant (b) comprend une Particule de Type Virus non vivant de l’antigène de nucléocapside du Virus de l’Hépatite B.

3. Formulation de vaccin selon la revendication 1, dans laquelle le composant (b) comprend une Particule de Type Virus non vivant de l’antigène de nucléocapside du Virus du Papillome Humain.

4. Formulation de vaccin selon la revendication 1, dans laquelle le composant (b) comprend une Particule de Type Virus non vivant de l’antigène de nucléocapside du Virus de l’Hépatite C.

5. Utilisation (a) de l’Antigène de surface de l’Hépatite B et (b) d’au moins un antigène de nucléocapside viral pour la fabrication d’une formulation de vaccin pour l’administration nasale, destinée au traitement ou à la prévention d’une infection virale, où ledit HBsAg et ledit antigène de nucléocapside viral ont la forme de Particules de Type Virus et agissent tous deux comme immunogène et comme adjuvant.

6. Utilisation selon la revendication 5, où le composant (b) comprend une Particule de Type Virus non vivant de l’antigène de nucléocapside du Virus de l’Hépatite B.

7. Utilisation selon la revendication 5, où le composant (b) comprend une Particule de Type Virus non vivant de l’antigène de nucléocapside du Virus du Papillome Humain.

8. Utilisation selon la revendication 5, où le composant (b) comprend une Particule de Type Virus non vivant de l’antigène de nucléocapside du Virus de l’Hépatite C.
**First schedule**

1-10μg HBcAg / acemannan 3mg/mL
2-10μg HBcAg / PBS 1X
3-10μg HBcAg / alum 0.5mg/mL

![Graph showing HBcAg Immunogenicity](image)

**Fig. 1**
Second Schedule

1. 10 μg HBsAg / PBS 1X
2. 10 μg HBsAg / acemannan 3 mg/mL
3. 10 μg HBsAg / 10 μg HBCAg / PBS 1X
4. 10 μg HBsAg / Alum 0.5 mg/mL

![Adjuvant effect of HBCAg]

**Fig. 2**
Third schedule

1- 5μg HBsAg / PBS 1X
2- 5μg HBsAg / 5μg HBCAg
3- 5μg HBsAg / 10μg HBCAg
4- 5μg HBsAg / 20μg HBCAg
5- 5μg HBsAg / acemannan 3mg/mL
6- 5μg HBsAg / alum 0.5mg/mL

Fig. 3
Fourth Schedule: Synergism at mucosal level.

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**Fig. 4** Composition, per groups, in the upper part of the figure.
**Fifth Schedule**

1-10μg HCV NC/ PBS 1X  
2-5μg HBsAg/ PBS 1X  
3-10μg HBsAg/ 10μg HCV NC / PBS 1X

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**Fig. 5**