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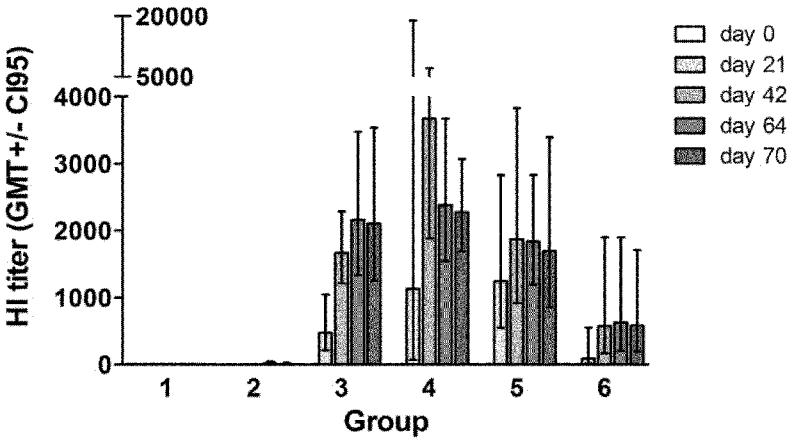
(54) Title: VACCINE COMPOSITION FOR NAIVE SUBJECTS

Figure 1



(57) Abstract: The invention relates to nasally-administered vaccine compositions effective in naive subjects such as children. Further, the vaccine composition is suitable for vaccinating the general population during a pandemic. One aspect of the invention is directed to the paediatric use of the vaccine of the invention including a vaccine effective in children against seasonal influenza virus strains. A further aspect of the invention is directed to subjects of all age groups when the composition is for pandemic use.

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VACCINE COMPOSITION FOR NAIVE SUBJECTS

Field of Invention

The invention relates to nasally-administered vaccine compositions effective in naive subjects such as children. Further, the vaccine composition is suitable for vaccinating the general population during a pandemic.

Background of the Invention

Influenza vaccines currently in general use are based on live virus or inactivated virus, and inactivated virus vaccines can be based on whole virus, "split" virus, subunit proteins or on purified surface antigens (including haemagglutinin and neuraminidase).

The socioeconomic impact of influenza and its medical burden in healthy young children has been increasingly recognized. Moreover, children have the highest attack rates of influenza during epidemic periods, and transmit influenza viruses in the community to other risk groups.

Healthy young children have an increased risk of influenza infection because they do not have a fully developed immune system. Infants are in their first three months of life susceptible to infections that are not common in older individuals (such as Streptococcus agalactiae) and infants rely on maternal antibody for the first few month of life. Infants do not respond to certain vaccines in the same way as adults and are unable to produce effective antibodies to polysaccharide antigens until around 5 years of age. The immune system grows and develops with the child and does not fully resemble that of an adult until puberty, when sex hormones may be responsible for the full maturation of the child's immune system.

The American Advisory Committee on Immunization Practices (ACIP) has recommended annual influenza vaccination for all children aged 6-59 months, because children aged 6-23 months are at substantially increased risk for influenza-related hospitalizations and children aged 24-59 months are at increased risk for influenza-related clinic and emergency department visits. The recommendation has been extended for seasonal influenza vaccination for all persons aged ≥ 6 months

who do not have contraindications. The U.S. food and drug administration categorizes pediatric subpopulation according to the following age ranges. The newborn population range from birth to 1 month of age. The infant population range from 1 month to 2 years of age. The child population range from 2 years to 12 years of age. The adolescent population range from 12 to 21years of age.In Europe, some countries have issued similar recommendations as the ACIP, although with a more restricted position with regard to universal immunization of young children. The European Medicines Agency categorizes paediatric medicines according to the following populations. The newborn population includes pre-term to term and up to 28 days. The infant population are from 1 month to 23 months. The child population are form 2 years to 11 years. Adolescents are from 12 years to 18 years.

Studies have shown that conventional parenteral vaccines have limited ability to induce satisfactory protective immunity in unprimed (naïve) children, especially the very young ones. ACIP has recommended a two-dose vaccination regimen in immunologically naive very young children, but more recently such recommendation has been extended to children aged up to 8 years of age, because of the accumulating evidence indicating that 2 doses are required for protection in this population.

During inter-pandemic periods, influenza viruses that circulate are related to those from the preceding epidemics. The viruses spread among people with varying levels of immunity from infections earlier in life. Such circulation, in a phenomenon known as antigenic drift, over a period of usually 2-3 years, promotes the selection of new strains that have changed enough to cause an epidemic again among the general population. Drift variants may have different impacts in different communities, regions, countries or continents in any one year, although over several years their overall impact is often similar. Typical influenza epidemics cause increases in incidence of pneumonia and lower respiratory disease as witnessed by increased rates of hospitalisation and mortality.

At unpredictable intervals, novel influenza viruses emerge through a process known as “antigenic shift” and are able to cause pandemics. Antigenic shift is the process by which two or more different strains of a virus combine to form a new subtype

having a mixture of the surface antigens of the two or more original strains. Antigenic shift is a specific case of reassortment or viral shift that confers a phenotypic change. Thus, an influenza pandemic occurs when a new influenza virus appears against which the human population has no pre-existing immunity. The general population will when an antigenic shift occurs be naive to the new virus strain.

Antigenic shift is contrasted with antigenic drift, which is the natural mutation over time of known strains of influenza which may lead to a loss of immunity, or in vaccine mismatch. Antigenic drift occurs in all types of influenza including influenza virus A, influenza B and influenza C. Antigenic shift, however, occurs only in influenza virus A because it infects more than just humans.

During a pandemic, antiviral drugs will not be sufficient or effective enough to cover the needs and the number of individuals at risk of potentially life-threating influenza disease. The development of suitable vaccines is essential in order to achieve protective antibody levels in immunologically naive subjects.

These problems may be countered by adjuvantation and/or optimal vaccine delivery the aim of which is to increase immunogenicity of the vaccine in order to be able to decrease the antigen content and thus increase the number of vaccine doses available. The use of an adjuvant may also help prime the immune system against an antigen in a population with no pre-existing immunity to the specific influenza strain. An adjuvant may also enhance the delivery of the vaccine and thereby decrease the amount of antigen needed to induce an immune response. The vaccine delivery and/or the route of vaccination might be of high importance. Most influenza vaccines are delivered parenterally and therefore mainly induce immunity against influenza in the blood. However, influenza viruses enter our bodies through our nose or mouth i.e. through mucosal membranes. By delivering influenza vaccine to the nose one can induce influenza-specific immunity in both the mucosa and in the blood. This might be of benefit when aiming to induce protective immunity against influenza, especially in individuals with no prior immunity to the influenza vaccine strain or to any influenza.

New non-live vaccines, such as a vaccine based on a whole inactivated virus or on part from an inactivated virus, able to induce protective immunity against influenza disease in individuals with no pre-existing immunity to the vaccine antigen are needed. Individuals without sufficient pre-existing immunity to influenza and/or with weakened immune status include immuno-compromised individuals, young children, elderly and large parts of the world wide population (or all) in case of a pandemic. The present invention is directed particularly to children with limited or no pre-existing immunity to viral antigens. This group especially is in need of a safe, non-live vaccine that can prime an immunological response against e.g. influenza. New vaccines that could be used as peri-pandemic vaccines to prime an immunologically naive population against a pandemic strain before or upon declaration of a pandemic are also needed. The present invention is directed particularly to naive populations and notably can be readily administered due to being formulated for nasal administration and only containing inactivated virus or parts of viruses, thus not requiring medically trained personnel. Formulations of vaccine antigens with potent adjuvants allow for enhancing immune responses.

Summary of the Invention

It is an object of the invention to provide vaccines that are able to prime an immune response and provide protective immunity against both seasonal and pandemic virus strains and other pathogenic organisms in subjects with no pre-existing immunity to the vaccine strain. One aspect of the invention is directed to the paediatric use of the vaccine of the invention including a vaccine effective in children against seasonal influenza virus strains. A further aspect of the invention is directed to subjects of all age groups when the composition is for pandemic use.

A first aspect of the present invention is directed to a composition comprising

i) one or more non-live antigens, and

ii) an adjuvant comprising:

one or more carboxylic acids,

an aqueous medium, and

ptionally one or more mono-glycerides

for use as an intranasally administered vaccine for use in naive subjects.

The composition may be formulated for use as a vaccine against all suitable pathogens. Thus the composition may be formulated as a vaccine for any suitable viral strain or bacteria. The composition may be formulated for use as an influenza vaccine for intranasal administration. The invention was developed for use as a vaccine for the intranasal immunization against pathogenic infections e.g. influenza in subjects with limited or no pre-existing immunity to the vaccine strain.

A second aspect of the present invention is directed to a composition for use as an intranasally administered vaccine to pediatric immuno-compromised subjects, the composition comprising

one or more non-live influenza virus antigens, and
an adjuvant comprising:
one or more carboxylic acids,
an aqueous medium, and
optionally one or more mono-glycerides.

A third aspect of the invention is directed to a composition comprising

i) one or more non-live antigens, and
ii) an adjuvant comprising:
one or more carboxylic acids,
an aqueous medium, and
optionally one or more mono-glycerides

for use as an intranasally administered vaccine for use in naive immuno-compromised patients.

A further aspect of the invention is directed to a composition, said composition comprising

i) one or more Streptococcus pneumoniae antigens, and
ii) an adjuvant comprising:
one or more carboxylic acids,
an aqueous medium, and
optionally one or more mono-glycerides

for use as an intranasally administered vaccine for use in naive subjects and/or immune-compromised patients for the prevention of infection with Streptococcus

pneumoniae or for reducing the severity of symptoms associated with an infection with Streptococcus pneumoniae

Brief description of the drawings

Figure 1: Development of HI antibody titers against H1N1 A/Ned/602/09 (A). Ferrets of group 1, 3-6 were intranasally inoculated by nasal drops on days 0, 21 and 42 and ferrets of group 2 were subcutaneously injected on days 21 and 42. HI antibody titers were determined in sera collected prior to the immunizations on day 0, 21 and 42 and after the last immunization on days 64 and 70.Group 1 (control, i.n. saline), group 2 (s.c. TIV), group 3 (i.n. Endocine™ adjuvanted split antigen at 5 µg HA), group 4 (i.n. Endocine™ adjuvanted split antigen at 15 µg HA), group 5 (i.n. Endocine™ adjuvanted split antigen at 30 µg HA) and group 6 (i.n. Endocine™ adjuvanted inactivated whole virus antigen at 15 µg HA). Bars represent geometric mean of 6 animals per group with 95% CI (GMT +/- CI95).

Figure 2: HI titers against distant viruses.
Ferrets of group 1, 3-6 were intranasally inoculated by nasal drops on days 0, 21 and 42 and ferrets of group 2 were subcutaneously injected on days 21 and 42. HI antibody titers were determined in sera collected prior to the immunizations on day 0, 21 and 42 and after the last immunization on days 64 and 70.Group 1 (control, i.n. saline), group 2 (s.c. TIV), group 3 (i.n. Endocine™ adjuvanted split antigen at 5 µg HA), group 4 (i.n. Endocine™ adjuvanted split antigen at 15 µg HA), group 5 (i.n. Endocine™ adjuvanted split antigen at 30 µg HA) and group 6 (i.n. Endocine™ adjuvanted inactivated whole virus antigen at 15 µg HA). Bars represent geometric mean of 6 animals per group with 95% CI (GMT +/- CI95). For GMT calculations, the ≤5 value was replaced with the absolute value 5.A: Antibody titers against H1N1 A/Swine/Ned/25/80. B: Antibody titers against H1N1 A/Swine/Italy/14432/76. C: Antibody titers against H1N1 A/New Jersey/08/76.

Figure 3: Development of VN antibody titers against H1N1 A/Ned/602/09.
Ferrets of group 1, 3-6 were intranasally inoculated by nasal drops on days 0, 21 and 42 and ferrets of group 2 were subcutaneously injected on days 21 and 42. VN antibody titers were determined in sera collected prior to the immunizations on day 0, 21 and 42 and after the last immunization on days 64 and 70. Group 1 (control,

i.n. saline), group 2 (s.c. TIV), group 3 (i.n. Endocine™ adjuvanted split antigen at 5 µg HA), group 4 (i.n. Endocine™ adjuvanted split antigen at 15 µg HA), group 5 (i.n. Endocine™ adjuvanted split antigen at 30 µg HA) and group 6 (i.n. Endocine™ adjuvanted inactivated whole virus antigen at 15 µg HA). Bars represent geometric mean of 6 animals per group with 95% CI (GMT +/- CI95).

Figure 4: Comparison of the vaccine ImmunoseTM FLU, here comprising 15 ug HA split influenza antigen with 20 mg/ml (2 %) EndocineTM ,of the present invention with other adjuvanted vaccine products, FluMist (live attenuated vaccine) and injectable vaccines in influenza naïve ferrets.

Table 3: Efficacy of Endocine™ formulated 2009 H1N1 vaccines in ferrets demonstrated by clinical, virological and gross-pathology parameters.

: Group 1 (control, i.n. saline), group 2 (s.c. TIV), group 3 (i.n. Endocine™ adjuvanted split antigen at 5 µg HA), group 4 (i.n. Endocine™ adjuvanted split antigen at 15 µg HA), group 5 (i.n. Endocine™ adjuvanted split antigen at 30 µg HA) and group 6 (i.n. Endocine™ adjuvanted inactivated whole virus antigen at 15 µg HA).

Clinical Scores. Survival, number of animals that survived up to 4 dpi; fever (^{0}C), maximum temperature increase presented as average with standard deviation, number of animals in which fever was observed in parentheses, (*), body temperature of 1 animal in group 4 was not available due to malfunction of the recorder; % body weight loss between 0 and 4 dpi presented as average with standard deviation, number of animals with body weight loss in parentheses.

Virology. Virus shedding in nose and throat swab samples, area under the curve (AUC) for titration results 1-4 dpi, number of animals showing 1 or more virus positive swab in parentheses; virus load in lung and turbinates ($log_{10}TCID_{50}$ /g) on 4 dpi presented as average with standard deviation, or the lower limit of detection in case all animals in the group were virus negative, number of animals with lung / turbinate virus in parentheses.

Gross pathology. % of estimated affected lung parenchyma by visual examination during necropsy on 4 dpi presented as average with standard deviation, number of animals with affected lung in parentheses; lung/body weight ratio ($x10^2$) on 4 dpi presented as average with standard deviation.

Table 4: Semi-quantitative scoring for histopathological parameters on 4 dpi.

[a]: Group 1 (control, i.n. saline), group 2 (s.c. TIV), group 3 (i.n. Endocine™ adjuvanted split antigen at 5 µg HA), group 4 (i.n. Endocine™ adjuvanted split antigen at 15 µg HA), group 5 (i.n. Endocine™ adjuvanted split antigen at 30 µg HA) and group 6 (i.n. Endocine™ adjuvanted inactivated whole virus antigen at 15 µg HA).

Histopathology. Semi-quantitative scoring for histopathological parameters on 4 dpi. Extent of alveolitis/alveolar damage, score: 0, 0%; 1, 25%; 2, 25-50%; 3, > 50 %; severity of alveolitis, score: no inflammatory cells (0); few inflammatory cells (1); moderate numbers of inflammatory cells (2); many inflammatory cells (3); alveolar oedema, alveolar haemorrhage and type II pneumocyte hyperplasia were scored as positive slides (no=0, yes=1); All histopathology results are presented as average with standard deviation.

Detailed description of the invention

In describing the embodiments of the invention specific terminology will be resorted to for the sake of clarity. However, the invention is not intended to be limited to the specific terms so selected, and it is understood that each specific term includes all technical equivalents which operate in a similar manner to accomplish a similar purpose.

The term “naïve subjects” means subjects immunologically naïve to a pathogen i.e. subjects that have not been vaccinated or exposed to a given pathogen and therefore has no pre-existing immunity to that pathogen.

The term “influenza naive subjects” means subjects immunologically naïve to a specific influenza virus i.e. subjects that have not been vaccinated or exposed to a specific influenza and therefore has no pre-existing immunity to that influenza strain. For influenza it means infants and children when vaccinating against seasonal influenza and means entire populations when peri-pandemic and pandemic periods, including infants, children, adults, and the elderly.

The term "pediatric subjects" refers to children under the age of 21 and include the following subpopulations newborn population from the day of birth to 1 month of age, infants from 1 month to 2 years of age, child from 2 years to 12 years of age and adolescent from 12 years to 21 years of age.

The term "peri-pandemic period" refers to the time period surrounding a pandemic. Given pandemics are time periods officially identified by WHO, the term refers to the time period immediately prior to the official recognition of the pandemic and immediately following a pandemic, during which time vaccination is recommended.

The term "non-live antigens" refers to antigens derived from inactivated, non-live pathogens including viruses e.g.whole inactivated viruses, split antigens, subunit antigens, recombinant antigens or peptides or bacteria or parasites.

The term "Immunose™ FLU" refers to a composition comprising non-live influenza antigen and Endocine™.

The term "Endocine™" refers to an adjuvant comprising equimolar amounts of glycerol monooleate and oleic acid

The one or more non-live influenza virus antigens in the composition of the invention can be from one or more influenza strain, A, B and/or C strain. A vaccine composition that is able to prime an immune response and provide protective immunity against pandemic influenza strains normally only contains antigens from one influenza A strain (monovalent) whereas a vaccine composition that is able to prime an immune response and provide protective immunity against seasonal influenza strains normally contains antigens from three or more different strains (trivalent or quadrivalent). Most commonly two different influenza A strains and one or more influenza B strains.

The invention is directed to a vaccine composition surprisingly found to be highly effective against subjects naive to influenza viral strains, such as children (younger than 8 years old) and persons during a peri-pandemic or pandemic period. Children

are often naive to influenza strains circulating seasonally whereas all persons are considered naive during a pandemic.

The invention is further directed to a method of immunization before or during an epidemic or pandemic period comprising intranasally administering a vaccine composition comprising a composition of the invention as well as to a method of immunization of paediatric subjection comprising intranasally administering a vaccine composition comprising a composition of the invention and still further directed to a method of immunization of naïve subjects comprising intranasally administering a vaccine composition comprising a composition of the invention.

The invention is directed to infants, children and adolescent populations as these populations, when naïve, are less responsive when it comes to common vaccine strategies. The immune system in infants and children are not fully developed and they therefore mount a less efficient immune response to conventional parenteral vaccine strategies. However, the present invention offers a special opportunity for infants and children as a unique lymphoid tissue in the upper respiratory tract is present at birth and well developed early in childhood. The pharyngeal lymphoid tissue known as the adenoid (or nasopharyngeal tonsil) is located in the pharynx of children and is part of Waldeyer's ring which comprises the nasopharyngeal tonsil (adenoid(s)), the pair of palatine tonsils, the pair of tubal tonsils and the lingual tonsils. The adenoid is active in building up the immune system and starts to disappear during adolescence. Nasal vaccine delivery may therefore be of particular advantages for infants and children. Pediatric subpopulations may be defined either as by the U.S. Food and Drug Administration or as by the European Medicines Agency or as a combination of the two.

In one embodiment the composition is for use as an intranasal administered vaccine for pediatric use. In one embodiment the composition is for use as an intranasal administered vaccine in newborn ((term and pre-term) with an age up to 28 days). In one embodiment the composition is for use as an intranasal administered vaccine in infants (with an age of 1 month to 23 months). In one embodiment the composition is for use as an intranasal administered vaccine in children (with an age of 2 years

to 11 years). In one embodiment the composition is for use as an intranasal administered vaccine in adolescent (with an age of 12 years to 18 years).

There is a need for a safe vaccine suitable for small children with limited or no pre-existing immunity to e.g. influenza and for naive subjects in general that induces protective immunity against e.g. influenza disease.

Live attenuated virus vaccines are associated with safety concerns. Flumist® has not been approved, due to these safety issues, for use in small children under 2 years of age. Paradoxically, these are most often naive subjects which are particularly vulnerable to influenza, and belong to a high risk group for influenza. Flumist® is approved for older children but is a live attenuated virus vaccine.

It has surprisingly been found that intranasal administration of adjuvanted non-live influenza vaccines induced very high immune responses and subsequent complete protection against influenza disease in ferrets with no pre-existing immunity to the vaccine antigen. Both the whole and split non-live antigen vaccines gave superior results over the injected commercially available influenza vaccine, Fluarix®.

The composition of the invention does not utilize a live attenuated virus but rather non-live influenza virus antigens. Moreover, it can be administered intranasally. Intranasal administration is particularly suitable for pediatric administration in infants and children due to the presence of the pharyngeal lymphoid tissue known as the adenoid. The intranasal administration of the composition of the invention allows for its generalized use and administration without specialized training, such as throughout the population during peri-pandemic and pandemic periods by self-administration. The use of non-live influenza virus antigens allows for its use in small children without the safety concerns associated with live attenuated virus vaccines. The inventors have developed a vaccine efficacious in naive subjects which may be intranasally administered, thereby having the above-mentioned advantages and meeting an important need for vulnerable populations and classes of patients.

The invention is directed, in a first aspect, to a composition, said composition comprising

i) one or more non-live antigens, and
ii) an adjuvant comprising:
one or more carboxylic acids,
an aqueous medium, and
optionally one or more mono-glycerides
for use as an intranasally administered vaccine for use in naive subjects.

In another aspect the invention is directed to a composition comprising
i) one or more non-live virus antigens, and
ii) an adjuvant comprising:
one or more carboxylic acids,
an aqueous medium, and
optionally one or more mono-glycerides
for use as an intranasally administered vaccine for use in naive subjects.

The composition of the invention is suitable for use as a vaccine against infectious pathogens e.g. virus and bacteria. The composition of the invention is suitable for an influenza vaccine for intranasal administration. The composition of the invention is directed for use as a vaccine for the intranasal immunization against influenza in naïve subjects.

The influenza viruses consist of three types A, B, and C. Influenza A viruses infect a wide variety of birds and mammals, including humans, horses, pigs, ferrets, and chickens. Influenza B is present in humans, ferrets and seals and influenza C is present in humans dogs and pigs. Animals infected with Influenza A often act as a reservoir for the influenza virus, by generating pools of genetically and antigenically diverse viruses which are transmitted to the human population. Transmission may occur through close contact between humans and the infected animals, for example, by the handling of livestock. Transmission from human to human may occur through close contact, or through inhalation of droplets produced by coughing or sneezing.

The outer surface of the influenza A virus particle consists of a lipid envelope which contains the glycoproteins hemagglutinin (HA) and neuraminidase (NA). The HA

glycoprotein is comprised of two subunits, termed HA1 and HA2. HA contains a sialic acid binding site, which binds to sialic acid found on the outer membrane of epithelial cells of the upper and lower respiratory tract, and is absorbed into the cell via receptor mediated endocytosis. Once inside the cell, the influenza virus particle releases its genome, which enters the nucleus and initiates production of new influenza virus particles. NA is also produced, which cleaves sialic acid from the surface of the cell to prevent recapture of released influenza virus particles. The virus incubates for a short period, roughly five days in a typical case, although the incubation period can vary greatly. Virus is secreted approximately one day prior to the onset of the illness, and typically lasts up to three to five days. Typical symptoms include fever, fatigue, malaise, headache, aches and pains, coughing, and sore throat. Some symptoms may persist for several weeks post infection.

Different strains of influenza virus are characterized primarily by mutations in the HA and NA glycoproteins, and thus HA and NA are used to identify viral subtypes (i.e., H5N1 indicates HA subtype 5 and NA subtype 1). As such, influenza vaccines often target the HA and NA molecules. Conventional influenza virus vaccines often utilize whole inactivated viruses, which possess the appropriate HA and/or NA molecule. Alternatively, recombinant forms of the HA and NA proteins or their subunits may be used as vaccines. The antigen in the vaccine composition may be inactivated antigens such as e.g. whole inactivated viruses, split antigens, subunit antigens, recombinant antigens or peptides. The term “antigen” or “immunogen” is defined as anything that can serve as a target for an immune response. The term also includes protein antigens, recombinant protein components, virus like particles (VLPs) as well as genetically engineered RNA or DNA, which – when injected into the cells of the body - the "inner machinery" of the host cells "reads" the DNA and uses it to synthesize the pathogen's proteins. Because these proteins are recognised as foreign, when they are processed by the host cells and displayed on their surface, the immune system is alerted, which then triggers a range of immune responses. The term also includes material, which mimic inactivated bacteria or viruses or parts thereof. The immune response can be cellular and/or humoral and be detected in systemic and/or mucosal compartments.

However, influenza is an RNA virus and is thus subject to frequent mutation, resulting in constant and permanent changes to the antigenic composition of the virus. The antigenic composition refers to portions of the polypeptide which are recognized by the immune system, such as antibody binding epitopes. Small, minor changes to the antigenic composition are often referred to as antigenic drift. Influenza A viruses are also capable of "swapping" genetic materials from other subtypes in a process called reassortment, resulting in a major change to the antigenic composition referred to as antigenic shift. Because the immune response against the viral particles relies upon the binding of antibodies to the HA and NA glycoproteins, frequent changes to the glycoproteins reduce the effectiveness of the immune response acquired against influenza viruses over time, eventually leading to a lack of immunity. The ability of influenza A to undergo a rapid antigenic drift and shift can often trigger influenza epidemics due to the lack of pre-existing immunity to the new strain.

The American Advisory Committee on Immunization Practices (ACIP) has recommended annual influenza vaccination for all children aged 6-59 months, because children aged 6-23 months are at substantially increased risk for influenza-related hospitalizations and children aged 24-59 months are at increased risk for influenza-related clinic and emergency department visits. The recommendation has been extended for seasonal influenza vaccination to all persons ages ≥ 6 months. Accordingly, the composition of the invention is for use as a vaccine for intranasal administration to children aged 18 years and under, particular aged 12 and under. Typically, the children are less than 8 years of age, such as 6 years old or less. An important intended class of patients for the vaccine of the invention is children, particularly children of 2 months to less than 9 years of age, typically children of age 3 months to less than 9 years old, such as of age 6 months to less than 8 years old, most typically of age 6 month to less than 7 years old, such as of age 6 months to less than 72 months, or of age 6 months to 60 months, or of age 6 months to 24 months. The composition of the invention is intended, at least in part, as a vaccine for paediatric use.

The features of an influenza virus strain that give it the potential to cause a pandemic outbreak are: it contains a new haemagglutinin compared to the

haemagglutinin in the recently circulating strains, which may or may not be accompanied by a change in neuraminidase subtype; it is capable of being transmitted horizontally in the human population; and it is pathogenic for humans. A new haemagglutinin may be one which has not been evident in the human population for an extended period of time, probably a number of decades, such as H2. Or it may be a haemagglutinin that has not been circulating in the human population before, for example H5, H9, H7 or H6 which are found in birds. In either case the majority, or at least a large proportion of, or even the entire population has not previously encountered the antigen and is immunologically naive to it.

The invention is directed to infants, children and adolescent populations as these populations, when naive, are less responsive when it comes to common vaccine strategies. The immune system in infants and children are not fully developed and they therefore mount a less efficient immune response to conventional parenteral vaccine strategies. However, the present invention offers a special opportunity for infants and children as a unique lymphoid tissue in the upper respiratory tract is present at birth and well developed early in childhood. The pharyngeal lymphoid tissue known as the adenoid (or nasopharyngeal tonsil) is located in the pharynx of children and is part of Waldeyer's ring which comprises the nasopharyngeal tonsil (adenoid(s)), the pair of palatine tonsils, the pair of tubal tonsils and the lingual tonsils. The adenoid is active in building up the immune system and starts to disappear during adolescence. Nasal vaccine delivery may therefore be of particular advantages for infants and children. Pediatric subpopulations may be defined either as by the U.S. Food and Drug Administration or as by the European Medicines Agency or as a combination of the two.

The U.S. food and drug administration categorizes pediatric subpopulation according to the following age ranges. The newborn population range from birth to 1 month of age. The infant population range from 1 month to 2 years of age. The child population range from 2 years to 12 years of age. The adolescent population range from 12 to 21years of age. The European Medicines Agency categorizes paediatric medicines according to the following populations. The newborn population includes pre-term to term and up to 28 days. The infant population are from 1 month to 23

months. The child population are form 2 years to 11 years. Adolescents are from 12 years to 18 years.

In one embodiment the composition is for use as an intranasal administered vaccine for pediatric use. In one embodiment the composition is for use as an intranasal administered vaccine in newborn ((term and pre-term) with an age up to 28 days) or alternatively for use in newborn from day of birth to an age of 1 month. In one embodiment the composition is for use as an intranasal administered vaccine in infants with an age of 1 month to 23 months or alternatively with an age of 1 month to 2 years. In one embodiment the composition is for use as an intranasal administered vaccine in children with an age of 2 years to 11 years or alternatively with an age of 2 years to 12 years. In one embodiment the composition is for use as an intranasal administered vaccine in adolescent with an age of 12 years to 18 years alternatively with an age of 12 years to 21 years.

The vaccine of the invention is particularly directed to naive subjects, eg children below 8 years of age during seasonal influenza epidemics. The composition of the invention is also intended, as a vaccine for use in all age groups during pandemic or peri-pandemic periods.

The composition is therefore particularly directed to naive subjects. The naive subjects may be children under 18 years old, such as children 0 to 18 years, particularly children aged 12 and under. Typically, the children are less than 8 years of age, such as 6 years old or less. An important intended class of patients for the vaccine of the invention is particularly children of 2 months to less than 9 years of age, typically children of age 3 months to less than 9 years old, such as of age 6 months to less than 8 years old, most typically of age 6 month to less than 7 years old, such as of age 6 months to less than 72 months, or of age 6 months to 60 months or of age 6 months to 24 months. The composition of the invention is intended, at least in part, as a vaccine for pediatric use.

The naive subjects may be of all age groups when the composition is particularly directed to a vaccine for use during pandemic or peri-pandemic periods.

Intranasal administration is intended to mean administration to the nose by any mode of administration such as by spraying the vaccine into the nasal cavity or by administering the vaccine via pipette or similar device by dripping the vaccine into the nasal cavity or onto the nasal mucosal wall.

The composition advantageously comprises one or more non-live influenza virus antigens rather than live attenuated virus. As stated, this avoids safety concerns both in the selection of the patient class but also in terms of production, distribution, nasal administration, handling and disposal. The non-live influenza virus antigen may be selected from the group consisting of whole inactivated virus, split virus, subunit influenza antigen and recombinant antigens. The use of recombinant proteins can be used to increase the titer of neutralizing antibodies produced against a challenge with the virus. The glycosylation of HA plays an important role in the ability of the immune response to elict an antibody response and the virus ability to evade the immune system. Hence recombinant HA proteins can be generated containing heterogeneous complex-type glycans as well as recombinant proteins which are monoglycosylated or non-glycosylated with increased immunogenicity.

Preferably, the non-live influenza virus antigen is a split antigen or a subunit influenza antigen, more preferably a split antigen.

The influenza A genome contains 11 genes on eight pieces of RNA, encoding for 11 proteins: hemagglutinin (HA), neuraminidase (NA), nucleoprotein (NP), M1, M2, NS1, NS2(NEP: nuclear export protein), PA, PB1 (polymerase basic 1), PB1-F2 and PB. Non-live influenza virus antigens may be selected from any one protein or combination of proteins from the virus.

The composition of the invention may comprise any inactivated influenza virus. As understood by the person skilled in the art, the influenza virus varies from season to season and also by geographic area and populations in which they infect. The present invention is directed to vaccines comprising an adjuvant of the invention and non-live influenza virus antigens from one or more influenza virus. The non-live influenza antigen used in the vaccine composition of the invention will be any antigenic material derived from an inactivated influenza virus. For instance, it may

comprise inactivated whole virus particles. Alternatively, it may comprise disrupted virus (split virus) wherein for instance an immunogenic protein, for example M2 ion channel protein, or glycoproteins are retained. Purified preparations of influenza membrane glycoproteins, haemagglutinin (HA) and/or neuraminidase (NA) may be used as the antigenic material in the vaccine composition. A vaccine composition according to the invention may comprise one or more types of antigenic materials. The influenza type virus used to prepare the vaccine composition will, of course, depend on the influenza against which a recipient of the vaccine is to be protected.

For example, the non-live influenza virus antigen comprises one or more antigens of, for instance, the genetic backbone of one or more of the following influenza viruses: A/Ann Arbor/6/60 (A/AA/6/60), B/Ann Arbor/1/66 virus, the FluMist MDV-A (ca A/Ann Arbor/6/60), the FluMist MDV-B (ca B/Ann Arbor/1/66), A/Leningrad/17 donor strain backbone, and PR8.

In another specific example, the vaccine compositions of the invention comprise a non-live influenza virus antigen of, for instance, an HA or an NA polypeptide sequence (or at least 90% identical or at least 95% identical to such sequences) from one or more of the following: B/Yamanashi; A/New Caledonia; A/Sydney; A/Panama; B/Johannesburg; B/Victoria; B/Hong Kong; A/Shandong/9/93; A/Johannesburg/33/94; A/Wuhan/395/95; A/Sydney/05/97; A/Panama/2007/99; A/Wyoming/03/2003; A/Texas/36/91; A/Shenzhen/227/95; A/Beijing/262/95; A/New Caledonia/20/99; B/Ann Arbor/1/94; B/Yamanashi/166/98; B_Johannesburg.sub.--5.sub.--99; BVictoria/504/2000; B/Hong Kong/330/01; B_Brisbane.sub.--32.sub.--2002; B/Jilin/20/03; an H1N1 influenza A virus, an H3N2 influenza A virus, H9N2 influenza A virus, an H5N1 influenza A virus, an H7N9 influenza A virus ; an influenza B virus; and a pandemic influenza strain (either designated by WHO or not circulating in the human population).

In one embodiment the influenza virus strain may be of one or more of the strains included in the 2013/2014 vaccine: such as an A/California/7/2009 (H1N1)-like virus, an (H3N2) virus antigenically like the cell-propagated prototype virus A/Victoria/361/2011 or A/Texas/50/2012 and a B/Massachusetts/2/2012–like (Yamagata lineage) virus.

In one embodiment the influenza virus strain may be of one or more of the strains previously recommended by the WHO for use in an influenza vaccine.

In one embodiment the influenza virus strain or strains may be a strain from a Quadrivalent influenza vaccine and contain antigens from any four of the following five influenza virus strains; an A/California/7/2009 (H1N1)-like virus, an (H3N2) virus antigenically like the cell-propagated prototype virus A/Victoria/361/2011 or A/Texas/50/2012 and a B/Massachusetts/2/2012–like (Yamagata lineage) virus, a B/Brisbane/60/2008–like (Victoria lineage) virus.

The adjuvant of the composition of the invention is critical for its suitability for intranasal administration and for its efficacy. A suitable adjuvant for intranasal administration may be an adjuvant that comprises optionally a monoester of glycerol in combination with a fatty acid, or it may be a combination of fatty acids. Carboxylic acids used in such adjuvants comprise long chain (C4-C30) alkyl, alkenyl or alkynyl carboxylic acids which may optionally be branched or unbranched, cyclic or acyclic, optionally having single, double or multiple unsaturation (double or triple bond) which may further optionally be of different kind.

Monoglycerides used in such adjuvants may be carboxylic acid esters of glycerin, wherein the carboxylic acids may be long chain (C4-C30) alkyl, alkenyl or alkynyl carboxylic acids which may optionally be branched or unbranched, optionally having single, double or multiple unsaturation (double or triple bond) which may further optionally be of different kind.

The concentration of monoglyceride in a vaccine composition may be in the range of e.g. about 1 to about 50 mg/ml, such as, e.g. from about 1 to about 25 mg/ml, from about 5 to about 15 mg/ml or about 10 mg/ml.

The concentration of fatty acid in a vaccine composition may be in the range of e.g. about 0.5 to about 50 mg/ml, such as, e.g. from about 1 to about 25 mg/ml, from about 1 to about 15 mg/ml, from about 1 to about 10 mg/ml, from about 2 to about 8

mg/ml or about 6-7 mg/ml. In one embodiment the molar basis of the concentration of a fatty acid in the vaccine composition corresponds to the concentration (on a molar basis) of the monoglyceride.

Any combination of the concentration ranges mentioned above for monoglyceride and fatty acid is within the context of the present application. Moreover, the broadest range mentioned gives a preferred range, and then the range is narrowed to the most preferred range.

The inventors of the present invention have found that adjuvants as described above and disclosed in WO 2012/042003 (which is hereby included in its entirety by reference) are particularly useful when vaccination is performed via the nasal route, e.g. administration to the mucosa of the nasal cavity. The inventors have found that use of such adjuvants in vaccination via the nasal route improves the immune response upon vaccination. The inventors have found the use of such adjuvants safe and tolerable in several species including humans.

Accordingly, the composition may comprise mono-glycerides which are glycerides mono-esterified with carboxylic acids selected from the group consisting of lauric acid (C12), myristic acid (C14), palmitic acid (C16), palmitoleic acid (C16:1), oleic acid (C18:1), linoleic acid (C18:2), stearic acid, hexanoic acid, caprylic acid, decanoic acid (capric acid), arachidic acid, behenic acid, lignoceric acid, alpha-linolenic acid, stearidonic acid, eicosapentaenoic acid, docosahexaenoic acid, gamma-linolenic acid, dihomo- gamma-linolenic acid, arachidonic acid, erucic acid, nervonic acid.

In a further embodiment, the mono-glycerides are glycerides mono-esterified with carboxylic acids selected from the group consisting of palmitoleic acid (C16:1), oleic acid (C18:1) and linoleic acid (C18:2).

Preferably, the mono-glyceride is glyceride mono-esterified with oleic acid (glyceryl oleate).

The adjuvant preferably comprises one or more carboxylic acids selected from the group consisting of lauric acid, myristic acid, palmitic acid, palmitoleic acid, oleic acid, linoleic acid stearic acid, hexanoic acid, caprylic acid, decanoic acid (capric acid), arachidic acid, behenic acid, lignoceric acid, alpha-linolenic acid, stearidonic acid, eicosapentaenoic acid, docosahexaenoic acid, gamma-linolenic acid, dihomo-gamma- linolenic acid, arachidonic acid, erucic acid and nervonic acid. Preferably, the one or more carboxylic acids are selected from the group consisting of oleic acid and lauric acid.

In a combination of suitable embodiments, the adjuvant comprises glyceryl oleate, oleic acid and an aqeuous medium. The vaccine composition of the present invention can also comprise additional pharmaceutical excipients. Such pharmaceutical excipients can be:

1. Agents to control the tonicity/osmolarity of the vaccine. Such agents are e.g. physiological salts like sodium chloride. Other physiological salts are potassium chloride, potassium dihydrogen phosphate, disodium phosphate, magnesium chloride etc. Such agent could also be other ionic substances which influence the ionic strength and stability. The osmolality of the vaccine may be adjusted to a value in a range from about 200 to about 400 mOsm/kg, preferably in a range from about 240 to about 360 mOsm/kg or the osmolality must be close to the physiological level e.g. in the physiological range from about 290 to about 310 mOsm/kg.

2. Agents to adjust the pH of or to buffer the vaccine composition. Normally, pH of the vaccine composition is in a range of from about 5 to about 8.5. Suitable pH adjusting agents or buffer substances include hydrochloric acid, sodium hydroxide (to adjust pH) as well as phosphate buffer, Tris buffer, citrate buffer, acetate buffer, histidine buffer etc. (to buffer the vaccine).

3. Other additives like e.g. surface-active agents, antioxidants, chelating agents, antibacterial agents, viral inactivators, preservatives, dyes, anti-foaming agents, stabilizers or surface active agents, or combinations thereof.

The surface-active agent may be hydrophilic, inert and biocompatible, such as, e.g., poloxamers such as e.g. Pluronic F68 or Pluronic 127.

The antibacterial agents may be e.g. amphotericin or any derivative thereof, chlorotetracyclin, formaldehyde or formalin, gentamicin, neomycin, polymyxin B or any derivative thereof, streptomycin or any combination thereof.

The antioxidants may be e.g. ascorbic acid or tocopherol or any combination thereof.

The viral inactivators may be e.g. formalin, beta-propiolactone, UV-radiation, heating or any combination thereof.

The preservatives may be e.g. benzethonium chloride, EDTA, phenol, 2-phenoxyethanol or thimerosal or any combination thereof. EDTA has also been shown to be a chelating agent, an antioxidant and a stabilizer.

The dyes may be e.g. any indicators (such as e.g. phenol red) or brilliant green or any combination thereof.

The anti-foaming agents may be e.g. polydimethylsilozone.

The surfactants may be e.g. anionic, cationic or non-ionic or zwitterionic, such as e.g. polyoxyethylene and derivatives thereof, polysorbates (such as e.g. polysorbate 20 or polysorbate 80), Tween 80, poloxamers (such as e.g Pluronic F68) or any combination thereof.

Typically, the concentration of monoglyceride in a vaccine composition is in an amount in the range of about 0.1 g to about 5.0 g per 100 mL, or in the range of about 0.1 g about 2.0 g per 100 ml, or about 0.5 g to about 2.0 g, such as 0.5 g to about 1.5 g per 100 mL of the vaccine composition.

Furthermore, the concentration of the one or more carboxylic acids is in an amount in the range of about from 0.1 g to about 5.0 g per 100 mL, or in the range of about

0.1 g to about 2.0 g per 100 mL or about 0.5 g to about 2.0 g, such as 0.5 g to about 1.5 g per 100 mL of the vaccine composition.

The one or more monoglycerides together with one or more carboxylic acids in an vaccine composition may be in an amount of at the most 10% w/v, or at the most 5% w/v, or at the most 4% w/v, or at the most 3% w/v, or at the most 2% w/v or at the most 1 % w/v or at the most 0.5 % w/v or at the most 0.1 % w/v or at the most 0.05 % w/v.

The adjuvant may comprise a combination of lipids selected from the group consisting of mono-olein, oleic acid, lauric acid, and soybean oil. In one suitable embodiment, the adjuvant comprises oleic acid, lauric acid in Tris buffer. Suitably, this embodiment comprises 0.25 g to 0.75 g of oleic acid, 0.25 g to 0.75 g of lauric acid in 7-15 mL of Tris buffer (pH 7-9). A specific example comprises 0.4 g to 0.5 g of oleic acid, 0.3 g to 0.4 g of lauric acid in 8-10 mL of 0.1 M Tris buffer (pH 7-9). In a further suitable embodiment, the adjuvant comprises oleic acid and mono-olein in Tris buffer. Suitably, this embodiment comprises 0.25 g to 0.75 g of oleic acid, 0.25 g to 0.75 g of mono-olein in 7-15 mL of Tris buffer. A specific example comprises 0.3 g to 0.4 g of oleic acid, 0.4 g to 0.5 g of mono-olein in 8-10 mL of 0.1 M Tris buffer (pH 7-9). A further embodiment comprises 0.5 g to 0.25 g of mono-olein, 0.5 g to 0.25 g of oleic acid, and 0.25 g to 0.75 g of soybean oil in 7-15 mL of Tris buffer. A specific example of this embodiment comprises 0.1 g to 0.2 g of mono-olein, 0.8 g to 1.5 g of oleic acid, and 0.5 g to 0.6 g of soybean oil in 8-12 mL of Tris buffer (pH 7-9).

Three types of adjuvants were used successfully in the examples below: Example adjuvant A comprising 0.4 g to 0.5 g of oleic acid, 0.3 g to 0.4 g of lauric acid in 8-10 mL of 0.1 M Tris buffer (pH 7-9); Example adjuvant B comprising 0.3 g to 0.4 g of oleic acid, 0.4 g to 0.5 g of mono-olein in 8-10 mL of 0.1 M Tris buffer (pH 7-9); and Example adjuvant C comprising 0.1 g to 0.2 g of mono-olein, 0.8 g to 1.5 g of oleic acid, and 0.5 g to 0.6 g of soybean oil in 8-12 mL of Tris buffer (pH 7-9). These adjuvants are typically prepared in w/v concentration of 2-12% lipid content (6 g -12 g per 100 mL), most typically from 3-10%, such as 4%, 5%, 6%, 7, 8%, or 9%. These concentrations are those of the adjuvant mix itself. This adjuvant is then

mixed with the antigen containing composition in 2:1 to 1:8 ratios, such as, for example, in a 1:1 ratio so as to provide a 4% lipid content vaccine composition when commencing from an adjuvant with an 8% lipid concentration. Typically, the lipid content in the vaccine composition of the invention is 0.5% to 6% w/v, typically as 1% to 6% w/v, more typically 1% to 4%.

The Example B composition is an Endocine™ formulation comprising equimolar amounts of glycerol monooleate and oleic acid and has been found to be exceptionally effective in naive subjects. In a highly preferred embodiment, this 8% lipid formulation is diluted with the antigen containing compositions so as to provide a vaccine composition with a lipid concentration of 1-4% w/v.

As stated, the composition is suitable for use in a method for immunization during a peri-pandemic or pandemic period comprising intranasally administering the vaccine composition of the invention. The method for immunization during a peri-pandemic or pandemic period can be used for subjects of all age. The invention further relates to a method of immunization during seasonal epidemics of paediatric subjects comprising intranasally administering a vaccine composition as described.

As stated, the invention is directed to a method of immunization of naïve subjects comprising intranasally administering a vaccine composition.

The Examples below show the efficacy of this vaccine composition in naive subjects.

The surprisingly efficacy in eliciting an immune response in naïve individuals implies that the vaccine of the invention is able to elicit immune response in individuals who have a weakened immune system in terms of being able to respond to invasive pathogens such as vira where they do not already have strong pre-existing immunity. A composition of the invention is therefore suitable for immuno-compromised individuals. Accordingly, a further aspect of the invention is directed to a vaccine composition comprising adjuvanted non-live influenza antigens intranasally administered to pediatric immune-compromised patients, including

those with HIV; subjects taking immunosuppressant drugs, recent organ recipients; premature babies, and post-operative patients.

This aspect relates to a composition, said composition comprising

i) one or more non-live antigens, and

ii) an adjuvant comprising:
one or more carboxylic acids,
an aqueous medium, and
optionally one or more mono-glycerides.

for use as an intranasally administered vaccine in pediatric immuno-compromised patients.

Immuno-compromised individuals have an increased susceptibility to opportunistic pathogens e.g.influenza virus and are at an increased risk for hospitalization and death from influenza. Immuno-compromised individuals and in particular pediatric immune-compromised individuals may be a suitable patient class for immunization with a composition of the present invention. On embodiment of the present invention may therefore be a composition comprising

i) one or more non-live influenza virus antigens, and

ii) an adjuvant comprising:
one or more carboxylic acids,
an aqueous medium, and
optionally one or more mono-glycerides.

for use as an intranasally administered vaccine in pediatric immuno-compromised patients.

A surprising effect of the present invention as illustrated by example 2 is that the composition of the present invention is able to reduce virus shedding. Children shed more virus than immune-competent healthy adults, which leads to increased virus spreading to other people in their proximity. The present invention may therefore be suitable for treating paediatric populations such as infants, children and adolescents. The present invention may be suitable for preventing virus spreading by a pediatric population. In one embodiment the composition of the present invention is for use in pediatric subjects such as infants, children and the adolescents. In one embodiment

the composition of the present invention is for use in naive subjects for reducing virus shedding. In one embodiment the composition of the present invention in for use in influenza naive subjects for reducing virus shedding. Further, a composition of the present invention may be particularly suitable for containing a pandemic by reducing virus spreading. In one embodiment a composition of the present invention is for use in naive subjects for reducing virus shedding in a pandemic zone. In one embodiment a composition of the present invention is for use in naive subjects for reducing virus shedding during a peri-pandemic period. In one embodiment a composition of the present invention is for use in the pediatric subjects for reducing virus shedding during a peri-pandemic period.

A method of immunization against influenza in pediatric immuno-compromised patients by intranasal administration of a composition as described supra is an interesting aspect of the surprising result.

The composition is typically for use as an intranasally administered vaccine to pediatric immuno-compromised subjects against influenza. The pediatric immune-compromised subjects are suitably selected from the group consisting of people who are HIV infected; subjects taking immunosuppressant drugs, such as recent organ recipients; premature babies, and post-operative patients.

A further aspect of the invention is directed to a vaccine for use in naive subjects and pediatric immuno-compromised patients. The adjuvant of the invention has demonstrated its efficacy in influenza naive subjects. This renders it suitable for both naive patient classes and pediatric immune-compromised patients.

Accordingly, a further aspect of the invention is directed to a composition for use as an intranasally administered vaccine for use in naive subjects and pediatric immuno-compromised patients, said composition comprising

i) one or more non-live antigens, and

ii) an adjuvant comprising:
one or more carboxylic acids,
an aqueous medium, and
optionally one or more mono-glycerides.

Suitable types of vaccines for immunization of naive subjects and pediatric immuno-compromised patients comprise, according to the present invention, an antigen of the respectively relevant pathogen intended to be immunized or treated by vaccine This includes, without being limited to, immunogens derived from viruses selected from the group consisting of hepatitis B, hepatitis A, hepatitis C, hepatitis D & E virus, Non-A/Non-B Hepatitis virus, pox and smallpox viruses, polio virus, measles virus, human immunodeficiency virus (HIV), enteroviruses, retroviruses, respiratory syncytial virus, rotavirus, human papilloma virus, varicella-zoster virus, yellow fever virus, SARS virus, animal viruses, herpes viruses, cytomegalovirus, varicella zoster, Epstein Barr virus, para-influenza viruses, adenoviruses, coxsakie viruses, picorna viruses, rhinoviruses, rubella virus, papovirus, and mumps virus. Some non-limiting examples of known viral antigens other than the Influenza virus antigens mentioned above may include the following: antigens derived from HIV-I such as tat, nef, gpl20 or gpl[beta]O, gp40, p24, gag, env, vif, vpr, vpu, rev or part and/or combinations thereof; antigens derived from human herpes viruses such as gH, gL gM gB gC gK gE or gD or or part and/or combinations thereof or Immediate Early protein such as ICP27, ICP47, ICP4, ICP36 from HSVI or HSV2; antigens derived from cytomegalovirus, especially human cytomegalovirus such as gB or derivatives thereof; antigens derived from Epstein Barr virus such as gp350 or derivatives thereof; antigens derived from Varicella Zoster Virus such as gp I, 11, 111 and IE63; antigens derived from a hepatitis virus such as hepatitis B, hepatitis C or hepatitis E virus antigen (e.g. env protein El or E2, core protein, NS2, NS3, NS4a, NS4b, NS5a, NS5b, p7, or part and/or combinations thereof of HCV); antigens derived from human papilloma viruses (for example HPV6, 11, 16, 18, e.g. LI, L2, EI, E2, E3, E4, E5, E6, E7, or part and/or combinations thereof); antigens derived from other viral pathogens, such as Respiratory Syncytial virus (e.g F and G proteins or derivatives thereof), parainfluenza virus, measles virus, mumps virus, flaviviruses (e. g. Yellow Fever Virus, Dengue Virus, Tick-borne encephalitis virus, Japanese Encephalitis Virus) or part and/or combinations thereof.

The composition of the invention may comprise non-live antigens of the following viruses but are not limited to: non-live antigens from Herpes zoster, HIB, Pertussis, Polio, Tetanus, Diphteria, Hepatitis A, Seasonal Influenza, Influenza A, Influenza B,

Respiratory syncytial virus (RSV), Human metapneumovirus (hMPV), Human papillomavirus (HPV), Rotavirus, Norovirus, Human immunodeficiency virus (HIV), Herpes simplex, and/or Parainfluenza virus (OIV), Rhino virus, Severe acute respiratory syndrome (SARS), Coronaviruses, Herpes zoster/varicella, Hepatitis A-E, Hantavirus, and/or Cytomegalovirus, or mixtures thereof,

The compostion of the invention may comprise non-live antigens of the following bacteria but are not limited to : non-live antigens from Pneumococci, Meningococci, Haemophilus influenzae b,(Hib) Bacillus anthracis, Chlamydia trachomatis, Pseudomonas aeruginosa, Mycobacterium tuberculosis, Diphtheria, Escherichia coli. Group Streptococcus, Neisseria gonorrhoeae, Bordetella pertussis or mixtures thereof,

The antigens may be e.g. whole non-live antigens such as e.g. whole inactivated viruses. The antigen may also be part of a pathogen such as e.g. part of an inactivated virus. The antigen components that may be used are, but not limited to, for example, viral, bacterial, mycobaterial or parasitic antigens. Bacterial pathogens may be e.g. Mycobacteria causing tuberculosis and leprosy, pneumocci, aerobic gram negative or gram-positive bacilli, mycoplasma, staphylococcal infections, streptococcal infections, Helicobacter pylori, salmonellae, Bordetella pertussis and chlamydiae. The diseases may also be bacterial infections such as infections caused by Mycobacteria causing tuberculosis and leprosy, pneumocci, aerobic gram negative bacilli, mycoplasma, staphyloccocal infections, streptococcal infections, Helicobacter pylori, salmonellae, diphtheria, Bordetella pertussis causing whooping cough, and chlamydiae.

Preferred types of vaccines for immunization of naive subjects and immune-compromised patients may be selected from the group consisting of pneumococcal vaccine, Hepatitis A-E vaccine, Meningococci vaccine, Haemophilus influenzae b (Hib) vaccine, Diphtheria vaccine and DTaP vaccine (protects from diphtheria, tetanus, and pertussis (whooping cough)).

The diseases may also be parasitic such as, e.g. malaria, leishmaniasis, trypanosomiasis, toxoplasmosis, schistosomiasis, filariasis or various types of cancer such as, e.g. breast cancer, stomach cancer, colon cancer, rectal cancer,

cancer of the head and neck, renal cancer, malignant melanoma, laryngeal cancer, ovarian cancer, cervical cancer, prostate cancer.

The diseases may also be allergies due to house dust mite, pollen and other environmental allergens and autoimmune diseases such as, e.g. systemic lupus erythematosis.

The antigen in the vaccine composition may be whole non-live antigens such as e.g. whole inactivated viruses, split non-live antigens or subunit non-live antigens. Inactivation processes are well known in the art such as heat inactivation, irradiation inactivation by UV-light or in activation by formalin inactivation or treatment with beta-propiolactone.

The composition of the invention are for use as vaccines for immunization of naive subjects and pediatric immuno-compromised patients. The pediatric immune-compromised patients are suitably selected from the group consisting of people who are HIV infected subjects; subjects taking immunosuppressant drugs, such as recent organ recipients; premature babies, and post-operative patients. The naive subjects may be children under 18 years old, such as children 0 to 18 years, particularly children aged 12 and under. Typically, the children are less than 8 years of age, such as 6 years old or less. An important intended class of patients for the vaccine of the invention is particularly children of 2 months to less than 9 years of age, typically children of age 3 months to less than 9 years old, such as of age 6 months to less than 8 years old, most typically of age 6 month to less than 7 years old, such as of age 6 months to less than 72 months, or of age 6 months to 60 months or of age 6 months to 24 months. The composition of the invention is intended, at least in part, as a vaccine for pediatric use.

The naive subjects may be of all age groups when the composition is particularly directed to a vaccine for use during pandemic or peri-pandemic period.

Streptococcus pneumoniae is a major cause of morbidity and mortality worldwide with an estimated 1.6 million people dying of invasive pneumococcal disease (IPD) each year (WHO, 2002). IPD occurs most commonly among the very young (<24

months) and the elderly (>65 years); the elderly have the highest IPD mortality rates. Currently, four vaccines are available for the prevention of infection with Streptococcus pneumoniae. No intranasal vaccines are available for Streptococcus pneumonia.

One interesting embodiment of the invention is directed to an intranasal alternative for the prevention of infection with Streptococcus pneumoniae, directed particularly at infants, children, adolescents and other Streptococcus pneumoniae naive subjects. The composition of the invention does not utilize live attenuated bacteria but rather non-live streptococcus pneumonia antigens. The surprisingly efficacy of the vaccine of the invention is a result of the adjuvant used and the surprising result was specific for naive subjects. Similar results are anticipated also for immuno-compromised subjects.

Accordingly, a further aspect of the invention is directed to a composition for use as an non-live intranasally administered vaccine for use in naive subjects and pediatric immune-compromised patients for the prevention of infection with Streptococcus pneumoniae or for reducing the severity of symptoms associated with an with Streptococcus pneumonia infection, said composition comprising

i) one or more Streptococcus pneumoniae antigens, and

ii) an adjuvant comprising:
one or more carboxylic acids,
an aqueous medium, and
optionally one or more mono-glycerides.

The immuno-compromised patients are suitably selected from the group consisting of infants, children and adolescent who are; HIV infected subjects; subjects taking immunosuppressant drugs, such as recent organ recipients; premature babies, and post-operative patients

An important embodiment of the invention is directed to a vaccine against pneumococcal infection for the prevention of and/or reducing of the symptoms of disease states selected from the group consisting of bronchitis, pneumonia, septicemia, pericarditis, meningitis and peritonitis.

One embodiment is related to the use of pneumococcal vaccine, such as a pneumococcal polysaccharide vaccine (PPV) in pediatric subjects, particular for use in
subjects from 4 weeks of age to 6 years of age (e.g. to subjects that are immunologically naïve to pneumococcal antigens and with immune systems not fully developed.

The vaccine composition according to the invention may further comprise pharmaceutically acceptable excipients such as e.g. a medium which may be an aqueous medium further comprising a surface-active agent, which may be hydrophilic and inert and biocompatible, such as, e.g., poloxamers such as e.g. Pluronic F68 or Pluronic 127.

A pneumococcal vaccine according to present invention may further comprise antibacterial agents, antioxidants, viral inactivators, preservatives, dyes, stabilizers, anti-foaming agents, surfactants (non-ionic, anionic or cationic) as described herein, or any combination thereof. The antibacterial agents may be e.g. amphotericin or any derivative thereof, chlorotetracyclin, formaldehyde or formalin, gentamicin, neomycin, polymyxin B or any derivative thereof, streptomycin or any combination thereof. The antioxidants may be e.g. ascorbic acid or tocopherol or any combination thereof. The pathogenic e.g. viral and/or bacterial inactivators may be e.g. formalin, beta-propiolactone, UV-radiation, heating or any combination thereof.

When describing the embodiments of the present invention, the combinations and permutations of all possible embodiments have not been explicitly described. Nevertheless, the mere fact that certain measures are recited in mutually different dependent claims or described in different embodiments does not indicate that a combination of these measures cannot be used to advantage. The present invention envisages all possible combinations and permutations of the described embodiments.

Examples

Example 1

Objective

The objective of the present study was to investigate the immunogenicity and protective efficacy of intranasally administered adjuvant-formulated influenza split antigen and adjuvant-formulated killed whole influenza virus antigen in the ferret model, according to the present invention.

The vaccine based on H1N1/California/2009 split antigen (vaccine A) was studied with antigen doses of 5, 15, or 30 µg HA and the vaccine based on H1N1/California/2009 killed whole virus antigen (vaccine B) was studied with an antigen dose of 15 µg HA. Vaccine efficacy was studied using wild-type H1N1 A/The Netherlands/602/2009 virus as challenge.

The Endocine™ adjuvant comprised equimolar amounts of glycerol monooleate and oleic acid with a final concentration of 20mg/ml (2 %) in the vaccine composition. In this experiment Immunose™ FLU means non-live influenza antigens mixed with Endocine™.

Experimental groups Immunization phase

Table 1

Group number	Number of animals	Test substance	Antigen dose (µg HA)	Route of immunization
1	6	Saline	0	Nasal
2	6	Fluarix®	15 per strain	Subcutaneous
3	6	Vaccine A	5	Nasal
4	6	Vaccine A	15	Nasal
5	6	Vaccine A	30	Nasal
6	6	Vaccine B	15	Nasal

Vaccine preparation and administration

Saline: 0.9% saline pH 5-5.5.

Fluarix® : Parenteral vaccine (composed of A/California/7/2009(H1N1)-like, A/Perth/16/2009(H3N2)-like and B/Brisbane/60/2008-like vaccine strains at 15 µg

HA of each vaccine strain in 0.5ml). Animals of group 2 were vaccinated subcutaneously at day 21 and 42 with 0.5ml Fluarix (GlaxoSmithKline Biologicals).

Vaccine A: Influenza vaccine nasal drops, 5, 15 and 30 µg HA / 0.2 ml, adjuvant formulation comprising an Endocine formulation of equimolar amounts of glycerol monooleate and oleic acid (pH 8, in Tris 0.1M) with a final concentration of 20mg/ml in the vaccine compositionl; H1N1/California/2009 split antigen.

Vaccine B: Influenza vaccine nasal drops, 15 µg HA / 0.2 ml, adjuvant formulation comprising an Endocine formulation of equimolar amounts of glycerol monooleate and oleic acid (pH 8, in Tris 0.1M) with a final concentration of 20mg/ml in the vaccine composition, H1N1/California/2009 killed whole virus antigen.

Ferrets

Healthy female ferrets (Mustela putorius furo: outbred), approximately 12 months of age, with body weights of 760-1210 g and seronegative for antibodies against circulating influenza viruses B, A/H1N1, A/H3N2 and A/pH1N1 as demonstrated by hemagglutination inhibition (HI) assay were used. Animals were housed in normal cages, in groups of maximal 8 animals during the pre-immunization phase and in study groups of 6 animals during the immunization phase. The study groups were transferred to negatively pressurized glovebox isolator cages on the day of challenge. During the whole study animals were provided with commercial food pellets and water ad libitum.

Immunization

Five groups of six ferrets received three intranasal immunizations (droplets: 100 µl in each nostril, using a pipet with filtertip) under anesthesia with ketamine and domitor at days 0, 21 and 42. Animals of group 1 received 200 µl of steril physiological saline (0,9% saline pH5-5,5). Groups 3, 4 and 5 were intranasally immunized with 200 µl Endocine™ formulated H1N1/California/2009 split antigen containing 5, 15 and 30 µg HA, respectively. Group 6 was intranasally immunized with 200 µl Endocine™ formulated H1N1/California/2009 whole virus antigen containing 15 µg HA. Control group 1 received 200 µl of saline intranasally. One group of six ferrets (group 2) were vaccinated subcutaneously at day 21 and 42 with 0.5 ml Fluarix® (GlaxoSmithKline Biologicals), season 2010/2011, a non-adjuvanted trivalent influenza vaccine (TIV) that contained 15 µg HA of each vaccine strain.

Blood samples for serum preparation were collected prior immunization on days 0, 21 and 42 and before challenge on study days 64 and 70.

Challenge virus preparation and administration

On study day 70, all animals were challenged with a field isolate of influenza virus (H1N1 strain A/The Netherlands/602/2009) by the intratracheal route. To prepare the challenge virus, the H1N1 A/The Netherlands/602/2009 challenge stock (7.8 log10 TCID50/ml) was diluted in ice-cold PBS to a concentration of 3.3 x 105 TCID50/ml. All animals were challenged intratracheally with 3 ml of the challenge virus preparation containing 106 TCID50, administered with a small catheter into the trachea using a tracheoscope and released just above the bifurcation. Preparation and administration of the challenge virus were performed under BSL3 conditions. One day after challenge a sample of the remaining challenge virus dilution was titrated on Madin-Darby canine kidney (MDCK) cells to confirm the infectivity of the virus. Back titration of the challenge dilution one day after the inoculation showed that the material still contained 4.8 log10 TCID50.

Procedures and sample collection

Several procedures were performed on the ferrets over the course of the experiment. For implantation of temperature sensors, immunizations, viral challenge and computed tomography (CT) imaging the animals were anesthetized with a cocktail of ketamine (4-8 mg/kg: i.m.; Alfasan, Woerden, The Netherlands) and domitor (0.1 mg/kg: i.m.; Orion Pharma, Espoo, Finland). For sampling (blood, swabs and nasal washes) and euthanasia by exsanguination, the animals were anesthetized with ketamin. Two weeks prior to the start of the experiment, a temperature logger (DST micro-T ultrasmall temperature logger; Star-Oddi, Reykjavik, Iceland) was placed in the peritoneal cavity of the ferrets. This device recorded body temperature of the animals every 10 minutes. Ferrets were weighed prior to each immunization (days 0, 21 and 42) and on the days of challenge and euthanasia (days 70 and 74). Animals of groups 1, 2 and 4 were monitored by CT imaging on days 64, 71, 72, 73 and 74. Blood samples were collected prior to the immunization on days 0, 21 and 42, on day 64 and before challenge on day 70. Nose and throat swabs were collected prior challenge on day 70 and on each day after challenge.

Collection of blood samples and serum

Blood samples were collected and split in 2 equal volumes. One volume, used to isolate PBMC, was immediately transferred to a tube containing EDTA anti-coagulant. The other volume, used to collect serum, was transferred to a serum tube containing clot activator. All serum tubes were centrifuged at ca. 2000 xg for 10 minutes at room temperature. Serum was aliquoted in 0.1ml samples and stored at ca. -80 °C.

Isolation of PBMC and plasma

Blood samples, used to isolate PBMC, were immediately transferred to a tube containing EDTA anti-coagulant, centrifuged at 880x G for 5 min, the plasma was stored at ca. -80 °C. The cell pellet was resuspended in 3.5 ml wash buffer (D-PBS: lot#: RNBB7791, V-CMS: 10700395 and EDTA:lot#: 079K8712, V-CMS: 10700037), layered on 3ml lymphoprep and centrifuged at 800x G for 30 minutes. After centrifugation the cell containing interface was collected, transferred to a new tube and 4 times washed in wash buffer. Centrifugation at 600 xg, 465 xg and 350 xg for 10 min and at 250 xg for 15 min was involved in the subsequent washing steps. After the last wash step, the cell pellet was resuspended, put on ice for at least 10 min, resupended in 1 ml ice cold freeze medium (RPMI lot# 1MB078, 20 % FCS VC# 201110194, 10% DMSO VC # 10700203), transferred to an ampoule, and stored at -80 oC.

Serology

Antibody titers against H1N1 A/The Netherlands/602/2009 and 2 distant viruses H1N1 A/Swine/Ned/25/80 and H1N1 A/Swine/Italy/14432/76 were determined by hemagglutination inhibition assay (HI) and virus neutralization assay (VN). Antibody titers against the distant virus H1N1 A/New Jersey/08/76 were determined by hemagglutination inhibition assay.

HI assay

The HI assay is a standard binding assay based on the ability of influenza virus hemagglutinin specific antibodies to block influenza induced agglutination of red blood cells. The samples were pre-treated with cholera filtrate (obtained from Vibrio

cholerae cultures) in order to remove non-specific anti-hemagglutinin activity. Following an incubation for 16 hours at 37°C the cholera filtrate was inactivated by incubating the samples for 1 hour at 56°C. Serial two-fold dilutions of the samples were made in phosphate buffered sulphate (PBS) (in duplicate 96-wells plates starting with a dilution of 1:20) and when the samples showed a-specific hemagglutination, they were pre-treated with turkey erythrocytes. After removal of these erythrocytes the samples were incubated with a fixed concentration of 4 hemagglutination units (HAU) of the concerning influenza virus for 1 hour at 4°C. Finally, the plates were scored independently for inhibition of hemagglutination, as shown by sedimentation of the erythrocytes. Trending ferret control sera were included in all runs.

VN assay

The VN assay is a standard assay based on the ability of a subset of influenza virus-specific antibodies to neutralize the virus such that there will be no virus replication in the cell culture. The samples were heat-inactivated for 30 minutes at 56°C and subsequently serial two-fold dilutions of the samples were made in infection medium (Eagles minimal essential medium supplemented with 20 mM Hepes, 0.075% sodium bicarbonate, 2 mM L-Glutamine, 100 IU/ml of penicillin and streptomycin, 17.5 µg/ml trypsin and 2.3 ng/ml amphotericin B) in triplicate in 96-wells plates starting with a dilution of 1:8. The sample dilutions were then incubated with 25-400 TCID50 of the concerning virus for 1 hour at 37oC, 5% CO2. After completion of the 1 hour incubation period the virus-antibody mixtures were transferred to plates with Madine Darby Canine Kidney (MDCK) cell culture monolayers that were 95-100% confluent. These plates were than incubated for 1 hour at 37°C, 5% CO2, and the virus-antibody mixtures were subsequently removed and replaced by infection medium. After an incubation period of 6 days at 37°C, 5% CO2 the plates were read using turkey erythrocytes to detect the presence of influenza virus hemagglutinin. The VN titers were calculated according to the method described by Reed and Muench (Reed, L.J.; Muench, H. (1938). "A simple method of estimating fifty percent endpoints". The American Journal of Hygiene 27: 493–497).

Virus replication in the upper and lower respiratory tract

On days 0, 1, 2, 3 and 4 after challenge, nose and throat swabs were taken from the animals under anesthesia. Four days after challenge, the ferrets were euthanized by exsanguination under anesthesia after which full-body gross-pathology was performed and tissues were collected. Samples of the right nose turbinate and of all lobes of the right lung and the accessory lobe were collected and stored at –80°C until further processing. Turbinate and lung samples were weighed and subsequently homogenized with a FastPrep-24 (MP Biomedicals, Eindhoven, The Netherlands) in Hank's balanced salt solution containing 0.5% lactalbumin, 10% glycerol, 200 U/ml penicillin, 200 µg/ml streptomycin, 100 U/ml polymyxin B sulfate, 250 µg/ml gentamycin, and 50 U/ml nystatin (ICN Pharmaceuticals, Zoetermeer, The Netherlands) and centrifuged briefly before dilution.

After collection, nose and throat swabs were stored at -80°C in the same medium as used for the processing of the tissue samples. Quadruplicate 10-fold serial dilutions of lung and swab supernatants were used to determine the virus titers in confluent layers of MDCK cells as described previously (Rimmelzwaan GF et al.,J Virol Methods 1998 Sep;74(1)57-66).

Antibody titer results

Serum levels of antibodies were determined on days 0, 21, 42, 64, and 70 prior to each immunization. Titers against H1N1 A/The Netherlands/602/2009 and 2 distant viruses (H1N1 A/Swine/Ned/25/80 and H1N1 A/Swine/Italy/14432/76 were determined by hemagglutination inhibition assay (HI) and virus neutralization assay (VNT). Antibody titers against the distant virus H1N1 A/New Jersey/08/76) were determined by hemagglutination inhibition assay (HI).

HI antibody titers - Homologous: H1N1 A/The Netherlands/602/2009

The geometric mean HI titers are depicted in Figure 1. The ≤ 5 value was replaced with the corresponding absolute value 5 for calculation of the geometric mean. All pre-sera (day 0) were HI antibody negative (titer: ≤5).

Analysis of the HI titers by group revealed the following results:

Group 1 (Saline; infection control)

All serum samples were HI antibody negative.

Group 2 (Fluarix®; parenteral control)

One serum sample collected after the first immunization (day 42) was low HI antibody positive (titer: 13). Low HI titers (range 13-70) were detected after the second immunization in sera of five out of six animals.

Group 3 (Vaccine A, 5 µg HA; intranasal)

All samples collected after the first immunization were HI antibody positive (day 21; GMT: 477, range 160-1120). HI antibody titers increased considerably after the second immunization (day 42; GMT: 1669, range 1120-2560) and in four out of six animals also after the third immunization (day 64; GMT: 2158, range 1280-3840). Samples collected on day 70 (day of challenge) showed HI titers comparable to those measured at day 64 (day 70; GMT: 2103, range 1120-3840).

Group 4 (Vaccine A, 15 µg HA; intranasal)

Five out of six samples collected after the first immunization were HI antibody positive (day 21; GMT: 1130 range, 5-5760). All samples collected after the second immunization were HI antibody positive; HI antibody titers increased considerably in five animals (day 42; GMT: 3673, range, 1120-5760). The third immunization did not result in increased HI antibody titers (day 64; GMT: 2386, range 1920-4480). Samples collected on day 70 (day of challenge) showed HI titers comparable to those measured at day 64 (day 70; GMT: 2281, range 1280-2560).

Group 5 (Vaccine A, 30 µg HA; intranasal)

All samples collected after the first immunization were HI antibody positive (day 21; GMT: 1249, range 400-3200). HI antibody titers increased in five out of six animals after the second immunization (day 42; GMT: 1874, range 640-3840) and in two animals also after the third immunization (day 64; GMT: 1837 range 1280-3200). Samples collected on day 70 (day of challenge) showed HI titers comparable to those measured at day 64 (day 70; GMT: 1699, range 640-3200).

Group 6 (Vaccine B, 15 µg HA; intranasal)

Five out of six samples collected after the first immunization were HI antibody positive (day 21; GMT: 87, range 5-1280). HI antibody titers increased considerably in all animals after the second immunization (day 42;GMT: 577, range 100-2880) and in two animals also after the third immunization (day 64; GMT: 626, range 160-2560). Samples collected on day 70 (day of challenge) showed HI titers comparable to those measured at day 64 (day 70; GMT: 583, range 160-2240).

Heterologous: H1N1 A/Swine/Ned/25/80, H1N1 A/Swine/Italy/14432/76 and H1N1 A/New Jersey/08/76

HI antibody titers against the distant viruses H1N1 A/Swine/Ned/25/80, H1N1 A/Swine/Italy/14432/76 and H1N1 A/New Jersey/08/76 were detected. The geometric mean HI titers against the distant viruses are depicted in Figure 2. The ≤ 5 value was replaced with the corresponding absolute value 5 for calculation of the geometric mean. All pre-sera (day 0) were HI antibody negative (titer: ≤5). Cross-reactive HI antibody titers were considerably lower than homologous H1N1 A/The Netherlands/602/2009 HI antibody titers.

Analysis of the HI titers by group revealed the following results:

Group 1 (Saline; infection control)

All serum samples were HI antibody negative, except one. One sample collected on day 64 showed a very low HI antibody titer of 7.5 against H1N1 A/Swine/Italy/14432/76.

Group 2 (Fluarix®; parenteral control)

All samples were H1N1 A/Swine/Ned/25/80 and H1N1 A/Swine/Italy/14432/76 HI antibody negative. Low HI titers against H1N1 A/New Jersey/08/76 were detected in three out of six animals after the first immunization in sera collected on days 42.

Group 3 (Vaccine A, 5 µg HA; intranasal)

All animals developed cross-reactive HI antibodies against the three distant viruses. The highest titers were measured after the second and/or third immunization. H1N1 A/Swine/Ned/25/80 HI antibody titers (GMT) on days 21, 42, 64 and 70 were 6 (range 5-7.5), 24 (range 5-60), 32 (range 20-80) and 19 (range 5-70), respectively. H1N1 A/Swine/Italy/14432/76 HI antibody titers (GMT) on days 21, 42, 64 and 70

were 16 (range 5-50), 38 (range 10-80), 63 (range 40-160) and 42 (range 20-120), respectively. H1N1 A/New Jersey/08/76 HI antibody titers (GMT) on days 21, 42, 64 and 70 were 5, 26 (range 7.5-70), 39 (range 5-80) and 29 (range 20-50), respectively.

Group 4 (Vaccine A, 15 µg HA; intranasal)

All animals developed cross-reactive HI antibodies against the three distant viruses after the second immunization. The third immunization did not result in increased HI titers. H1N1 A/Swine/Ned/25/80 HI antibody titers (GMT) on days 21, 42, 64 and 70 were 42 (range 5-90), 239 (range 20-1120), 88 (range 50-160) and 75 (range 40-160), respectively. H1N1 A/Swine/Italy/14432/76 HI antibody titers (GMT) on days 21, 42, 64 and 70 were 78 (range 5-280), 327 (range 35-1280), 153 (range 80-320) and 105 (range 70-160), respectively. H1N1 A/New Jersey/08/76 HI antibody titers (GMT) on days 21, 42, 64 and 70 were 25 (range 5-80), 176 (range 60-400), 64 (range 40-140) and 63 (range 40-160), respectively.

Group 5 (Vaccine A, 30 µg HA; intranasal)

All animals except one developed cross-reactive HI antibodies against H1N1 A/Swine/Ned/25/80. All animals developed cross-reactive HI antibodies against H1N1 A/Swine/Italy/14432/76 and H1N1 A/New Jersey/08/76. The highest titers were measured after the second and/or third immunization. H1N1 A/Swine/Ned/25/80 HI antibody titers (GMT) on days 21, 42, 64 and 70 were 23 (range 5-80), 41 (range 5-320), 42 (range 5-320) and 34 (range 5-320), respectively. H1N1 A/Swine/Italy/14432/76 HI antibody titers (GMT) on days 21, 42, 64 and 70 were 39 (range 5-160), 54 (range 5-640), 78 (range 20-720) 50 (range 5-480), respectively. H1N1 A/New Jersey/08/76 HI antibody titers (GMT) on days 21, 42, 64 and 70 were 9 (range 5-30), 40 (range 5-400), 35 (range 5-160) and 27 (range 5-160), respectively.

Group 6 (Vaccine B, 15 µg HA; intranasal)

All animals developed cross-reactive HI antibodies against H1N1 A/Swine/Italy/14432/76. All animals except one developed cross-reactive HI antibodies against H1N1 A/Swine/Ned/25/80 and all animals except one developed cross-reactive HI antibodies against H1N1 A/New Jersey/08/76. The highest titers

were measured after the second and/or third immunization. H1N1 A/Swine/Ned/25/80 HI antibody titers (GMT) on days 21, 42, 64 and 70 were 7 (range 5-40), 19 (range 5-80), 15 (range 5-80) and 9 (range 5-40), respectively. H1N1 A/Swine/Italy/14432/76 HI antibody titers (GMT) on days 21, 42, 64 and 70 were 9 (range 5-160), 32 (range 5-160), 27 (range 5-160), 15 (range 5-80), respectively. H1N1 A/New Jersey/08/76 HI antibody titers (GMT) on days 21, 42, 64 and 70 were 8 (range 5-80), 47 (range 10-240), 19 (range 5-140) and 13 (range 5-80), respectively.

VN antibody titers:

Homologous: H1N1 A/The Netherlands/602/2009

VN antibody titers were measured in serum samples from all experimental animals. The geometric mean VN titers are depicted in Figure 3. All pre-sera (day 0) were VN antibody negative (titer: ≤ 8).

Analysis of the VN titers by group revealed the following results:

Group 1 (Saline; infection control)

All serum samples were VN antibody negative, except one collected on day 42 that measured ≤ 64.

Group 2 (Fluarix®; parenteral control)

All serum samples were VN antibody negative.

Group 3 (Vaccine A, 5 µg HA; intranasal)

Four out of six samples collected after the first immunization were low VN antibody positive (day 21; GMT: 19 range, 8-64). All samples collected after the second immunization were VN antibody positive. VN antibody titers increased considerably in five animals after the second immunization (day 42; GMT: 242, range, 64-859) and after the third immunization (day 64; GMT: 995, range 362-2436). Samples collected on day 70 (day of challenge) showed comparable, or lower VN titers than those measured at day 64 (day 70; GMT: 535, range 304-859).

Group 4 (Vaccine A, 15 µg HA; intranasal)

Five out of six samples collected after the first immunization were VN antibody positive (day 21; GMT: 147 range, 8-724). All samples collected after the second immunization were VN antibody positive. VN antibody titers increased considerably in five animals after the second immunization (day 42; GMT: 2376, range, 64-8192) and in two animals after the third immunization (day 64; GMT: 1688, range 662-4871). Samples collected on day 70 (day of challenge) showed VN titers comparable to those measured at day 64 (day 70; GMT: 1581, range 351-3444).

Group 5 (Vaccine A, 30 µg HA; intranasal)
All samples collected after the first immunization were VN antibody positive (day 21; GMT: 74, range 11-627). VN antibody titers increased considerably in five out of six animals after the second immunization (day 42; GMT: 504, range 41-3435) and in three out of six animals after the third immunization (day 64; GMT: 1673 range 724-4884). Samples collected on day 70 (day of challenge) showed VN titers comparable to those measured at day 64 (day 70; GMT: 1699, range 304-5793).

Group 6 (Vaccine B, 15 µg HA; intranasal)
Two out of six samples collected after the first immunization were low VN antibody positive (day 21; GMT: 12, range 8-64). All samples collected after the second immunization were VN antibody positive (day 42;GMT: 78, range 32-304). VN antibody titers increased after the third immunization (day 64; GMT: 242, range 113-747). Samples collected on day 70 (day of challenge) showed comparable, or lower VN titers than those measured at day 64 (day 70; GMT: 177, range 91-362).

Heterologous: H1N1 A/Swine/Ned/25/80, H1N1 A/Swine/Italy/14432/76. VN antibody titers against the distant viruses H1N1 A/Swine/Ned/25/80 and H1N1 A/Swine/Italy/14432/76 were tested (data not shown). All groups 3, 4, 5, and 6 outperformed groups 1 and 2 on days 42, 64 and 70.

Example 2

For all experimental animals certain clinical and pathological parameters were determined, i.e. mortality, body temperature, body weight, aerated lung volumes, viral load in turbinates and lungs, viral shedding in upper respiratory tract, Macroscopic pathologic examination post mortem of lung weight, mean percentage

of lesion affected lung tissue. Microscopic examination of inflammation parameters of nasal turbinates and lungs. Animal groups 3, 4 and 5 outperformed groups 1 and 2 in all macroscopic and in most microscopic parameters tested (data not shown).

Virus replication in the upper and lower respiratory tract

On days 0, 1, 2, 3 and 4 after challenge, nose and throat swabs were taken from the animals under anesthesia. Four days after challenge, the ferrets were euthanized by exsanguination under anesthesia after which full-body gross-pathology was performed and tissues were collected. Samples of the right nose turbinate and of all lobes of the right lung and the accessory lobe were collected and stored at –80°C until further processing. Turbinate and lung samples were weighed and subsequently homogenized with a FastPrep-24 (MP Biomedicals, Eindhoven, The Netherlands) in Hank's balanced salt solution containing 0.5% lactalbumin, 10% glycerol, 200 U/ml penicillin, 200 µg/ml streptomycin, 100 U/ml polymyxin B sulfate, 250 µg/ml gentamycin, and 50 U/ml nystatin (ICN Pharmaceuticals, Zoetermeer, The Netherlands) and centrifuged briefly before dilution.

After collection, nose and throat swabs were stored at -80°C in the same medium as used for the processing of the tissue samples. Quadruplicate 10-fold serial dilutions of lung and swab supernatants were used to determine the virus titers in confluent layers of MDCK cells as described previously (Rimmelzwaan GF et al.,J Virol Methods 1998 Sep;74(1)57-66).

Gross-pathology and histopathology

The animals were necropsied according to a standard protocol, as previously described (van den Brand JM et al.,PLoS One 2012;7(8)e42343) . In short, the trachea was clamped off so that the lungs would not deflate upon opening the pleural cavity allowing for an accurate visual quantification of the areas of affected lung parenchyma. Samples for histological examination of the left lung were taken and stored in 10% neutral-buffered formalin (after slow infusion with formalin), embedded in paraffin, sectioned at 4 µm, and stained with haematoxylin and eosin (HE) for examination by light microscopy. Samples were taken in a standardized way, not guided by changes observed in the gross pathology. Semi-quantitative assessment of influenza virus-associated inflammation in the lung was performed as described previously (Table 4) (Munster VJ et al.,Science 2009 Jul

24;325(5939):481-3). All slides were examined without knowledge of the identity or treatment of the animals.

Virus load in lung and upper respiratory tract Results

All ferrets of control groups 1 (i.n. saline) and 2 (parenteral TIV) showed high titers of replication competent virus in lung (mean titers; 5.7 and 5.5 log10TCID50/ gram tissue, respectively) and nasal turbinates (mean titers: 7.2 and 6.9 log10TCID50/ gram tissue, respectively) (Table 3). Ferrets of groups 3, 4 and 5 (i.n. Endocine™ adjuvanted split antigen pH1N1/09 vaccines) had no detectable infectious virus in their lungs and nasal turbinates. Ferrets of group 6 (i.n. Endocine™ adjuvanted whole virus at 15 µg HA) had no detectable infectious virus in their lungs and with a mean titer of 4.1 log10TCID50/ gram tissue a significant lower virus titer in the nasal turbinates as compared to control group 1 ($p=0.02$).

Intranasal immunization with Endocine™ adjuvanted pH1N1/09 vaccines reduced virus titers in swabs taken from the nose and throat as compared to saline or TIV administration. Virus loads expressed as area under the curve (AUC) in the time interval of 1-4 dpi, in nasal and throat swabs are shown in Table 3. Virus loads in nasal swabs of groups 3, 4 and 5 (i.n. Endocine™ adjuvanted split antigen at 5, 15 and 30 µg HA, respectively), but not of groups 2 and 6 were significant lower than in group 1 (group 1 versus groups 3-5; $p \leq 0.03$). Virus loads in throat swabs of group 1 and 2 were comparable and significant higher than in groups 3, 4, 5 and 6 ($p \leq 0.03$).

Gross-pathology and histopathology Results

Reduced virus replication in groups intranasally immunized with the Endocine™ adjuvanted pH1N1/09 vaccines corresponded with a reduction in gross-pathological changes of the lungs (Table 3).

The macroscopic post-mortem lung lesions consisted of focal or multifocal pulmonary consolidation, characterized by well delineated reddening of the parenchyma. All ferrets in control group 1 (i.n. saline) and group 2 (parenteral TIV) showed affected lung tissue with a mean percentage of 50% and 37%, respectively and corresponded with a mean relative lung weight (RLW) of 1.5 and 1.3, respectively (Table 3). In contrast, lungs in groups 3, 4, 5 and 6 (i.n. Endocine™ adjuvanted pH1N1/09 vaccines) were much less affected with mean percentages of

affected lung tissue of 7-8%. The RLWs in these four Endocine™vaccinated groups were in line with these observations (in a close range of 0.8 to 0.9).

The pulmonary consolidation corresponded with an acute broncho-interstitial pneumonia at microscopic examination. It was characterized by the presence of inflammatory cells (mostly macrophages and neutrophils) within the lumina and walls of alveoli, and swelling or loss of lining pneumocytes. In addition protein rich oedema fluid, fibrin strands and extravasated erythrocytes in alveolar spaces and type II pneumocyte hyperplasia were generally observed in the more severe cases of alveolitis. The histological parameters that were scored are summarized in Table 4. The most severe alveolar lesions were found in the control groups 1 (i.n. saline) and 2 (parenteral TIV). All parameters of alveolar lesions scored lowest in group 5, but in fact the differences between the groups 3, 4, 5 and 6 were not significant.

Conclusively, in lungs - The intratracheal challenge with H1N1 influenza A/Netherlands/602/2009 virus in this ferret model resulted in a slight to severe pneumonia. However, several animals, all from vaccinated groups, were not affected by macroscopically discernable lung lesions at all. Based on the macroscopic post-mortem evaluation of lung lesions (estimated % of lung affected), vaccinated (vaccine-A 15 µg HA) group 4 and vaccinated (vaccine-A 30 µg HA) group 5 equally suffered the least lung lesions with both a very low score of 7%, directly followed by vaccinated (vaccine-A 5 µg HA) group 3 and vaccinated (vaccine-B 15 µg HA) group 6 with both 8%. Placebo-PBS-treated group 1 animals suffered the most lung lesions with a marked mean score of 50%. Parenterally vaccinated control group 2 suffered slightly less but still prominent lung lesions with a mean 37%. The mean relative lung weights (RLW) were evidently in accordance with these estimated percentages of affected lung tissue, corroborating the validity of these estimated percentages of affected lung tissue.

The results of the microscopic examination of the lungs confirmed, for the majority of assessed parameters of lung lesions, the best scores for highest dosed vaccinated (vaccine-A 30 µg HA) group 5, and a gradual progression in respiratory lesions correlated to the decrease of HA dose of vaccine-A (groups 3 and 2, respectively). Vaccination with vaccine-B 15 µg HA practically equaled the results of lowest dose vaccine-A 5 µg HA (group 3). Placebo-PBS-treatment (group 1) scored by far the

worst throughout all assessed histopathological parameters, closely followed by parenterally vaccinated control group 2. Remarkably, all intranasally vaccinated animals (groups 3, 4, 5, and 6) were protected from alveolar haemorrhage.

Overall conclusions - In conclusion therefore, based on the averaged pathology scores in this ferret virus challenge model, the vaccination with vaccine-A 30 µg HA (group 5) performed the best and resulted in the least respiratory laesions, whereas the placebo-PBS-treatment performed the worst and resulted in the most respiratory lesions. Vaccination with vaccine-A 15 µg HA (group 4) performed just slightly less compared to group 5, followed by vaccination with vaccine-A 5 µg HA (group 3) that performed practically similar compared to vaccination with vaccine-B 15 µg HA (group 6). All intranasally vaccinated animals, regardless of the dose and type of vaccine, were protected from alveolar haemorrhage. Parenteral control vaccination (group 2) performed poorly with marked respiratory lesions and just marginally better compared to the placebo-PBS-treatment (group 1).

Example 3:

The Table 2 below and Figure 4 compare the vaccine of the present invention with other products, FluMist and injectable vaccines in naïve ferrets.

Table 2

Vaccine from	Ferrets (naïve)	Dose	Route	Vaccine strain (H1N1)	Evaluation strain (H1N1)	NT titer evaluation
GSK * (GSK H1N1)	N=6	15ug HA, unadjuvanted	IM	A/California/7/09	A/The Netherlands/602/09	Before challenge (after 2 vacc)
GSK *	N=6	15ug HA, $AS03_A$	IM			
Novartis # (Novartis TIV)	N=3	15ug HA, unadjuvanted	IM	A/Brisbane/59/07	A/California/7/09	Before challenge (after 2 vacc)
Medimmune # (pandemic LAIV)	N=3	$1x10^7$ $TCID_{50}$	IN	A/California/7/09 (*ca*)		
GSK ¤ (GSK TIV)	N=6	15ug HA, unadjuvanted	SC	A/California/7/09	A/The Netherlands/602/09	Day 42 (after 2 vacc)
Eurocine Vaccines ¤ (Immunose ™ FLU)	N=6	15ug HA, Endocine 20mg/ml	IN			

* Baras et al. Vaccine 29 (2011) 2120-2126

Chen et al. JID 2011:203

¤ Eurocine Vaccines: the present study

GSK monovalent pandemic vaccine (GSK H1N1), Novartis trivalent inactivated vaccine (Novartis TIV), GSK trivalent inactivated vaccine (GSK TIV) groups had a neutralization titer (NT) titer below 15.

The results show that a vaccine composition of the present invention, ImmunoseTM FLU, here comprising 15 µg HA split influenza antigen with 20 mg/ml (2 %) EndocineTM shows similar neutralizing titers to Medimmune´s pandemic LAIV vaccine FluMist (see figure 5) and superior titers to injected vaccines whereas the non-adjuvanted TIV gives poor response.

Abbreviations used in examples:

HA Influenza virus hemagglutinin protein

TCID50 Tissue culture infectious dose 50 %

PBMC Peripheral blood mononuclear cells

HI Influenza hemagglutination inhibition assay

SOP Standard Operation Procedure

PBS Phosphate buffered saline

EDTA Ethylene diamine tetraacetic acid

GMT Geometric mean titers (used to express serological data)

FCS Fetal Calf Serum (culture medium supplement)

VN Virus neutralization assay

DMSO Dimethyl Sulfoxide

Claims

1. A composition comprising
one or more non-live antigens, and
an adjuvant comprising:
one or more carboxylic acids,
an aqueous medium, and
optionally one or more mono-glycerides
for use as an intranasally administered vaccine for use in naive subjects.

2. A composition for use according to any of the preceding claims, wherein said vaccine is for pediatric use.

3. A composition for use according to any of the preceding claims, wherein said vaccine is for use in influenza naive subjects.

4. A composition for use according to any of the preceding claims, wherein said composition is for use in naive subjects for reducing virus shedding.

5. A composition for use according to any of the preceding claims, wherein said vaccine is for use in children.

6. A composition for use according to any of the preceding claims, wherein the children are less than 8 years of age, such as 6 years old or less.

7. A composition for use according to any of the preceding claims, wherein the children are of age 2 months to less than 9 years old, such as of age 6 months to less than 8 years old, preferably of age 6 month to less than 7 years old.

8. A composition for use according to any of the preceding claims for use as an influenza vaccine.

9. A composition for use according to any of the preceding claims, wherein said vaccine is for immunization against influenza in influenza naïve subjects.

10. A composition for use according to any of the preceding claims wherein the naive subjects are selected from the group consisting of children less than 12 years of age and persons of any age in a pandemic zone.

11. A composition for use according to any of the preceding claims, wherein said vaccine is for immunization against a peri-pandemic or pandemic infectious pathogen.

12. A composition for use according to any of the preceding claims, wherein said vaccine is for immunization against a pandemic infectious pathogen in infants, children and adults.

13. A composition for use according to any of the preceding claims, wherein said vaccine is for immunization against an infectious pathogen in infants, children and adults.

14. A composition for use according to any of the preceding claims wherein the non-live influenza virus antigen is selected from the group consisting of a whole inactivated virus, a split virus, and a subunit influenza antigen.

15. A composition for use according to any of the preceding claims wherein the non-live influenza virus antigen is a split antigen.

16. A composition for use according to any of the preceding claims, wherein the one or more mono-glycerides are glycerides mono-esterified with carboxylic acids selected from the group consisting of lauric acid (C12), myristic acid (C14), palmitic acid (C16), palmitoleic acid (C16: 1), oleic acid (C18: 1), linoleic acid (C18:2), stearic acid, hexanoic acid, caprylic acid, decanoic acid (capric acid), arachidic acid, behenic acid, lignoceric acid, alpha-linolenic acid, stearidonic acid, eicosapentaenoic acid, docosahexaenoic acid, gamma-linolenic acid, dihomo-gamma-linolenic acid, arachidonic acid, erucic acid, nervonic acid.

17. A composition for use according to any of the preceding claims, wherein the one or more mono-glycerides are glycerides mono-esterified with carboxylic acids selected from the group consisting of from palmitoleic acid (C16: 1), oleic acid (C18: 1) and linoleic acid (C18:2).

18. A composition for use according to any of the preceding claims, wherein the one or more mono-glyceride is glyceride mono-esterified with oleic acid (glyceryl oleate).

19. A composition for use according to any of the preceding claims, wherein the one or more carboxylic acids are selected from the group consisting of lauric acid, myristic acid, palmitic acid, palmitoleic acid, oleic acid, linoleic acid stearic acid, hexanoic acid, caprylic acid, decanoic acid (capric acid), arachidic acid, behenic acid, lignoceric acid, alpha-linolenic acid, stearidonic acid, eicosapentaenoic acid, docosahexaenoic acid, gamma-linolenic acid, dihomo-gamma- linolenic acid, arachidonic acid, erucic acid and nervonic acid.

20. A composition for use according to any of the preceding claims wherein the one or more carboxylic acids are selected from the group consisting of oleic acid and lauric acid.

21. A composition for use according to any of the preceding claims, wherein the carboxylic acid is oleic acid.

22. A composition according to any of the preceding claims, wherein the adjuvant comprises glyceryl oleate, oleic acid and an aqeuous medium.

23. A composition for use according to any of the preceding claims, wherein the vaccine composition comprises monoglycerides in an amount in the range of about 0.1 g to about 5.0 g per 100 mL, or in the range of about 0.1 g about 2.0 g per 100 ml, or about 0.5 g to about 2.0 g, such as 0.5 g to about 1.5 g per 100 mL of the vaccine composition.

24. A composition for use according to any of the preceding claims, wherein the vaccine composition comprises carboxylic acids is in an amount in the range of.

about from 0.1 g to about 5.0 g per 100 mL, or in the range of about 0.1 g to about 2.0 g per 100 mL or about 0.5 g to about 2.0 g, such as 0.5 g to about 1.5 g per 100 mL of the vaccine composition.

25. A composition for use according to any of the preceding claims, wherein one or more monoglycerides together with one or more carboxylic acids in an adjuvant mix is at the most 10% w/v, or at the most 5% w/v, or at the most 4% w/v, or at the most 3% w/v, or at the most 2% w/v or at the most 1 % w/v of the vaccine.

26. A method of immunization during a peri-pandemic or pandemic period comprising intranasally administering a therapeutically effective amount of a vaccine composition comprising a composition according to any one of claims 1- 25.

27. A method of immunization of pediatric subjects comprising intranasally administering a therapeutically effective amount of a vaccine composition comprising a composition according to any one of claims 1- 25.

28. A method of immunization of naïve subjects comprising intranasally administering a therapeutically effective amount of a vaccine composition comprising a composition according to any one of claims 1- 25.

29. A composition for use as an intranasally administered vaccine to pediatric immuno-compromised subjects, the composition comprising

one or more non-live influenza virus antigens, and

an adjuvant comprising:

one or more carboxylic acids,

an aqueous medium, and

optionally one or more mono-glycerides.

30. A composition according to claim 29, for use as an intranasally administered vaccine to pediatric immuno-compromised subjects against influenza.

31. A composition according to any of claim 29 to 30 wherein the pediatric immune-compromised subjects are selected from the group consisting of people who are HIV

infected subjects; subjects taking immunosuppressant drugs, recent organ recipients; premature babies, and post-operative patients.

32. A composition comprising

i) one or more non-live antigens, and

ii) an adjuvant comprising:

one or more carboxylic acids,

an aqueous medium, and

optionally one or more mono-glycerides

for use as an intranasally administered vaccine for use in naive immuno-compromised patients.

33. A composition for use according to claim 32, for use as an intranasally administered vaccine to naive immuno-compromised subjects against influenza.

34. A composition for use according to any of claim 32 to 33 wherein the pediatic immune-compromised subjects are selected from the group consisting of people with HIV infected subjects; subjects taking immunosuppressant drugs, recent organ recipients; premature babies, and post-operative patients.

35. A composition, said composition comprising

i) one or more Streptococcus pneumoniae antigens, and

ii) an adjuvant comprising:

one or more carboxylic acids,

an aqueous medium, and

optionally one or more mono-glycerides

for use as an intranasally administered vaccine for use in naive subjects and/or immune-compromised patients for the prevention of infection with Streptococcus pneumoniae or for reducing the severity of symptoms associated with an infection with Streptococcus pneumoniae

Figures

Figure 1

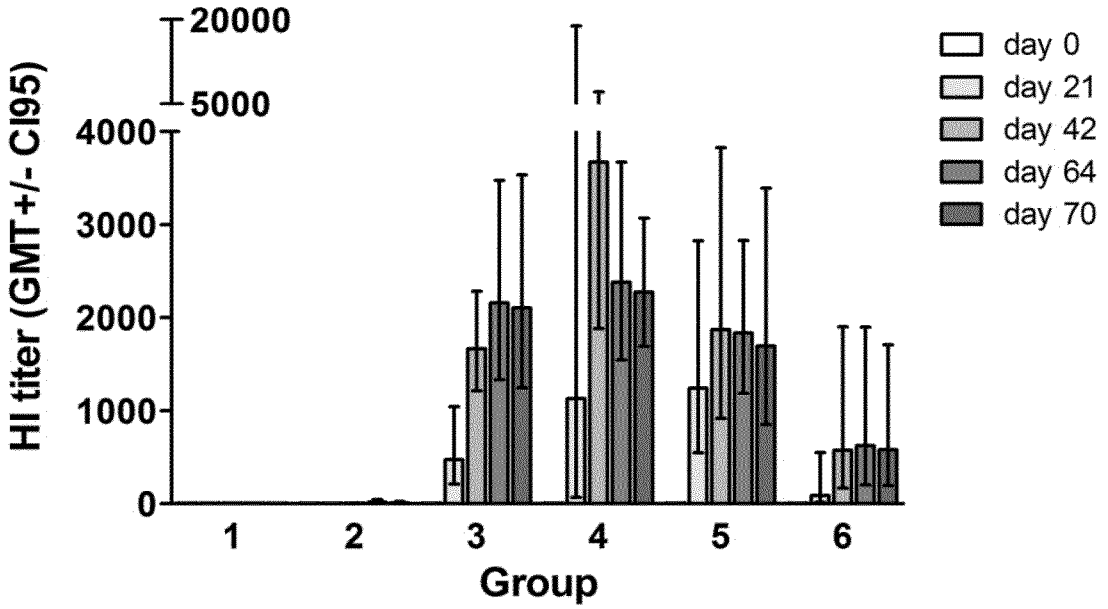

Figure 2

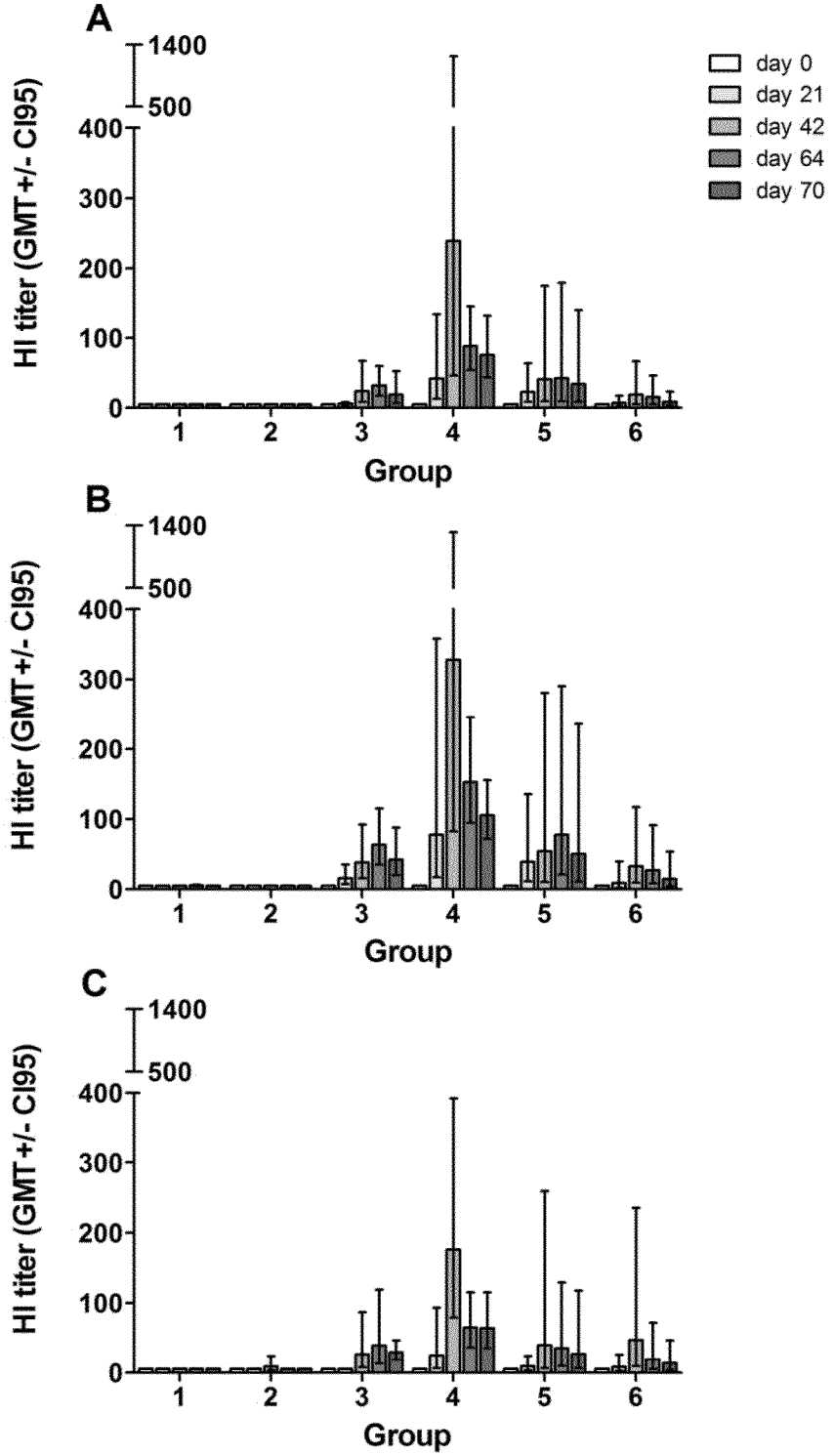
A
B
C
HI titer (GMT +/- CI95)
1400
500
400
300
200
100
0
1
2
3
4
5
6
Group
day 0
day 21
day 42
day 64
day 70

Figure 3

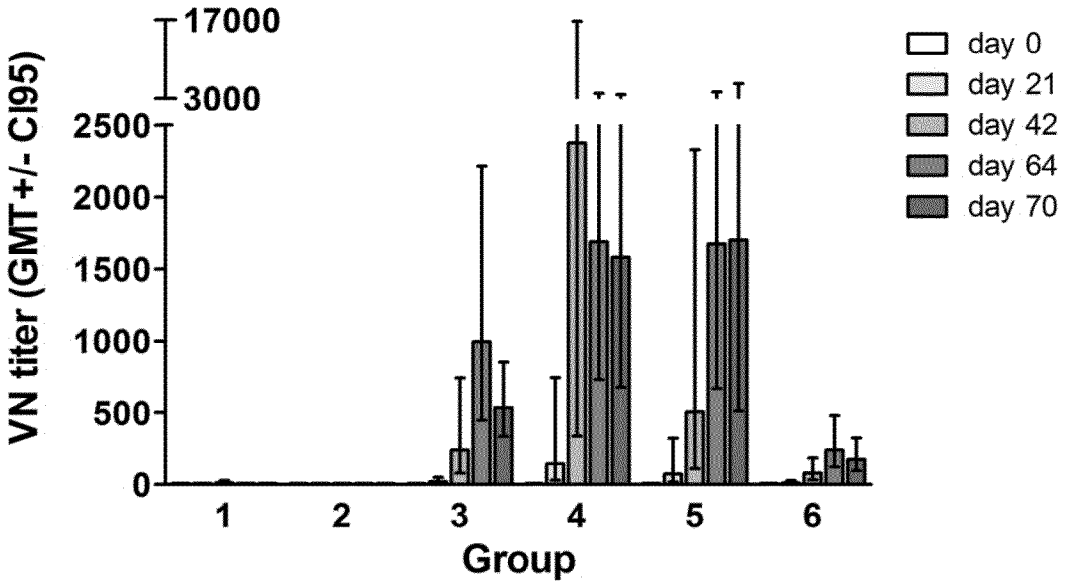
17000
3000
2500
2000
1500
1000
500
0
VN titer (GMT +/- CI95)
1
2
3
4
5
6
Group
day 0
day 21
day 42
day 64
day 70

Figure 4

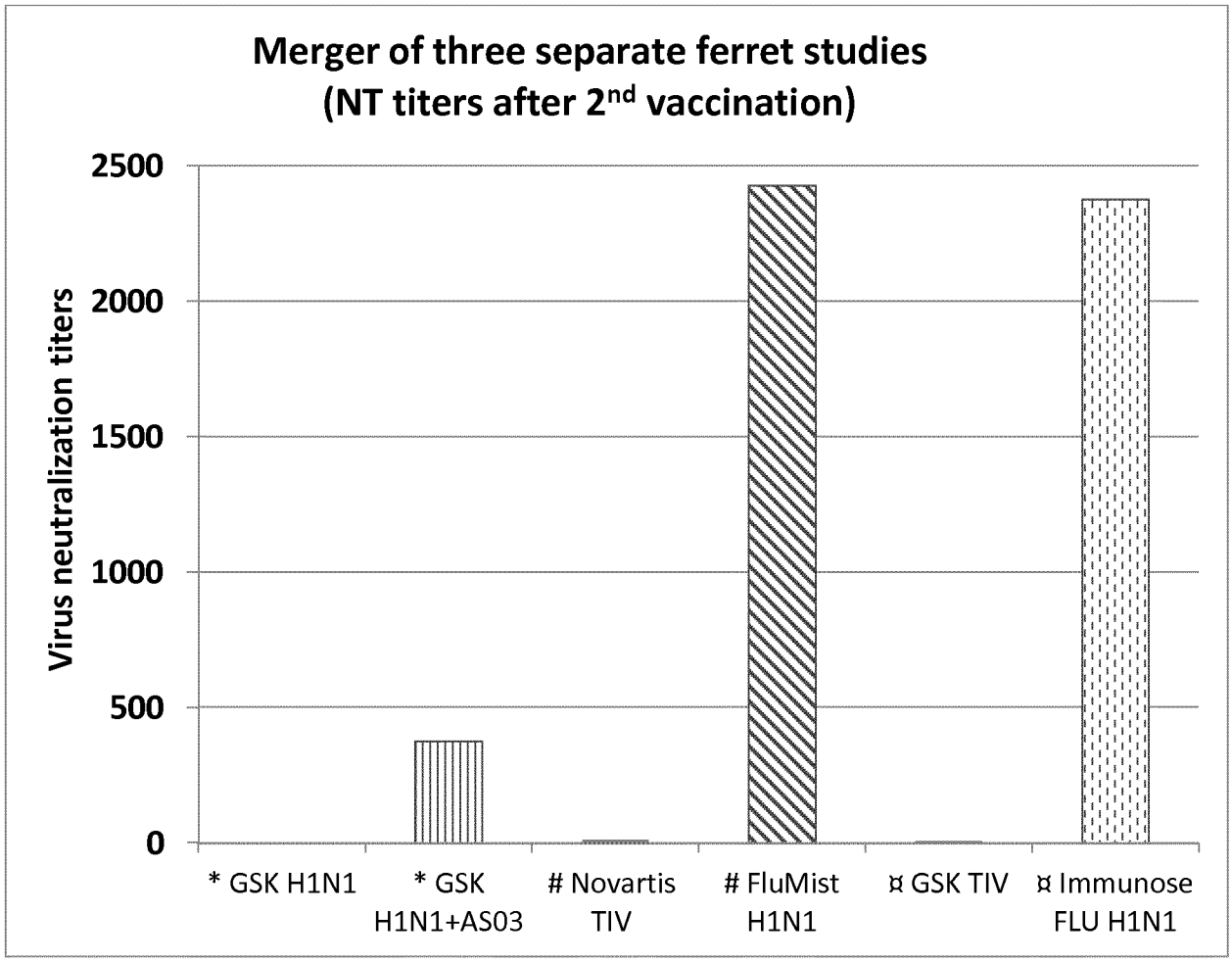
Merger of three separate ferret studies
(NT titers after 2nd vaccination)
Virus neutralization titers
2500
2000
1500
1000
500
0
* GSK H1N1
* GSK H1N1+AS03
# Novartis TIV
# FluMist H1N1
¤ GSK TIV
¤ Immunose FLU H1N1

Table 3

		Group [a]					
		1	2	3	4	5	6
Clinical score	Survival	6/6	5/6	6/6	6/6	6/6	6/6
	Fever	1.7±0.6 (6/6)	1.1±0.4 (6/6)	1.3±0.3(6/6)	1.2±0.6(4/5*)	1.1±0.6(6/6)	1.3±0.2(6/6)
	Body weight loss	18.0±4.6 (6/6)	11.5±2.1 (6/6)	-2.2±2.6 (1/6)	1.7±1.5 (4/6)	2.7±3.3 (4/6)	4.7±3.1 (6/6)
Virology	Lung virus load [$log_{10}TCID_{50}$/g]	5.7±0.5 (6/6)	5.5±0.9 (6/6)	≤1.5 (0/6)	≤1.4 (0/6)	≤1.3 (0/6)	≤1.3 (0/6)
	Turbinates virus load [$log_{10}TCID_{50}$/g]	7.2±2.4 (6/6)	6.9±1.5 (6/6)	≤1.9 (0/6)	≤1.7 (0/6)	≤1.7 (0/6)	4.1±2.7 (3/6)
	Virus shedding in nasal swabs	2.6 (5/6)	1.2 (4/6)	0.058 (1/6)	0.0 (0/6)	0.0 (0/6)	1.4 (3/6)
	Virus shedding in throat swabs	10 (6/6)	10 (6/6)	0.0 (1/6)	0.14 (1/6)	0.0 (1/6)	4.2 (5/6)
Gross pathology	Affected lung tissue [%]	50±25 (6/6)	37±21 (6/6)	8±4 (5/6)	7±5 (4/6)	7±5 (4/6)	8±4 (5/6)
	Relative lung weight	1.5±0.5	1.3±0.1	0.8±0.1	0.8±0.1	0.8±0.2	0.9±0.1

Table 4

		Group [a]					
		1	2	3	4	5	6
Histopathology	Extent of alveolitis/alveolar damage (score 0-3)	2.08±0.74 (6/6)	1.88±0.54 (6/6)	0.42±0.52 (3/6)	0.08±0.20 (1/6)	0.04±0.10 (1/6)	0.42±0.41 (4/6)
	Severity of alveolitis (score 0-3)	2.04±0.68 (6/6)	1.63±0.31 (6/6)	0.50±0.69 (3/6	0.08±0.20 (1/6)	0.04±0.10 (1/6)	0.46±0.46 (4/6)
	Alveolar oedema (% slides positive)	29±29 (4/6)	21±19 (4/6)	4±10 (1/6)	0±0 (0/6)	0±0 (0/6)	8±13 (2/6)
	Alveolar haemorrhage (% slides positive)	21±40 (2/6)	17±26 (2/6)	0±0 (0/6)	0±0 (0/6)	0±0 (0/6)	0±0 (0/6)
	Type II pneumocyte hyperplasia (% slide positive)	42±34 (4/6)	46±37 (4/6)	8±20 (1/6)	4±10 (1/6)	0±0 (0/6)	4±10 (1/6)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/077006

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/145 A61K39/39 A61K39/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C12N

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2012/042003 A1 (EUROCINE VACCINES AB [SE]; SCHROEDER ULF [SE]; ARWIDSSON HANS [SE]) 5 April 2012 (2012-04-05)	1-29,31, 32,34
Y	the whole document	30,33,35

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

[X] See patent family annex.

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"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 February 2014	03/03/2014

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

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PETERSSON P ET AL: "The Eurocine<(>R) L3 adjuvants with subunit influenza antigens induce protective immunity in mice after intranasal vaccination", VACCINE, ELSEVIER LTD, GB, vol. 28, no. 39, 7 September 2010 (2010-09-07), pages 6491-6497, XP027267112, ISSN: 0264-410X [retrieved on 2010-07-15]	1,3,4,8, 9,11,14, 16-26,28
Y	abstract table 1 page 6497, left-hand column, paragraph 2 page 6492, right-hand column	2,5-7, 10,27, 29-31
A,P	FALKEBORN TINA ET AL: "Endocine(TM), N3OA and N3OASq; Three Mucosal Adjuvants That Enhance the Immune Response to Nasal Influenza Vaccination.", PLOS ONE 2013, vol. 8, no. 8, 2013, page e70527, XP009172690, ISSN: 1932-6203 the whole document	1-35
Y	SUSANNA ESPOSITO ET AL: "Different influenza vaccine formulations and adjuvants for childhood influenza vaccination", VACCINE, vol. 29, no. 43, 4 August 2011 (2011-08-04), pages 7535-7541, XP028306518, ISSN: 0264-410X, DOI: 10.1016/J.VACCINE.2011.08.012 [retrieved on 2011-08-02] the whole document	2,5-7, 10,27, 29-31
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BUONAGURO ET AL: "DNA-VLP prime-boost intra-nasal immunization induces cellular and humoral anti-HIV-1 systemic and mucosal immunity with cross-clade neutralizing activity", VACCINE, ELSEVIER LTD, GB, vol. 25, no. 32, 20 July 2007 (2007-07-20) , pages 5968-5977, XP022162332, ISSN: 0264-410X, DOI: 10.1016/J.VACCINE.2007.05.052 page 5969, right-hand column, paragraph 4	1,3,11, 16-26,28
A	HINKULA J ET AL: "A novel DNA adjuvant, N3, enhances mucosal and systemic immune responses induced by HIV-1 DNA and peptide immunizations", VACCINE, ELSEVIER LTD, GB, vol. 24, no. 21, 22 May 2006 (2006-05-22), pages 4494-4497, XP028010738, ISSN: 0264-410X, DOI: 10.1016/J.VACCINE.2005.08.015 [retrieved on 2006-05-22] page 4494, left-hand column, paragraph 2 page 4495, left-hand column, paragraph 1	1-35
X	SCHRODER U ET AL: "Nasal and parenteral immunizations with diphtheria toxoid using monoglyceride/fatty acid lipid suspensions as adjuvants", VACCINE, ELSEVIER LTD, GB, vol. 17, no. 15-16, 9 April 1999 (1999-04-09), pages 2096-2103, XP004165061, ISSN: 0264-410X, DOI: 10.1016/S0264-410X(98)00408-3 abstract tables 4,5,6	1,3, 16-26,28
Y	GLUECK T: "Vaccinate your immunocompromised patients!", RHEUMATOLOGY, OXFORD UNIVERSITY PRESS, LONDON, GB, vol. 45, no. 1, 1 January 2006 (2006-01-01), pages 9-10, XP002460594, ISSN: 1462-0324, DOI: 10.1093/RHEUMATOLOGY/KEI237 the whole document	33,35

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ESPOSITO S ET AL: "Vaccinations in children with cancer", VACCINE, ELSEVIER LTD, GB, vol. 28, no. 19, 26 April 2010 (2010-04-26), pages 3278-3284, XP026997103, ISSN: 0264-410X [retrieved on 2010-03-10] page 3281, left-hand column, paragraph 6-8 page 3281, right-hand column, paragraph 3 -----	33,35
Y	HANA HAKIM ET AL: "Immunogenicity and safety of inactivated monovalent 2009 H1N1 influenza A vaccine in immunocompromised children and young adults", VACCINE, ELSEVIER LTD, GB, vol. 30, no. 5, 25 November 2011 (2011-11-25), pages 879-885, XP028436167, ISSN: 0264-410X, DOI: 10.1016/J.VACCINE.2011.11.105 [retrieved on 2011-12-05] abstract page 884, left-hand column, paragraph 3 -----	33,35

4

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2013/077006

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		KR 20130130711 A	02-12-2013
		US 2013243817 A1	19-09-2013
		WO 2012042003 A1	05-04-2012

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(87)PCT国际申请的公布数据
WO2014/095943 EN 2014.06.26
(71)申请人 优若森疫苗公司
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(74)专利代理机构 北京集佳知识产权代理有限公司 11227
代理人 郑斌 彭鲲鹏
(51)Int.Cl.
A61K 39/145(2006.01)
A61K 39/39(2006.01)
A61K 39/00(2006.01)

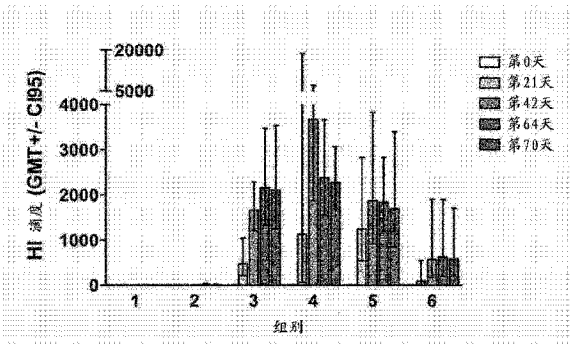
权利要求书3页 说明书26页 附图6页

(54)发明名称

用于首次免疫对象的疫苗组合物

(57)摘要

本发明涉及在首次免疫对象（例如儿童）中有效的经鼻施用疫苗组合物。此外，所述疫苗组合物适合于在大流行期间对一般群体进行疫苗接种。本发明的一个方面涉及本发明疫苗（包括在儿童中针对季节性流感病毒毒株有效的疫苗）的儿科用途。本发明的另一个方面涉及当组合物用于大流行应用时的所有年龄组的对象。



CN 104870010 A

1. 组合物，其包含：

一种或更多种非活抗原，以及

包含以下的佐剂：

一种或更多种羧酸、

水性介质、和

任选的一种或更多种单酰甘油，

所述组合物用作用于首次免疫对象的鼻内施用疫苗。

2. 根据前述权利要求中任一项所述应用的组合物，其中所述疫苗用于儿科应用。

3. 根据前述权利要求中任一项所述应用的组合物，其中所述疫苗用于流感首次免疫对象。

4. 根据前述权利要求中任一项所述应用的组合物，其中所述组合物用于首次免疫对象以减少病毒脱落。

5. 根据前述权利要求中任一项所述应用的组合物，其中所述疫苗用于儿童。

6. 根据前述权利要求中任一项所述应用的组合物，其中所述儿童小于8岁，例如为6岁或更小。

7. 根据前述权利要求中任一项所述应用的组合物，其中所述儿童为2月龄至小于9岁，例如6月龄至小于8岁，优选6月龄至小于7岁。

8. 根据前述权利要求中任一项所述应用的组合物，其用作流感疫苗。

9. 根据前述权利要求中任一项所述应用的组合物，其中所述疫苗用于在流感首次免疫对象中针对流感进行免疫。

10. 根据前述权利要求中任一项所述应用的组合物，其中所述首次免疫对象选自小于12岁的儿童和大流行地区中任意年龄的人。

11. 根据前述权利要求中任一项所述应用的组合物，其中所述疫苗用于针对近大流行或大流行感染性病原体进行免疫。

12. 根据前述权利要求中任一项所述应用的组合物，其中所述疫苗用于在婴儿、儿童和成年人中针对大流行感染性病原体进行免疫。

13. 根据前述权利要求中任一项所述应用的组合物，其中所述疫苗用于在婴儿、儿童和成年人中针对感染性病原体进行免疫。

14. 根据前述权利要求中任一项所述应用的组合物，其中所述非活流感病毒抗原选自完整灭活病毒、裂解病毒和亚单位流感抗原。

15. 根据前述权利要求中任一项所述应用的组合物，其中所述非活流感病毒抗原是裂解抗原。

16. 根据前述权利要求中任一项所述应用的组合物，其中所述一种或更多种单酰甘油是经选自以下的羧酸单酯化的甘油酯：月桂酸（C12）、豆蔻酸（C14）、棕榈酸（C16）、棕榈油酸（C16:1）、油酸（C18:1）、亚油酸（C18:2）、硬脂酸、己酸、辛酸、癸酸（羊蜡酸）、花生酸、山萮酸、木蜡酸、α-亚麻酸、硬脂四烯酸、二十碳五烯酸、二十二碳六烯酸、γ-亚麻酸、二高-γ-亚麻酸、花生四烯酸、芥酸、神经酸。

17. 根据前述权利要求中任一项所述应用的组合物，其中所述一种或更多种单酰甘油是经选自以下的羧酸单酯化的甘油酯：棕榈油酸（C16:1）、油酸（C18:1）和亚油酸（C18:

2)。

18. 根据前述权利要求中任一项所述应用的组合物，其中所述一种或更多种单酰甘油是经油酸单酯化的甘油酯（油酸甘油酯）。

19. 根据前述权利要求中任一项所述应用的组合物，其中所述一种或更多种羧酸选自：月桂酸、豆蔻酸、棕榈酸、棕榈油酸、油酸、亚油酸、硬脂酸、己酸、辛酸、癸酸（羊蜡酸）、花生酸、山萮酸、木蜡酸、α－亚麻酸、硬脂四烯酸、二十碳五烯酸、二十二碳六烯酸、γ－亚麻酸、二高－γ－亚麻酸、花生四烯酸、芥酸和神经酸。

20. 根据前述权利要求中任一项所述应用的组合物，其中所述一种或更多种羧酸选自油酸和月桂酸。

21. 根据前述权利要求中任一项所述应用的组合物，其中所述羧酸是油酸。

22. 根据前述权利要求中任一项所述应用的组合物，其中所述佐剂包含油酸甘油酯、油酸和水性介质。

23. 根据前述权利要求中任一项所述应用的组合物，其中疫苗组合物包含以下量的单酰甘油：每 100mL 所述疫苗组合物约 0.1g 至约 5.0g，或者每 100mL 所述疫苗组合物约 0.1g 至约 2.0g 或约 0.5g 至约 2.0g，例如每 100mL 所述疫苗组合物 0.5g 至约 1.5g。

24. 根据前述权利要求中任一项所述应用的组合物，其中疫苗组合物包含以下量的羧酸：每 100mL 所述疫苗组合物约 0.1g 至约 5.0g，或者每 100mL 所述疫苗组合物约 0.1g 至约 2.0g 或约 0.5g 至约 2.0g，例如每 100mL 所述疫苗组合物 0.5g 至约 1.5g。

25. 根据前述权利要求中任一项所述应用的组合物，其中佐剂混合物中一种或更多种单酰甘油与一种或更多种羧酸一起为所述疫苗的至多 10％ w/v、或至多 5％ w/v、或至多 4％ w/v、或至多 3％ w/v、或至多 2％ w/v 或至多 1％ w/v。

26. 在近大流行期或大流行期期间进行免疫的方法，其包括鼻内施用治疗有效量的疫苗组合物，所述疫苗组合物包含根据权利要求 1 至 25 中任一项所述的组合物。

27. 对儿科对象进行免疫的方法，其包括鼻内施用治疗有效量的疫苗组合物，所述疫苗组合物包含根据权利要求 1 至 25 中任一项所述的组合物。

28. 对首次免疫对象进行免疫的方法，其包括鼻内施用治疗有效量的疫苗组合物，所述疫苗组合物包含根据权利要求 1 至 25 中任一项所述的组合物。

29. 用作儿科免疫低下对象的鼻内施用疫苗的组合物，所述组合物包含：

一种或更多种非活流感病毒抗原，以及

包含以下的佐剂：

一种或更多种羧酸、

水性介质、和

任选的一种或更多种单酰甘油。

30. 根据权利要求 29 所述应用的组合物，其用作儿科免疫低下对象的针对流感的鼻内施用疫苗。

31. 根据权利要求 29 至 30 中任一项所述的组合物，其中所述儿科免疫低下对象选自以下的人：感染 HIV 的对象、正服用免疫抑制药物的对象、近期器官接受者、早产婴儿和手术后的患者。

32. 组合物，其包含：

i) 一种或更多种非活抗原，以及

ii) 包含以下的佐剂：

一种或更多种羧酸、

水性介质、和

任选的一种或更多种单酰甘油，

所述组合物用作用于首次免疫之免疫低下患者的鼻内施用疫苗。

33. 根据权利要求 32 所述应用的组合物，其用作首次免疫之免疫低下对象的针对流感的鼻内施用疫苗。

34. 根据权利要求 32 至 33 中任一项所述应用的组合物，其中儿科免疫低下对象选自以下的人：感染 HIV 的对象、正服用免疫抑制药物的对象、近期器官接受者、早产婴儿和手术后的患者。

35. 组合物，所述组合物包含：

i) 一种或更多种肺炎链球菌 (Streptococcus pneumoniae) 抗原，以及

ii) 包含以下的佐剂：

一种或更多种羧酸、

水性介质、和

任选的一种或更多种单酰甘油，

所述组合物用作用于首次免疫对象和 / 或免疫低下患者的鼻内施用疫苗，用以预防肺炎链球菌感染或用以降低与肺炎链球菌感染相关症状的严重程度。

用于首次免疫对象的疫苗组合物

技术领域

[0001] 本发明涉及在首次免疫(naive)对象(例如儿童)中有效的经鼻施用的疫苗组合物。此外,所述疫苗组合物适合于在大流行期间对一般群体进行疫苗接种。

背景技术

[0002] 目前,普遍使用的流感疫苗基于活病毒或灭活病毒,并且灭活病毒疫苗可基于完整病毒、"裂解(split)"病毒、亚单位蛋白或者基于纯化的表面抗原(包括血凝素和神经氨酸酶)。

[0003] 已逐渐认识到了流感的社会经济影响及其在健康年幼儿童中的医疗负担。此外,儿童在大流行期间的流感发病率最高,并将社区中的流感病毒传播至其他风险群体。

[0004] 因为健康年幼儿童没有充分发育的免疫系统,所以他们具有提高的流感感染风险。婴儿在其出生后前三个月易受在较年长个体中不常见的感染(如无乳链球菌(Streptococcus agalactiae))影响,并且婴儿在其出生后前几个月中依赖于母体抗体。婴儿不以与成人相同的方式对某些疫苗作出应答并且在约5岁之前都不能产生多糖抗原的有效抗体。免疫系统随儿童一起生长并发育,并且在青春期(性激素可负责儿童免疫系统完全成熟的时候)之前与成年人的免疫系统不完全相似。

[0005] 美国免疫实践咨询委员会(American Advisory Committee on Immunization Practices,ACIP)推荐每年对所有6至59月龄的儿童进行流感疫苗接种,原因是6至23月龄的儿童的流感相关住院治疗的风险是显著提高的,并且24至59月龄的儿童的流感相关门诊和急诊就诊的风险是提高的。对于季节性流感疫苗接种,该推荐延长至没有禁忌的≥6月龄的所有人。美国食品和药物管理局根据以下年龄范围对儿科亚群进行了分类。新生儿群体为从出生至1月龄。婴儿群体为1月龄至2岁。儿童群体为2岁至12岁。青少年群体为12岁至21岁。在欧洲,一些国家发布了与ACIP类似的推荐,但是关于年幼儿童的通用免疫的限制更多。欧洲药品管理局根据以下群体对儿科药品进行了分类。新生儿群体包括早产至足月产直至28天。婴儿群体为1月龄至23月龄。儿童群体为2岁至11岁。青少年为12岁至18岁。

[0006] 研究显示,常规的肠胃外疫苗在未接触过抗原的(首次免疫)儿童(尤其是非常小的儿童)中诱导满意的保护性免疫的能力有限。ACIP推荐在首次免疫的非常年幼儿童中进行两剂量的疫苗接种方案,但是最近,这样的推荐延长至年龄大至8岁的儿童,原因是越来越多的证据表明在该群体中实现保护需要2剂量。

[0007] 在大流行时期中,传播的流感病毒与来自之前流行的那些病毒相关。病毒在具有来自生命早期感染的不同水平免疫力的人群中蔓延。在通常2至3年的时期中,在称为抗原漂移(antigenic drift)的现象中的这种传播促进了对改变足以在一般群体中再次引起流行的新毒株的选择。漂移变体在任意一年中对不同的社区、地区、国家或大洲可具有不同影响,但是经过几年,其总体影响常常是相似的。典型的流感大流行使得肺炎和下呼吸道疾病的发生率提高,正如住院率和死亡率提高所证明的。

[0008] 每隔不可预测的间隔，新型流感病毒通过被称为“抗原转变 (antigenic shift)”的过程出现并且能够引起大流行。抗原转变是两种或更多种不同的病毒毒株组合以形成具有这两种或更多种原始毒株之表面抗原的混合物的新亚型。抗原转变是赋予表型改变的重配或病毒转变的具体情况。因此，当人群对其没有已有免疫力的新流感病毒出现时，则发生流感大流行。当抗原转变发生时，一般群体对于新病毒毒株将是首次免疫的。

[0009] 抗原转变与抗原漂移形成对照，抗原漂移是已知流感毒株随时间发生的可导致免疫力丧失或疫苗错配的天然突变。所有类型的流感病毒中都会发生抗原漂移，包括甲型流感病毒、乙型流感病毒和丙型流感病毒。但是，仅甲型流感病毒中发生抗原转变，原因是其不只是感染人。

[0010] 在大流行期间，抗病毒药将不足以或不足够有效以覆盖处于潜在威胁生命的流感疾病风险下的个体的需求和数目。必需开发合适的疫苗以便在首次免疫对象中实现保护性抗体水平。

[0011] 这些问题可通过佐剂化 (adjuvantation) 和 / 或最佳疫苗递送来对抗，所述佐剂化和 / 或最佳疫苗递送的目的在于提高疫苗的免疫原性，以便能够降低抗原含量并因此提高可用的疫苗数量。使用佐剂还可帮助在对特定流感毒株没有已有免疫力的群体中针对抗原刺激免疫系统。佐剂还可增强疫苗的递送并从而降低诱导免疫应答所需的抗原量。疫苗递送和 / 或疫苗接种途径可能具有很高的重要性。大多数流感疫苗肠胃外递送并因此主要在血液中诱导针对流感的免疫。然而，流感病毒通过我们的鼻或口（即，通过黏膜）进入我们的身体。通过将流感疫苗递送至鼻，在黏膜和血液中均可诱导流感特异性免疫。当目的是诱导针对流感的保护性免疫（尤其是在先前对流感疫苗株或对任意流感没有免疫力的个体中诱导）时，这可能是有益的。

[0012] 需要新的非活疫苗（例如基于完整灭活病毒或灭活病毒一部分的疫苗），其能够在对疫苗抗原没有已有免疫力的个体中诱导针对流感疾病的保护性免疫。对流感没有足够的已有免疫力和 / 或免疫状态变弱的个体包括免疫低下 (immuno-compromised) 个体、年幼儿童、老年人和大流行情况下全球群体中的大部分人（或所有人）。本发明特别地涉及对病毒抗原具有有限的或没有已有免疫力的儿童。该群组尤其需要可刺激针对例如流感的免疫应答的安全非活疫苗。还需要这样的新疫苗，其可在宣布大流行之前或宣布大流行时用作近大流行性 (peri-pandemic) 疫苗以针对大流行性毒株刺激首次免疫群体。本发明特别地涉及首次免疫群体，并且尤其是，因被配制成用于鼻施用并且仅包含灭活病毒或病毒一部分而可容易地进行施用，因此不需要经医疗培训的人员。疫苗抗原与有效佐剂的制剂允许增强免疫应答。

[0013] 发明概述

[0014] 本发明的一个目的是提供这样的疫苗：其能够刺激免疫应答并在对疫苗株没有已有免疫力的对象中提供针对季节性和流行性病毒毒株二者及其他病原生物体的保护性免疫。本发明的一个方面涉及本发明疫苗的儿科用途，本发明疫苗包括在儿童中对季节性流感病毒毒株有效的疫苗。本发明的另一个方面涉及当组合物用于大流行时使用时的所用年龄组的对象。

[0015] 本发明的第一方面涉及这样的组合物，其包含：

[0016] i) 一种或更多种非活抗原，以及

[0017] ii) 包含以下的佐剂：

[0018] 一种或更多种羧酸、

[0019] 水性介质、和

[0020] 任选的一种或更多种单酰甘油，

[0021] 所述组合物用作用于首次免疫对象的鼻内施用疫苗。

[0022] 所述组合物可被配制成用作针对所有合适病原体的疫苗。因此，所述组合物可被配制为用于任何合适的病毒毒株或细菌的疫苗。所述组合物可被配制成用作用于鼻内施用的流感疫苗。开发本发明用作用于针对病原体感染（例如，在对疫苗株具有有限的或没有已有免疫力的对象中）进行鼻内免疫的疫苗。

[0023] 本发明的第二方面涉及用作儿科免疫低下对象的鼻内施用疫苗的组合物，所述组合物包含：

[0024] 一种或更多种非活流感病毒抗原，以及

[0025] 包含以下的佐剂：

[0026] 一种或更多种羧酸、

[0027] 水性介质、和

[0028] 任选的一种或更多种单酰甘油。

[0029] 本发明的第三方面涉及这样的组合物，其包含：

[0030] i) 一种或更多种非活抗原，以及

[0031] ii) 包含以下的佐剂：

[0032] 一种或更多种羧酸、

[0033] 水性介质、和

[0034] 任选的一种或更多种单酰甘油，

[0035] 所述组合物用作用于首次免疫的免疫低下患者的鼻内施用疫苗。

[0036] 本发明的又一个方面涉及组合物，所述组合物包含：

[0037] i) 一种或更多种肺炎链球菌 (Streptococcus pneumoniae) 抗原，

[0038] 以及

[0039] ii) 包含以下的佐剂：

[0040] 一种或更多种羧酸、

[0041] 水性介质、和

[0042] 任选的一种或更多种单酰甘油，

[0043] 所述组合物用作用于首次免疫对象和 / 或免疫低下患者的鼻内施用疫苗，用以预防肺炎链球菌感染或用以降低与肺炎链球菌感染相关症状的严重程度。

[0044] 附图简述

[0045] 图 1：针对 H1N1 A/Ned/602/09(A) 的 HI 抗体滴度的演变。在第 0、21 和 42 天通过滴鼻剂对第 1、3 至 6 组的雪貂 (ferret) 进行鼻内接种，并且在第 21 和 42 天对第 2 组雪貂进行皮下注射。在第 0、21 和 42 天进行免疫之前并在第 64 和 70 天进行最后一次免疫之后所收集的血清中测定 HI 抗体滴度。第 1 组（对照，鼻内 (i.n.)，盐水），第 2 组（皮下 (s.c.)，TIV），第 3 组（鼻内，5 μg HA 的 Endocine™ 佐剂化裂解抗原），第 4 组（鼻内，15 μg HA 的 Endocine™ 佐剂化裂解抗原），第 5 组（鼻内，30 μg HA 的 Endocine ™ 佐剂化

裂解抗原）和第 6 组（鼻内，15μg HA 的 Endocine™佐剂化灭活完整病毒抗原）。条代表每组 6 只动物的几何平均数，95% CI(GMT+/-CI95)。

[0046] 图 2：针对不同病毒的 HI 滴度。

[0047] 在第 0、21 和 42 天通过滴鼻剂对第 1、3 至 6 组的雪貂进行鼻内接种，并且在第 21 和 42 天对第 2 组雪貂进行皮下注射。在第 0、21 和 42 天进行免疫之前并在第 64 和 70 天进行最后一次免疫之后所收集的血清中测定 HI 抗体滴度。第 1 组（对照，鼻内，盐水），第 2 组（皮下，TIV），第 3 组（鼻内，5μg HA 的 Endocine™佐剂化裂解抗原），第 4 组（鼻内，15μg HA 的 Endocine™佐剂化裂解抗原），第 5 组（鼻内，30μg HA 的 Endocine ™佐剂化裂解抗原）和第 6 组（鼻内，15μg HA 的 Endocine™佐剂化灭活完整病毒抗原）。条代表每组 6 只动物的几何平均数，95% CI(GMT+/-C195)。对于 GMT 计算，将≤ 5 的值替换为绝对值 5。A：针对 H1N1 A/ 猪 /Ned/25/80 的抗体滴度。B：针对 H1N1 A/ 猪 /Italy/14432/76。C：针对 H1N1 A/New Jersey/08/76 的抗体滴度。

[0048] 图 3：针对 H1N1 A/Ned/602/09 的 VN 抗体滴度的演变。

[0049] 在第 0、21 和 42 天通过滴鼻剂对第 1、3 至 6 组的雪貂进行鼻内接种，并且在第 21 和 42 天对第 2 组雪貂进行皮下注射。在第 0、21 和 42 天进行免疫之前并在第 64 和 70 天进行最后一次免疫之后所收集的血清中测定 VN 抗体滴度。第 1 组（对照，鼻内，盐水），第 2 组（皮下，TIV），第 3 组（鼻内，5μg HA 的 Endocine™佐剂化裂解抗原），第 4 组（鼻内，15μg HA 的 Endocine™佐剂化裂解抗原），第 5 组（鼻内，30μg HA 的 Endocine ™佐剂化裂解抗原）和第 6 组（鼻内，15μg HA 的 Endocine™佐剂化灭活完整病毒抗原）。条代表每组 6 只动物的几何平均数，95% CI(GMT+/-C195)。

[0050] 图 4：本发明的疫苗 ImmunoseTM FLU 与另一些佐剂化疫苗产品 FluMist（减毒活疫苗）和可注射疫苗在流感首次免疫雪貂中的比较，所述疫苗 ImmunoseTM FLU 包含 15μg HA 裂解流感抗原和 20mg/ml(2%)Endocine™。

[0051] 表 3：通过临床参数、病毒学参数和大体病理学 (gross-pathology) 参数证明的由 Endocine™配制的 2009H1N1 疫苗的效力。

[0052] ：第 1 组（对照，鼻内，盐水），第 2 组（皮下，TIV），第 3 组（鼻内，5μg HA 的 Endocine™佐剂化裂解抗原），第 4 组（鼻内，15μg HA 的 Endocine ™佐剂化裂解抗原），第 5 组（鼻内，30μg HA 的 Endocine™佐剂化裂解抗原）和第 6 组（鼻内，15μg HA 的 Endocine™佐剂化灭活完整病毒抗原）。

[0053] 临床评分。存活，存活直至 4dpi 的动物数；发热（℃），最大温度提高，表示为带有标准偏差的平均值，括号中为观察到发热的动物数，（*），第 4 组中 1 只动物的体温因记录器故障而没有获得；0 至 4dpi 之间的%体重减轻，表示为带有标准偏差的平均值，括号中为体重减轻的动物数。

[0054] 病毒学。鼻咽拭子样品中的病毒脱落，滴定结果 1 至 4dpi 中的曲线下面积 (AUC)，括号中为示出 1 个或更多个病毒阳性拭子的动物数；在 4dpi 肺和鼻甲中的病毒载量 ($log_{10}TCID_{50}/g$) 表示为带有标准偏差的平均值，或者在组中所有动物是病毒阴性时的检测下限，括号中为具有肺 / 鼻甲病毒的动物数。

[0055] 大体病理学。在 4dpi 尸体剖检期间通过目视检查所估计的受感染的肺实质的%，表示为带有标准偏差的平均值，括号中为具有受感染的肺的动物数；在 4dpi 的肺 / 体重比

($\times 10^2$) 表示为带有标准偏差的平均值。

[0056] 表 4 :在 4dpi 的组织病理学参数的半定量评分。

[0057] [a]:第 1 组 (对照,鼻内,盐水),第 2 组 (皮下, TIV),第 3 组 (鼻内,5 μg HA 的 Endocine™佐剂化裂解抗原),第 4 组 (鼻内,15 μg HA 的 Endocine ™佐剂化裂解抗原),第 5 组 (鼻内,30 μg HA 的 Endocine™佐剂化裂解抗原) 和第 6 组 (鼻内,15 μg HA 的 Endocine™佐剂化灭活完整病毒抗原)。

[0058] 组织病理学。在第 4dpi 的组织病理学参数的半定量评分。肺泡炎 / 肺泡损伤的程度,评分 :0,0% ;1,25% ;2,25%至 30% ;3,> 50% ;肺泡炎的严重程度,评分 :没有炎性细胞 (0),很少的炎性细胞 (1),中等数量的炎性细胞 (2),许多炎性细胞 (3) ;肺泡水肿、肺泡出血和 II 型肺细胞增生以阳性玻片评分 (无= 0,有= 1) ;所有的组织病理学结果表示为带有标准偏差的平均值。

[0059] 发明详述

[0060] 在描述本发明的实施方案时,为了清楚起见,将采用特定术语。但是,本发明不旨在受限于所选择的特定术语,并且应理解,每个特定术语包括以类似方式操作以实现类似目的的所有技术等同物。

[0061] 术语“首次免疫对象”意指对病原体为首次免疫的对象,即,未进行接种或未暴露于给定病原体并因此对所述病原体没有已有免疫力的对象。

[0062] 术语“流感首次免疫对象”意指对特定流感病毒为首次免疫的对象,即,未进行接种或未暴露于特定流感并因此对所述流感毒株没有已有免疫力的对象。对于流感,其在针对季节性流感接种时意指婴儿和儿童,并且在近大流行期和大流行期时意指整个群体,包括婴儿、儿童、成年人和老年人。

[0063] 术语“儿科对象”是指 21 岁以下的儿童并且包括以下亚群 :出生至 1 月龄的新生儿群体、1 月龄至 2 岁的婴儿、2 岁至 12 岁的儿童和 12 岁至 21 岁的青少年。

[0064] 术语“近大流行期”是指大流行附近的时期。给定大流行是由 WHO 官方认定的时期,该术语是指紧邻官方承认大流行前并紧邻大流行后的时期,期间推荐进行接种。

[0065] 术语“非活抗原”是指来源于灭活的非活病原体的抗原,所述病原体包括病毒如完整灭活病毒、裂解抗原、亚单位抗原、重组抗原或肽或细菌或寄生物。

[0066] 术语“Immunose™ FLU”是指包含非活流感抗原和 Endocine™的组合物。

[0067] 术语“Endocine™”是指包含等摩尔量的单油酸甘油酯和油酸的佐剂。

[0068] 本发明组合物中的一种或更多种非活流感病毒抗原可来自于一种或更多种流感毒株——甲型、乙型和 / 或丙型毒株。能够刺激免疫应答并提供针对大流行性流感毒株的保护性免疫的疫苗组合物通常仅包含来自一种甲型流感毒株的抗原 (单价),而能够引发免疫应答并提供针对季节性流感毒株的保护性免疫的疫苗组合物通常包含来自三种或更多种不同毒株的抗原 (三价或四价)。最常见的为两种不同的甲型流感毒株和一种或更多种乙型流感毒株。

[0069] 本发明涉及这样的疫苗组合物,出乎意料地发现,其对对流感病毒毒株为首次免疫的对象例如儿童 (小于 8 岁) 和近大流行或大流行期期间的人是高度有效的。儿童对季节性传播的流感毒株通常是首次免疫的,而所有人在大流行期间都被认为是首次免疫的。

[0070] 本发明还涉及在流行期或大流行期之前或期间进行免疫的方法,其包括鼻内施用

包含本发明组合物的疫苗组合物；以及对儿科对象进行免疫的方法，其包括鼻内施用包含本发明组合物的疫苗组合物；并且其还涉及对首次免疫对象进行免疫的方法，其包括鼻内施用包含本发明组合物的疫苗组合物。

[0071] 本发明涉及婴儿、儿童和青少年群体，因为这些群体在首次免疫时，在应用常见疫苗策略时的应答较小。婴儿和儿童的免疫系统还没有充分发育，因此，他们对常规的肠胃外疫苗策略产生免疫应答的效率较低。但是，本发明为婴儿和儿童提供了特别的机会，原因是上呼吸道中独特的淋巴组织在出生时就存在并且早在儿童时期就发育良好。被称为腺样体（或鼻咽扁桃体）的咽部淋巴组织位于儿童的咽部并且是沃尔德艾尔环 (Waldeyer's ring) 的一部分，所述沃尔德艾尔环包含鼻咽扁桃体（腺样体）、腭扁桃体对、咽鼓管扁桃体对和舌扁桃体。腺样体积极参与构建免疫系统并且在青春期开始消失。因此，经鼻疫苗递送对婴儿和儿童可具有特殊的优势。儿科亚群可由美国食品和药物管理局或者由欧洲药品管理局或者由二者的组合来限定。

[0072] 在一个实施方案中，所述组合物用作用于儿科应用的鼻内施用疫苗。在一个实施方案中，组合物用作新生儿（（足月产和早产）至 28 日龄）的鼻内施用疫苗。在一个实施方案中，所述组合物用作婴儿 (1 月龄至 23 月龄）的鼻内施用疫苗。在一个实施方案中，所述组合物用作儿童 (2 岁至 11 岁）的鼻内施用疫苗。在一个实施方案中，所述组合物用作青少年 (12 岁至 18 岁）的鼻内施用疫苗。

[0073] 需要诱导针对例如流感疾病的保护性免疫的安全疫苗，其适用于对例如流感具有有限的或没有已有免疫力的幼小儿童并且一般来说适用于首次免疫对象。

[0074] 减毒活病毒疫苗与安全问题相关。**Flumist®** 因为这些安全问题而没有被批准用于 2 岁以下的幼小儿童。自相矛盾的是，这些最常为特别易受流感影响并且属于流感高风险组的首次免疫对象。虽然**Flumist®**被批准用于较大儿童，但是其是减毒活病毒疫苗。

[0075] 出乎意料地发现，鼻内施用佐剂化非活流感疫苗在对疫苗抗原没有已有免疫力的雪貂中诱导了非常高的免疫应答，并随后诱导了针对流感疾病的完全保护。完整非活抗原疫苗和裂解非活抗原疫苗均给出了优于注射型可商购流感疫苗**Flumist®**的结果。

[0076] 本发明组合物不利用减毒活病毒，而是利用非活流感病毒抗原。而且，其可鼻内施用。鼻内施用特别适合于婴儿和儿童的儿科施用，原因是存在被称为腺样体的咽部淋巴组织。鼻内施用本发明组合物允许其普遍使用并且在没有专业培训的情况下施用，例如在近大流行期和大流行期期间在整个群体中自我施用。使用非活流感病毒抗原允许将其用于幼小儿童，而没有与减毒活病毒疫苗相关的安全问题。本发明人开发了在首次免疫对象中有效的疫苗，其可鼻内施用，从而具有上述优势并满足易感群体和患者类型的重要需求。

[0077] 在第一方面中，本发明涉及组合物，所述组合物包含：

[0078] i) 一种或更多种非活抗原，以及

[0079] ii) 包含以下的佐剂：

[0080] 一种或更多种羧酸、

[0081] 水性介质、和

[0082] 任选的一种或更多种单酰甘油，

[0083] 所述组合物用作用于首次免疫对象的鼻内施用疫苗。

[0084] 在另一个方面中，本发明涉及组合物，其包含：

[0085] i）一种或更多种非活病毒抗原，以及

[0086] ii）包含以下的佐剂：

[0087] 一种或更多种羧酸、

[0088] 水性介质、和

[0089] 任选的一种或更多种单酰甘油，

[0090] 所述组合物用作用于首次免疫对象的鼻内施用疫苗。

[0091] 本发明组合物适合于用作针对感染性病原体（例如病毒和细菌）的疫苗。本发明组合物适合于用于鼻内施用的流感疫苗。本发明组合物涉及用作用于在首次免疫对象中针对流感进行鼻内免疫的疫苗。

[0092] 流感病毒由三种类型（甲型、乙型和丙型）组成。甲型流感病毒感染多种鸟类和哺乳动物，包括人、马、猪、雪貂和鸡。乙型流感存在于人、雪貂和海豹中，并且丙型流感存在于人、狗和猪中。感染甲型流感的动物通常通过产生传播至人群的遗传多样性和抗原多样性病毒的合并物充当流感病毒的储库。传播可通过人与被感染动物之间的紧密接触发生，例如，通过处理家畜发生。人至人的传播可通过紧密接触，或者通过由咳嗽或喷嚏产生的微滴吸入发生。

[0093] 甲型流感病毒颗粒的外表面由包含糖蛋白血凝素 (HA) 和神经氨酸酶 (NA) 的脂质包膜组成。HA 糖蛋白包含两个亚单位，称为 HA1 和 HA2。HA 包含与上呼吸道和下呼吸道的上皮细胞外膜上发现的唾液酸结合的唾液酸结合位点，并且通过受体介导的内吞被吸收到细胞中。一旦在细胞中，流感病毒颗粒就释放其基因组，其进入细胞核中并引发新流感病毒颗粒的产生。还产生了 NA，其切割来自细胞表面的唾液酸以防止被释放的流感病毒颗粒被重新捕获。病毒孵育较短时间，在典型情况下为大致 5 天，但是孵育期可有很大不同。病毒在发病前大约一天时分泌并且通常持续多至三至五天。典型的症状包括发热、疲劳、不适、头痛、疼痛、咳嗽和咽喉痛。一些症状可在感染后持续数周。

[0094] 流感病毒的不同毒株的特征主要在于 HA 和 NA 糖蛋白的突变，因此 HA 和 NA 用于鉴定病毒亚型（即，H5N1 指示 HA 亚型 5 和 NA 亚型 1）。因此，流感疫苗通常靶向 HA 和 NA 分子。常规流感病毒疫苗通常采用具有适当 HA 和 / 或 NA 分子的完整灭活病毒。或者，HA 和 NA 蛋白或其亚单位的重组形式也可用作疫苗。疫苗组合物中的抗原可以是灭活抗原，例如完整灭活病毒、裂解抗原、亚单位抗原、重组抗原或肽。术语“抗原”或“免疫原”定义为可充当免疫应答靶标的任何物质。该术语还包括蛋白质抗原、重组蛋白组分、病毒样颗粒 (VLP) 以及基因工程 RNA 或 DNA，其在注射到身体的细胞中时，宿主细胞的“内部机制”“读取”DNA 并使用所述 DNA 合成病原体的蛋白质。因为这些蛋白质被识别为外来的，所以当其被宿主细胞加工并展示在其表面上时，使免疫系统警觉，然后引发一系列免疫应答。该术语还包括模拟灭活细菌或病毒或其一部分的物质。免疫应答可以是细胞的和 / 或体液的，并且可在全身和 / 或黏膜区室中检测到。

[0095] 然而，流感是 RNA 病毒并因此已发生频繁的突变，导致了病毒抗原性组成的恒定和永久变化。抗原性组成是指多肽中被免疫系统识别的部分，如抗体结合表位。抗原组成的小的微小变化也称为抗原漂移。甲型流感病毒还能够在被称为重配的过程中从其他亚型中“交换”遗传物质，导致抗原性组成的较大变化，称为“抗原转变”。因为针对病毒颗粒的

免疫应答依赖于抗体与 HA 和 NA 糖蛋白的结合，所以糖蛋白的频繁变化随时间降低了针对流感病毒所获得的免疫应答的有效性，最终导致缺乏免疫力。甲型流感发生迅速抗原漂移和抗原转变的能力因缺乏对新毒株的已有免疫力而可常常引发流感大流行。

[0096] 美国免疫实践咨询委员会 (ACIP) 推荐每年对所有 6 至 59 月龄的儿童进行流感疫苗接种，原因是 6 至 23 月龄儿童的流感相关住院治疗的风险是显著提高的，并且 24 至 59 月龄儿童的流感相关门诊和急诊就诊的风险提高。对于季节性流感疫苗接种，该推荐延长至≥ 6 月龄的所有人。因此，本发明组合物用作用于鼻内施用至 18 岁及以下（特别是 12 岁及以下）儿童的疫苗。通常来说，儿童小于 8 岁，例如为 6 岁或更小。对于本发明疫苗，一类重要的目的患者为儿童，特别是 2 月龄至小于 9 岁的儿童，典型为 3 月龄至小于 9 岁的儿童，例如 6 月龄至小于 8 岁的儿童，最典型地为 6 月龄至小于 7 岁的儿童，例如 6 月龄至小于 72 月龄、6 月龄至 60 月龄或 6 月龄至 24 月龄的儿童。本发明组合物至少部分地旨在用作用于儿科应用的疫苗。

[0097] 流感病毒毒株给予其造成大流行爆发的可能性的特征为 :相比于最近传播的毒株中的血凝素，其包含新血凝素，可能伴随有或可能不伴随有神经氨酸酶亚型的改变 ;其能够在人群中水平地传播 ;并且其对于人是病原性的。新血凝素可以是在较长时间（可能为数十年）中在人群中不明显存在的血凝素，例如 H2。或者，其可以是在鸟中发现的例如 H5、H9、H7 或 H6 之前未在人群中传播的血凝素。在任一情况下，大多数的、或至少大比例的或甚至整个群体之前没有遭遇过该抗原并且对于其来说是首次免疫的。

[0098] 本发明涉及婴儿、儿童和青少年群体，因为这些群体在首次免疫时，在应用常见疫苗策略时的应答较小。婴儿和儿童的免疫系统还没有充分发育，因此，他们对常规的肠胃外疫苗策略产生免疫应答的效率较低。但是，本发明为婴儿和儿童提供了特别的机会，原因是上呼吸道中独特的淋巴组织在出生时就存在并且早在儿童时期就发育良好。被称为腺样体（或鼻咽扁桃体）的咽部淋巴组织位于儿童的咽部并且是沃尔德艾尔环的一部分，所述沃尔德艾尔环包含鼻咽扁桃体（腺样体）、腭扁桃体对、咽鼓管扁桃体对和舌扁桃体。腺样体积极参与构建免疫系统并且在青春期开始消失。因此，鼻疫苗递送对婴儿和儿童可具有特殊的优势。儿科亚群可由美国食品和药物管理局或者由欧洲药品管理局或者由二者的组合来限定。

[0099] 美国食品和药物管理局根据以下年龄范围对儿科亚群进行了分类。新生儿群体为从出生至 1 月龄。婴儿群体为 1 月龄至 2 岁。儿童群体为 2 岁至 12 岁。青少年群体为 12 岁至 21 岁。欧洲药品管理局根据以下群体对儿科药品进行了分类。新生儿群体包括早产至足月产直至 28 天。婴儿群体为 1 月龄至 23 月龄。儿童群体为 2 岁至 11 岁。青少年为 12 岁至 18 岁。

[0100] 在一个实施方案中，所述组合物用作用于儿科应用的鼻内施用疫苗。在一个实施方案中，所述组合物用作新生儿（（足月产和早产）至 28 日龄）或者作为替代地，用于从出生至 1 月龄的新生儿的鼻内施用疫苗。在一个实施方案中，所述组合物用作用于 1 月龄至 23 月龄或者作为替代地 1 月龄至 2 岁婴儿的鼻内施用疫苗。在一个实施方案中，所述组合物用作 2 岁至 11 岁或者作为替代地 2 岁至 12 岁儿童的鼻内施用疫苗。在一个实施方案中，组合物用作 12 岁至 18 岁或者作为替代地 12 岁至 21 岁的青少年的鼻内施用疫苗。

[0101] 本发明疫苗特别地针对首次免疫对象，例如季节性流感大流行期间小于 8 岁的儿

童。本发明组合物还旨在作为在大流行期或近大流行期期间用于所有年龄组的疫苗。

[0102] 因此，组合物特别地针对首次免疫对象。首次免疫对象可以是 18 岁以下的儿童，例如 0 至 18 岁的儿童，特别是 12 岁及以下的儿童。通常来说，儿童小于 8 岁，例如为 6 岁或更小。对于本发明疫苗，一类重要的目的患者特别地为 2 月龄至小于 9 岁的儿童，通常为 3 月龄至小于 9 岁的儿童，例如 6 月龄至小于 8 岁的儿童，最通常为 6 月龄至小于 7 岁的儿童，例如 6 月龄至小于 72 月龄、6 月龄至 60 月龄或 6 月龄至 24 月龄的儿童。本发明组合物至少部分地旨在用作用于儿科应用的疫苗。

[0103] 当组合物特别地涉及在大流行期或近大流行期期间应用的疫苗时，首次免疫对象可以是任何年龄组。

[0104] 鼻内施用旨在意指通过任意的施用模式施用至鼻，例如通过将疫苗喷洒入鼻腔中或者通过将疫苗经由移液管或类似装置通过将疫苗滴入鼻腔中或鼻黏膜壁上来施用。

[0105] 组合物有利地包含一种或更多种非活流感病毒抗原，而不是减毒活病毒。如上所述，这不仅避免了患者类别选择的安全问题，而且避免了生产、分销、鼻施用、操作和处置方面的安全问题。非活流感病毒抗原可选自完整灭活病毒、裂解病毒、亚单位流感抗原和重组抗原。重组蛋白的使用可用于提高针对病毒攻击所产生的中和抗体的滴度。HA 糖基化在免疫系统引发抗体应答的免疫应答的能力和病毒逃避免疫系统的能力方面发挥着重要作用。因此，可产生其包含非均相复合物型多糖的重组 HA 蛋白，以及免疫原性提高的单糖基化或非糖基化重组蛋白。

[0106] 优选地，非活流感病毒抗原是裂解抗原或亚单位流感抗原，更优选裂解抗原。

[0107] 甲型流感基因组在 8 段 RNA 上包含 11 个基因，其编码 11 种蛋白质：血凝素 (HA)、神经氨酸酶 (NA)、核蛋白 (NP)、M1、M2、NS1、NS2(NEP：核输出蛋白 (nuclear export protein))、PA、PB1(聚合酶碱性蛋白 1)、PB1-F2 和 PB。非活流感病毒抗原可选自来自病毒任何一种蛋白质或蛋白质组合。

[0108] 本发明的组合物可包含任意的灭活流感病毒。如本领域技术人员所理解的，流感病毒一年四季不同并且随其感染的地理区域和群体也不同。本发明涉及包含本发明佐剂和来自一种或更多种流感病毒的非活流感病毒抗原的疫苗。本发明疫苗组合物中使用的非活流感抗原是来自灭活流感病毒的任意抗原性物质。例如，其可以包含灭活的完整病毒颗粒。或者，其可包含经破坏的病毒(裂解病毒)，其中例如保留了免疫原性蛋白质如 M2 离子通道蛋白或糖蛋白。流感膜糖蛋白、血凝素 (HA) 和 / 或神经氨酸酶 (NA) 的纯化制备物可用作疫苗组合物的抗原性物质。根据本发明的疫苗组合物可包含一种或更多种类型的抗原性物质。当然，用于制备疫苗组合物的流感型病毒将取决于待保护疫苗接受者所针对的流感。

[0109] 例如，非活流感病毒抗原包含例如以下流感病毒中一种或更多种的遗传骨架的一种或更多种抗原：A/Ann Arbor/6/60(A/AA/6/60)、B/Ann Arbor/1/66 病毒、FluMist MDV-A(ca A/Ann Arbor/6/60)、FluMist MDV-B(ca B/Ann Arbor/1/66)、A/Leningrad/17 供体毒株骨架和 PR8。

[0110] 在另一个具体实例中，本发明的疫苗组合物包含例如来自以下中一种或更多种的 HA 或 NA 多肽序列(或者与该序列具有至少 90％同一性或至少 95％同一性的序列)的非活流感病毒抗原：B/Yamanashi、A/New Caledonia、A/Sydney、A/Panama、B/Johannesburg、B/Victoria、B/Hong Kong、A/Shandong/9/93、A/Johannesburg/33/94、A/

Wuhan/395/95、A/Sydney/05/97、A/Panama/2007/99、A/Wyoming/03/2003、A/Texas/36/91、A/Shenzhen/227/95、A/Beijing/262/95、A/New Caledonia/20/99、B/Ann Arbor/1/94、B/Yamanashi/166/98、B_Johannesburg.sub.--5.sub.--99、BVictoria/504/2000、B/Hong Kong/330/01、B_Brisbane.sub.--32.sub.--2002、B/Jilin/20/03、H1N1 甲型流感病毒、H3N2 甲型流感病毒、H9N2 甲型流感病毒、H5N1 甲型流感病毒、H7N9 甲型流感病毒、乙型流感病毒和大流行性流感毒株（由 WHO 指定或者在人群中不传播）。

[0111] 在一个实施方案中，流感病毒毒株可以是包含在以下的 2013/2014 疫苗中的毒株中一种或更多种毒株：例如 A/California/7/2009(H1N1) 样病毒、抗原性如同经细胞繁殖的原型病毒 A/Victoria/361/2011 或 A/Texas/50/2012 的 (H3N2) 病毒、以及 B/Massachusetts/2/2012 样 (Yamagata 世系）病毒。

[0112] 在一个实施方案中，流感病毒毒株可以是先前 WHO 推荐用于流感疫苗的毒株中的一种或更多种。

[0113] 在一个实施方案中，流感病毒毒株可以是来自四价流感疫苗的毒株并且包含来自以下五种流感病毒毒株中任意四种的抗原：A/California/7/2009(H1N1) 样病毒、抗原性如同经细胞繁殖的原型病毒 A/Victoria/361/2011 或 A/Texas/50/2012 的 (H3N2)、和 B/Massachusetts/2/2012 样 (Yamagata 世系）病毒、以及 B/Brisbane/60/2008-like(Victoria 世系）病毒。

[0114] 本发明组合物的佐剂对于其对于鼻内施用的适合性和其效力是至关重要的。用于鼻内施用的合适佐剂可以是包含与脂肪酸组合的任选的单酰甘油的佐剂，或者其可以是脂肪酸的组合。所述佐剂中使用的羧酸包含 (C4-C30) 烷基、烯基或炔基羧酸，其可任选地为有支链或无支链，环或无环的，任选地具有单、双或多不饱和（双键或三键），其还可任选地为不同类型。

[0115] 所述佐剂中使用的单酰甘油可以是甘油的羧酸酯，其中所述羧酸可以是 (C4-C30) 烷基、烯基或炔基羧酸，其可任选地为有支链或无支链，任选地具有单、双或多不饱和（双键或三键），其还可任选地为不同类型。

[0116] 疫苗组合物中单酰甘油的浓度可以在例如约 1mg/ml 至约 50mg/ml（例如约 1mg/ml 至约 25mg/ml、约 5mg/ml 至约 15mg/ml 或约 10mg/ml）的范围中。

[0117] 疫苗组合物中脂肪酸的浓度可以在例如约 0.5mg/ml 至约 50mg/ml，（例如约 1mg/ml 至约 25mg/ml、约 1mg/ml 至约 15mg/ml、约 1mg/ml 至约 10mg/ml、约 2mg/ml 至约 8mg/ml 或约 6mg/ml 至约 7mg/ml）的范围中。在一个实施方案中，疫苗组合物中脂肪酸浓度的摩尔基础对应于单酰甘油的浓度（以摩尔为基础）。

[0118] 对于单酰甘油和脂肪酸，以上提及的浓度范围的任意组合在本申请的情况中。而且，所提及的最宽范围给出了优选范围，进而将范围变窄为最优选范围。

[0119] 本发明的发明人发现，当通过经鼻途径（如施用至鼻腔黏膜）来进行疫苗接种时，如上所述且如 WO 2012/042003（其通过引用整体并入本文）中所公开的佐剂是特别有用的。本发明人发现，在疫苗接种中经由经鼻途径使用这类佐剂在疫苗接种后改善了免疫应答。本发明人发现，使用这类佐剂在多种物种（包括人）中是安全且可耐受的。

[0120] 因此，组合物可包含经选自以下的羧酸单酯化的甘油酯：月桂酸 (C12)、豆蔻酸 (C14)、棕榈酸 (C16)、棕榈油酸 (C16:1)、油酸 (C18:1)、亚油酸 (C18:2)、硬脂酸、己酸、

辛酸、癸酸（羊蜡酸）、花生酸、山萮酸、木蜡酸、α-亚麻酸、硬脂四烯酸、二十碳五烯酸、二十二碳六烯酸、γ-亚麻酸、二高-γ-亚麻酸 (dihomo-gamma-linolenic acid)、花生四烯酸、芥酸、神经酸。

[0121] 在另一个实施方案中，单酰甘油是经选自以下的羧酸单酯化的甘油酯：棕榈油酸 (C16:1)、油酸 (C18:1) 和亚油酸 (C18:2)。

[0122] 优选地，单酰甘油是经油酸单酯化的甘油酯（油酸甘油酯）。

[0123] 佐剂优选地包含选自以下的一种或更多种羧酸：月桂酸、豆蔻酸、棕榈酸、棕榈油酸、油酸、亚油酸、硬脂酸、己酸、辛酸、癸酸（羊蜡酸）、花生酸、山萮酸、木蜡酸、α-亚麻酸、硬脂四烯酸、二十碳五烯酸、二十二碳六烯酸、γ-亚麻酸、二高-γ-亚麻酸、花生四烯酸、芥酸和神经酸。优选地，一种或更多种羧酸选自油酸和月桂酸。

[0124] 在一些合适实施方案的组合中，佐剂包含油酸甘油酯、油酸和水性介质。本发明的疫苗组合物还可包含另外的药物赋形剂。这样的药物赋形剂可以是：

[0125] 1. 控制疫苗的渗透压 (tonicity)/渗量 (osmolarity) 的物质。这类物质为例如生理盐如氯化钠。另一些生理盐为氯化钾、磷酸二氢钾、磷酸二钠、氯化镁等。这类物质还可以是影响离子强度和稳定性的其他离子物质。疫苗的渗量可调节至约 200 至约 400mOsm/kg，优选约 240 至约 360mOsm/kg 的值，或者渗量必须接近生理水平，例如在约 290 至约 310mOsm/kg 的生理范围中。

[0126] 2. 调节 pH 或缓冲疫苗组合物的物质。通常来说，疫苗组合物的 pH 在约 5 至约 8.5 的范围中。合适的 pH 调节剂或缓冲物质包括盐酸、氢氧化钠（以调节 pH）以及磷酸盐缓冲液、Tris 缓冲液、柠檬酸盐缓冲液、乙酸盐缓冲液、组氨酸缓冲液等（以缓冲疫苗）。

[0127] 3. 另一些附加剂，例如表面活性剂、抗氧化剂、螯合剂、抗菌剂、病毒灭活剂、防腐剂、染料、消泡剂、稳定剂或表面活性剂、或其组合。

[0128] 表面活性剂可以是亲水性的、惰性和生物相容性的，例如泊洛沙姆，例如普朗尼克 F68 或普朗尼克 127。

[0129] 抗菌剂可以是例如两性霉素或其任意衍生物、氯四环素、甲醛或福尔马林、庆大霉素、新霉素、多黏菌素 B 或其任意衍生物、链霉素或其任意组合。

[0130] 抗氧化剂可以是例如抗坏血酸或生育酚或其任意组合。

[0131] 病毒灭活剂可以是例如福尔马林、β-丙内脂、UV-辐射、加热或其任意组合。

[0132] 防腐剂可以是例如苯索氯铵、EDTA、酚、2-苯氧乙醇或硫柳汞或其任意组合。EDTA 也已被证明是螯合剂、抗氧化剂和稳定剂。

[0133] 染料可以是例如任何指示剂（例如酚红）或亮绿或其任意组合。

[0134] 消泡剂可以是例如聚二甲硅氧烷 (polydimethylsilozone)。

[0135] 表面活性剂可以是例如阴离子、阳离子或非离子或两性离子，例如聚氧化乙烯及其衍生物、聚山梨醇酯（例如聚山梨醇酯 20 或聚山梨醇酯 80）、吐温 80、泊洛沙姆（例如普朗尼克 F68）或其任意组合。

[0136] 通常来说，疫苗组合物中单酰甘油的浓度的量为每 100mL 所述疫苗组合物约 0.1g 至约 5.0g，或每 100mL 约 0.1g 至约 2.0g，或每 100mL 约 0.5g 至约 2.0g，例如每 100mL 0.5g 至约 1.5g。

[0137] 此外，一种或更多种羧酸的浓度的量为每 100mL 所述疫苗组合物的约 0.1g 至约

5.0g 或每 100mL 约 0.1g 至约 2.0g 或者约 0.5g 至约 2.0g，例如每 100mL 0.5g 至约 1.5g。

[0138] 疫苗组合物中一种或更多种单酰甘油与一种或更多种羧酸一起的量可以是至多 10% w/v、或至多 5% w/v、或至多 4% w/v、或至多 3% w/v、或至多 2% w/v 或至多 1% w/v 或至多 0.5% w/v 或至多 0.1% w/v 或至多 0.05% w/v。

[0139] 佐剂可包含选自单油精 (mono-olein)、油酸、月桂酸和大豆油的脂质的组合。在一个合适的实施方案中，佐剂包含 Tris 缓冲剂中的油酸、月桂酸。适当地，该实施方案包含溶于 7 至 15mL 的 Tris 缓冲剂 (pH 7 至 9) 中的 0.25g 至 0.75g 的油酸、0.25g 至 0.75g 的月桂酸。特定实例包含溶于 8 至 10mL 的 0.1M Tris 缓冲剂 (pH 7 至 9) 中的 0.4g 至 0.5g 的油酸、0.3g 至 0.4g 的月桂酸。在另一个合适的实施方案中，佐剂包含溶于 Tris 缓冲剂中的油酸和单油精。适当地，该实施方案包含溶于 7 至 15mL 的 Tris 缓冲剂中的 0.25g 至 0.75g 的油酸、0.25g 至 0.75g 的单油精。特定实例包含溶于 8 至 10mL 的 0.1M Tris 缓冲剂 (pH 7 至 9) 中的 0.3g 至 0.4g 的油酸、0.4g 至 0.5g 的单油精。另一个实施方案包含溶于 7mL 至 15mL 的 Tris 缓冲剂中的 0.5g 至 0.25g 的单油精、0.5g 至 0.25g 的油酸和 0.25g 至 0.75g 的大豆油。该实施方案的特定实例包含溶于 8 至 12mL 的 Tris 缓冲剂 (pH 7 至 9) 的 0.1g 至 0.2g 的单油精、0.8g 至 1.5g 的油酸和 0.5g 至 0.6g 的大豆油。

[0140] 三种类型的佐剂被成功地用于以下实例中：实例佐剂 A 包含 8 至 10mL 的 0.1M Tris 缓冲剂 (pH 7 至 9) 中的 0.4g 至 0.5g 的油酸、0.3g 至 0.4g 的月桂酸；实例佐剂 B 包含 8 至 10mL 的 0.1M Tris 缓冲剂 (pH 7 至 9) 中的 0.3g 至 0.4g 的油酸、0.4g 至 0.5g 的单油精；以及实例佐剂 C 包含 8 至 12mL 的 Tris 缓冲剂 (pH 7 至 9) 中的 0.1g 至 0.2g 的单油精、0.8g 至 1.5g 的油酸和 0.5g 至 0.6g 的大豆油。这些佐剂通常以 2%至 12%脂质含量 (每 100mL 6g 至 12g)，最通常 3%至 10%，例如 4%、5%、6%、7%、8%或 9%的 w/v 的浓度来制备。这些浓度是佐剂混合物自身的那些。然后该佐剂以 2 ： 1 至 1 ： 8 比例 (例如 1 ： 1 比例) 与包含抗原的组合物混合以从具有 8%脂质浓度的佐剂开始时提供 4%脂质含量的疫苗组合物。通常来说，本发明的疫苗组合物中脂质含量为 0.5% w/v 至 6% w/v，通常为 1% w/v 至 6% w/v，更通常为 1%至 4%。

[0141] 实例 B 组合物是包含等摩尔量的单油酸甘油酯和油酸的 Endocine™ 制剂并且已经发现在首次免疫对象中非常有效。在一个高度优选的实施方案中，该 8%脂质制剂用包含抗原的组合物来稀释以提供脂质浓度为 1% w/v 至 4% w/v 的疫苗组合物。

[0142] 如上所述，所述组合物适用于在包括鼻内施用本发明的疫苗组合物的近大流行或大流行期期间进行免疫的方法。近大流行或大流行期期间用于免疫的方法可用于所有年龄的对象。本发明还涉及在包括鼻内施用上述疫苗组合物的儿科对象的季节性流行期间进行免疫的方法。

[0143] 如上所述，本发明涉及包括鼻内施用疫苗组合物的首次免疫对象的免疫方法。

[0144] 以下实例示出了该疫苗组合物在首次免疫对象中的效力。

[0145] 在首次免疫个体中引起免疫应答的出人意料的疗效意味着本发明的疫苗能够在具有衰弱的免疫系统的个体中引起免疫应答，在能够响应于侵袭性病原体 (例如病毒) 的方面，其已经没有强烈的已有免疫。因此，本发明的组合物适用于免疫低下的个体。因此，本发明的另一方面涉及包含鼻内施用给儿科免疫低下患者的佐剂化非活流感抗原的疫苗组合物，所述儿科免疫低下患者包括具有 HIV 的那些、正服用免疫抑制药物的对象、近期器

官接受者、早产婴儿和手术后的患者。

[0146] 该方面涉及组合物，所述组合物包含

[0147] i) 一种或更多种非活抗原，和

[0148] ii) 包含以下的佐剂：

[0149] 一种或更多种羧酸、

[0150] 水性介质，以及

[0151] 任选的一种或更多种单酰甘油，

[0152] 所述组合物用作儿科免疫低下患者的鼻内施用疫苗。

[0153] 免疫低下的个体对机会病原体例如流感病毒具有增强的敏感性并且提高了流感住院治疗和死亡的风险。免疫低下的个体并且特别是儿科免疫低下的个体可以是用本发明的组合物进行免疫的适当患者类型。因此，本发明的一个实施方案可以是包含以下的组合物

[0154] i) 一种或更多种非活流感病毒抗原，和

[0155] ii) 包含以下的佐剂：

[0156] 一种或更多种羧酸、

[0157] 水性介质，以及

[0158] 任选的一种或更多种单酰甘油，

[0159] 所述组合物用作儿科免疫低下患者的鼻内施用疫苗。

[0160] 由实例 2 举例说明的本发明出人意料的效果在于本发明的组合物能够降低病毒脱落 (virus shedding)。儿童比具有免疫能力的健康成年人脱落更多的病毒，这导致提高病毒传播至与其邻近的其他人。因此，本发明可适用于治疗儿科群体，如婴儿、儿童和青少年。本发明可适用于预防由儿科群体传播的病毒。在一个实施方案中，本发明的组合物用于儿科对象，例如婴儿、儿童和青少年。在一个实施方案中，本发明的组合物用于首次免疫对象以降低病毒脱落。在一个实施方案中，本发明的组合物用于流感首次免疫对象以降低病毒脱落。此外，本发明的组合物可特别地适用于通过降低病毒传播而限制大流行。在一个实施方案中，本发明的组合物用于首次免疫对象以降低大流行地区的病毒脱落。在一个实施方案中，本发明的组合物用于首次免疫对象以降低近大流行期期间病毒脱落。在一个实施方案中，本发明的组合物用于儿科对象以在近大流行期期间降低病毒脱落。

[0161] 通过鼻内施用上述组合物针对儿科免疫受损患者中流感的免疫方法是出人意料的结果的有趣方面。

[0162] 所述组合物通常用作针对流感向儿科免疫低下的对象鼻内施用的疫苗。儿科免疫低下的对象适当地选自以下人：感染 HIV 的人；正服用免疫抑制药物的对象，如近期器官接受者；早产婴儿和手术后的患者。

[0163] 本发明的另一方面涉及用于首次免疫对象和儿科免疫低下患者的疫苗。本发明的佐剂已经证明了其在流感首次免疫对象中的效力。这使得其适用于首次免疫患者类型和儿科免疫低下的患者二者。

[0164] 因此，本发明的另一方面涉及用作鼻内施用的疫苗的组合物，所述组合物用于首次免疫对象和儿科免疫低下患者，所述组合物包含

[0165] i) 一种或更多种非活抗原，和

[0166] ii) 包含以下的佐剂：

[0167] 一种或更多种羧酸、

[0168] 水性介质，以及

[0169] 任选的一种或更多种单酰甘油。

[0170] 根据本发明，用于首次免疫对象和儿科免疫低下患者免疫的合适类型的疫苗包括分别打算通过疫苗来免疫或治疗的相关病原体的抗原。这包括但不限于来自选自以下的病毒的免疫原：乙型肝炎、甲型肝炎、丙型肝炎、丁型和戊型肝炎病毒、非甲型 / 非乙型肝炎病毒、痘病毒和天花病毒、脊髓灰质炎病毒、麻疹病毒、人类免疫缺陷病毒 (HIV)、肠道病毒、逆转录病毒、呼吸道合胞病毒、轮状病毒、人乳头瘤病毒、水痘 - 带状疱疹病毒、黄热病病毒、SARS 病毒、动物病毒、疱疹病毒、巨细胞病毒、水痘带状疱疹、EB 病毒 (Epstein Barr virus)、副流感病毒、腺病毒、柯萨奇病毒、细小核糖核酸病毒、鼻病毒、风疹病毒、乳多空病毒 (papovirus) 和腮腺炎病毒。除了上述流感病毒抗原以外，已知病毒抗原的一些非限制性实例可包含以下：来自 HIV-I 的抗原，例如 tat、nef、gp120 或 gp1[β]0、gp40、p24、gag、env、vif、vpr、vpu、rev 或其一部分和 / 或组合；来自人疱疹病毒的抗原，例如 gH、gL gM gB gC gK gE 或 gD 或其一部分和 / 或组合，或者立即早期蛋白，例如来自 HSV1 或 HSV2 的 ICP27、ICP47、ICP4、ICP36；来自巨细胞病毒的抗原，尤其是人类巨细胞病毒，如 gB 或其衍生物；来自 EB 病毒的抗原，如 gp350 或其衍生物；来自水痘带状疱疹病毒的抗原，例如 gp I、11、111 和 IE63；来自肝炎病毒的抗原，例如乙型肝炎、丙型肝炎或戊型肝炎病毒抗原 (例如，HCV 的 env 蛋白 E1 或 E2、核心蛋白、NS2、NS3、NS4a、NS4b、NS5a、NS5b、p7 或其一部分和 / 或组合)；来自人乳头瘤病毒的抗原 (例如，HPV6、11、16、18，例如 L1、L2、E1、E2、E3、E4、E5、E6、E7 或其一部分和 / 或组合)；来自其他病毒病原体的抗原，例如呼吸道合胞病毒 (例如 F 和 G 蛋白或其衍生物)、副流感病毒、麻疹病毒、腮腺炎病毒、黄病毒 (例如，黄热病病毒、登革病毒、蜱传脑炎病毒、日本脑炎病毒) 或其一部分和 / 或组合。

[0171] 本发明的组合物可包含但是不限于以下病毒的非活抗原：来自带状疱疹、HIB、百日咳、脊髓灰质炎、破伤风、白喉、甲型肝炎、季节性流感、甲型流感、乙型流感、呼吸道合胞病毒 (RSV)、人偏肺病毒 (hMPV)、人乳头瘤病毒 (HPV)、轮状病毒、诺如病毒、人类免疫缺陷病毒 (HIV)、单纯疱疹和 / 或副流感病毒 (OIV)、鼻病毒、严重急性呼吸道综合征 (SARS)、冠状病毒、带状疱疹 / 水痘、甲型至戊型肝炎、汉坦病毒和 / 或巨细胞病毒的非活抗原或其混合物。

[0172] 本发明的组合物可包含但是不限于以下细菌的非活抗原：来自肺炎球菌、脑膜炎球菌、流感嗜血杆菌 b(Hib) 炭疽杆菌、砂眼衣原菌、铜绿假单胞菌、结核分枝杆菌、白喉、大肠杆菌、链球菌组、淋病奈瑟菌、百日咳博德特菌的非活抗原或其混合物。

[0173] 所述抗原可以是例如完整非活抗原，例如完整灭活病毒。所述抗原还可以是病原体的一部分，例如灭活病毒的一部分。可用的抗原组分为 (但不限于) 例如病毒、细菌、分枝杆菌或寄生物抗原。细菌病原体可以是例如，引起结核病和麻风病的分枝杆菌、肺炎球菌、需氧革兰氏阴性菌或革兰氏阳性菌、支原菌、葡萄球菌传染、链球菌传染、幽门螺杆菌、沙门菌、百日咳博德特菌和衣原体。疾病还可以是细菌传染，如由以下引起的传染：引起结核病和麻风病的分枝杆菌、肺炎球菌、需氧革兰氏阴性杆菌、支原菌、葡萄球菌感染、链球菌传染、幽门螺杆菌、沙门菌、白喉、引起百日咳的百日咳博德特菌以及衣原体。

[0174] 用于首次免疫对象和免疫低下患者免疫的疫苗的优选类型可选自肺炎球菌疫苗、甲型至戊型肝炎疫苗、脑膜炎球菌疫苗、流感嗜血杆菌 b(Hib) 疫苗、白喉疫苗和 DTaP 疫苗(保护免受白喉、破伤风和百日咳)。

[0175] 疾病还可以是寄生物性的,例如疟疾、利什曼病、锥虫病、弓形虫病、血吸虫病、丝虫病或多种类型的癌症,例如乳腺癌、胃癌、结肠癌、直肠癌、头颈部癌、肾癌、恶性黑色素瘤、喉癌、卵巢癌、子宫颈癌、前列腺癌。

[0176] 疾病还可以是由于室内尘螨、花粉和其他环境变应原的变态反应和自身免疫疾病,例如系统性红斑狼疮。

[0177] 疫苗组合物中的抗原可以是完整非活抗原,例如完整灭活病毒、裂解非活抗原或者亚单位非活抗原。本领域中公知的灭活方法例如热灭活、由 UV 光的辐照灭活或通过福尔马林灭活的灭活或用 β - 丙内脂处理。

[0178] 本发明的组合物用作首次免疫对象和儿科免疫低下患者免疫的疫苗。儿科免疫低下患者适当地选自以下人 :感染 HIV 的对象 ;正服用免疫抑制药物的对象,例如近期器官接受者 ;早产婴儿和手术后的患者。首次免疫对象可以是 18 岁以下的儿童,例如 0 至 18 岁的儿童,特别是 12 岁及以下的儿童。通常来说,所述儿童小于 8 岁,例如 6 岁或更小。用于本发明疫苗患者的重要预期类型特别地为 2 月龄至小于 9 岁的儿童,通常来说,3 月龄至小于 9 岁的儿童,例如 6 月龄至 8 岁的儿童,最通常来说,6 月龄至小于 7 岁的儿童,例如 6 月龄至小于 72 月龄的儿童或者 6 月龄至 60 月龄的儿童或者 6 月龄至 24 月龄的儿童。本发明的组合物旨在至少部分地用作儿科应用的疫苗。

[0179] 首次免疫对象可以是所有年龄组,当组合物特别地涉及大流行或近大流行期期间应用的疫苗。

[0180] 肺炎链球菌是侵入性肺炎球菌疾病 (invasive pneumococcal disease, IPD) 发病率和死亡率的主要原因,全世界每年有 160 万人死于侵入性肺炎球菌疾病 (IPD)(WHO, 2002)。IPD 最通常地发生在非常年轻 (< 24 月龄) 和老年人 (> 65 岁) 中 ;老年人具有最高的 IPD 死亡率。目前,四种疫苗可用于肺炎链球菌感染的预防。没有鼻内疫苗可用于链球性菌肺炎。

[0181] 本发明的一个有趣实施方案涉及鼻内作为替代地用于肺炎链球菌感染的预防,特别针对婴儿、儿童、青少年和其他肺炎链球菌首次免疫对象。本发明的组合物不使用活性减毒细菌,但是使用非活链球菌肺炎抗原。本发明疫苗的出人意料的效力是所使用的佐剂的结果并且出人意料的结果对首次免疫对象具有特异性。相似的结果也可期望用于免疫低下的对象。

[0182] 因此,本发明的另一方面涉及组合物,其用作用于在首次免疫对象和儿科免疫低下患者中使用的非活鼻内施用的疫苗,以预防肺炎链球菌的感染或者降低与链球菌肺炎感染相关症状的严重程度,所述组合物包含

[0183] i) 一种或更多种肺炎链球菌抗原,和

[0184] ii) 包含以下的佐剂 :

[0185] 一种或更多种羧酸、

[0186] 水性介质,以及

[0187] 任选的一种或更多种单酰甘油。

[0188] 免疫低下的患者适当地选自婴儿、儿童和青少年；感染 HIV 的对象；正服用免疫抑制药物的对象，例如近期器官接受者；早产婴儿和手术后的患者。

[0189] 本发明的一个重要实施方案涉及针对肺炎球菌感染的疫苗，用于预防和 / 或减轻选自支气管炎、肺炎、败血病、心包炎、脑膜炎和腹膜炎的疾病状态的症状。

[0190] 一个实施方案涉及使用肺炎球菌疫苗，例如儿科对象中肺炎球菌多糖疫苗 (pneumococcal polysaccharide vaccine，PPV)，特别是用于 4 周龄至 6 岁的对象（例如，对肺炎球菌抗原首次免疫并且其中免疫系统没有完全发育的对象）。

[0191] 根据本发明的疫苗组合物还可包含可药用赋形剂，例如可以是进一步包含表面活性剂的水性介质的介质，其可以是亲水性和惰性和生物相容的介质，例如泊洛沙姆，例如普朗尼克 F68 或普朗尼克 127。

[0192] 根据本发明的肺炎球菌疫苗还可包含本文中所述的抗菌剂、抗氧化剂、病毒灭活剂、防腐剂、染料、稳定剂、消泡剂、（非离子、阴离子或阳离子）表面活性剂或其任意组合。抗菌剂可以是例如两性霉素或其任意衍生物、氯四环素、甲醛或福尔马林、庆大霉素、新霉素、多黏菌素 B 或其任意衍生物、链霉素或其任意组合。抗氧化剂可以是例如抗坏血酸或生育酚或其任意组合。病原体例如病毒和 / 或细菌灭活剂可以是例如福尔马林、β - 丙内脂、UV- 辐照、加热或其任意组合。

[0193] 当描述本发明的一些实施方案时，所有可能实施方案的组合和排列还没有明确地描述。然而，在互不相同的从属权利要求中记载或在不同实施方案中描述的某些测量的这个事实并不表示这些措施的组合不可被有利地使用。本发明考虑所述实施方案的所有可能的组合和排列。

实施例

[0194] 实施例 1

[0195] 目标

[0196] 根据本发明，本研究的目标是探究雪貂模型中鼻内施用的佐剂配制的流感裂解抗原和佐剂配制的完整死流感病毒抗原的免疫原性和保护效力。

[0197] 用 5μg HA、15μg HA 或 30μg HA 的抗原剂量研究基于 H1N1/California/2009 裂解抗原的疫苗（疫苗 A）并且用 15μg HA 的抗原剂量研究基于 H1N1/California/2009 完整死病毒抗原（疫苗 B）的疫苗。使用野生型 H1N1 A/Netherlands/602/2009 病毒作为攻击物研究疫苗效力。

[0198] 包含等摩尔量的单油酸甘油酯和油酸的 Endocine™佐剂在疫苗组合物中的最终浓度为 20mg/ml(2％）。在该实施例中，Immunose™ FLU 是指与 Endocine™混合的非活流感抗原。

[0199] 实验组免疫阶段

[0200] 表 1

[0201]

组编号	动物数目	受试物质	抗原剂量（μg HA）	免疫途径
1	6	盐水	0	经鼻
2	6	Fluarix®	15/株	皮下
3	6	疫苗 A	5	经鼻
4	6	疫苗 A	15	经鼻
5	6	疫苗 A	30	经鼻
6	6	疫苗 B	15	经鼻

[0202] 疫苗制备和施用

[0203] 盐水：0.9％盐水 pH 5-5.5。

[0204] **Fluarix®**：肠胃外疫苗（由 A/California/7/2009(H1N1) 样疫苗株、A/Perth/16/2009(H3N2) 样疫苗株和 B/Brisbane/60/2008 样疫苗珠在 0.5ml 中每个疫苗株的 15μg HA 组成）。用 0.5ml Fluarix(GlaxoSmithKline Biologicals) 在第 21 天和第 42 天对第 2 组的动物进行皮下接种疫苗。

[0205] 疫苗 A：流感疫苗滴鼻剂，5、15 和 30μg HA/0.2ml，佐剂制剂，其包含等摩尔量的单油酸甘油酯和油酸的 Endocine 制剂 (pH 8，在 Tris 0.1M 中），在疫苗组合物中的最终浓度为 20mg/ml；H1N1/California/2009 裂解抗原。

[0206] 疫苗 B：流感疫苗滴鼻剂，15μg HA/0.2ml，佐剂制剂，其包含等摩尔量的单油酸甘油酯和油酸的 Endocine 制剂 (pH 8，Tris 0.1M 中），在疫苗组合物中的最终浓度为 20mg/ml，H1N1/California/2009 完整死病毒抗原。

[0207] 雪貂

[0208] 使用健康雌性雪貂 (Mustela putorius furo：远交群），约 12 月龄，体重为 760g 至 1210g 并且通过血凝抑制 (hemagglutination inhibition，HI) 测定证明对针对循环乙型流感病毒、A/H1N1、A/H3N2 和 A/pH1N1 的抗体呈血清反应阴性。动物饲养在标准笼子中，在免疫前阶段期间最多 8 只动物为组并且在免疫阶段期间以 6 只动物为研究组。将研究组在攻击当天转移至负压的手套箱隔离器笼。在整个研究期间，为动物自由地提供商业化颗粒饲料 (food pellet) 和水。

[0209] 免疫

[0210] 每组六只的五组雪貂在第 0、21 和 42 天用氯胺酮和美托咪定的麻醉下接受三次鼻内免疫（液滴：每个鼻孔中 100μl，使用具有过滤嘴的移液管）。第 1 组的动物接受 200μl 的无菌生理盐水 (0.9％盐水，pH 5 至 5.5)。第 3、4 和 5 组分别用包含 5、15 和 30μg HA 的 200μl Endocine™配制的 H1N1/California/2009 裂解抗原来进行鼻内免疫。第 6 组用包含 15μg HA 的 200μl Endocine™配制的 H1N1/California/2009 完整病毒抗原来进行鼻内免疫。对照组 1 鼻内接受 200μl 的盐水。一组的六只雪貂（第 2 组）在第 21 和 42 天用 0.5ml **Fluarix®** (GlaxoSmithKline Biologicals) 在季节 2010/2011 进行皮下疫苗接

种，非佐剂化三价流感疫苗（trivalent influenza vaccine，TIV）每个疫苗株包含 15μg HA。在第 0、21 和 42 天免疫之前并且在研究第 64 天和第 70 天攻击之前收集用于血清制备的血样品。

[0211] 攻击病毒的制备和施用

[0212] 在研究第 70 天，所有动物通过气管内途径用流感病毒的野外隔离物（H1N1 株 A/Netherlands/602/2009）进行攻击。为了制备攻击病毒，将 H1N1 A/Netherlands/602/2009 攻击储备液（7.8log10TCID50/ml）用冰冷 PBS 稀释成 3.3×10^5 TCID50/ml 的浓度。所有动物用 3ml 包含 10^6 TCID50 的攻击病毒制剂进行气管内攻击，使用气管镜用小导管施用至气管中并且正好在分叉处上方释放。在 BSL3 条件下，进行攻击病毒的制备和施用。在攻击之后一天，剩余攻击病毒稀释物的样品在 Madin-Darby 犬肾（MDCK）细胞上滴定以证实病毒的感染性。一天在接种之后攻击稀释剂的反滴定显示出所述材料仍然包含 4.8log10 TCID50。

[0213] 程序和样品收集

[0214] 实验过程中，雪貂上进行若干个程序。对于温度传感器的植入、免疫、病毒攻击和计算机断层扫描（CT）成像，将动物用氯胺酮（4-8mg/kg：i.m.；Alfasan，Woerden，Netherlands）和美托咪定（0.1mg/kg：i.m.；Orion Pharma，Espoo，Finland）的混合物（cocktail）来麻醉。通过放血来取样（血液、拭子和鼻洗液）和安乐死，用氯胺酮麻醉动物。在实验开始之前的两周，将温度记录器（DST 微-T 超小温度记录器；Star-Oddi，Reykjavik，Iceland）放在雪貂的腹膜腔中。该装置每隔 10 分钟记录动物的身体温度。在每次免疫之前（第 0、21 和 42 天）和攻击和安乐死当天（第 70 天和第 74 天），对雪貂进行称重。第 1、2 和 4 组的动物在第 64、71、72、73 和 74 天通过 CT 成像来监测。在第 0、21 和 42 天免疫之前，在第 64 天并且在第 70 天攻击之前收集血样品。在第 70 天攻击之前和攻击之后的每天收集鼻和咽喉拭子。

[0215] 血样品和血清的收集

[0216] 收集血样品并将其分成 2 个相同体积。将用于分离 PBMC 的一个体积直接转移至包含 EDTA 抗凝剂的管中。将用于收集血清的另一体积转移至包含凝结活化剂的血清管。在室温下所有血清管在约 2000×g 下离心 10 分钟。血清被等分成 0.1ml 的样品并在约 -80℃下储存。

[0217] PBMC 和血浆的分离

[0218] 将用于分离 PBMC 的血样品直接转移至包含 EDTA 抗凝剂的管中，在 880×G 下离心 5 分钟，在约 -80℃下储存血浆。将细胞沉淀重悬于 3.5ml 清洗缓冲液（D-PBS：lot#：RNBB7791，V-CMS：10700395 和 EDTA：lot#：079K8712，V-CMS：10700037）中，在 3ml 淋巴细胞分离剂（lymphoprep）中分层并在 800×G 下离心 30 分钟。在离心之后，收集含有界面的细胞，将其转移至新管并且用清洗缓冲液清洗 4 次。在随后的清洗步骤中包括在 600×g、465×g 和 350×g 下离心 10 分钟并且在 250×g 下离心 15 分钟。在最后清洗步骤之后，将细胞沉淀重悬，放在冰上至少 10 分钟，重悬于 1ml 冰冷的冻结介质（RPMI lot#1MB078，20％ FCS VC#201110194，10％ DMSO VC#10700203）中，转移至安瓿，并且在 -80℃下储存。

[0219] 血清学

[0220] 针对 H1N1 A/Netherlands/602/2009 与 2 个不同病毒 H1N1 A/ 猪 /Ned/25/80 和 H1N1 A/ 猪 /Italy/14432/76 的抗体滴度通过血细胞凝集抑制测定（HI）和病毒中和测定

(VN)进行确定。针对不同病毒H1N1 A/New Jersey/08/76的抗体滴度通过血细胞凝集抑制测定进行确定。

[0221] HI测定

[0222] HI测定是标准结合测定,基于用于阻碍红血细胞凝集所引起的流感的流感病毒血凝素特异性抗体的能力。将所述样品用霍乱滤液(获自霍乱弧菌(Vibrio cholerae)培养物)进行预处理以除去非特异性抗血凝素活性。随后,在37℃下孵育16小时,霍乱滤液通过将样品在56℃下孵育1小时来灭活。样品的连续两倍稀释在磷酸缓冲的硫酸盐(phosphate buffered sulphate,PBS)中进行(一式两份96孔板,从1 : 20稀释开始)并且当样品显示出特异性血细胞凝集时,其用火鸡的红血球进行预处理。在除去这些红血球之后,样品用固定浓度为4的关于流感病毒的血细胞凝集单元(hemagglutination unit,HAU)在4℃下孵育1小时。最后,单独地对板上血细胞凝集的抑制记分,其用红血球的沉降来示出。趋于雪貂对照血清包括在所有实验中。

[0223] VN测定

[0224] VN测定是标准测定,基于中和病毒的流感病毒特异性抗体子集的能力,使得在细胞培养中将不会存在病毒复制。样品在56℃下热灭活30分钟并且随后连续两倍稀释的样品一式三份在96孔板中于1 : 8的稀释开始于感染介质(补充有20mM Hepes、0.075%碳酸氢钠、2mM L-谷氨酰胺、100IU/ml的青霉素和链霉素、17.5μg/ml胰蛋白酶和2.3ng/ml两性霉素B的Eagles极限必须培养基)中制成。然后,样品稀释物在37℃下5% CO_2中用关于病毒的25-400 TCID50孵育1小时。1小时孵育期完成之后,将病毒-抗体混合物转移至具有为95%至100%汇合的Madine Darby犬肾(MDCK)细胞培养物单层的盘。然后这些板在37℃、5% CO_2下孵育1小时,并且随后除去病毒-抗体混合物并通过感染介质进行代替。在37℃、5% CO_2下6天孵育期之后,使用火鸡的红血球读板以检测流感病毒血凝素的存在。根据Reed和Muench(Reed,L.J.;Muench,H.(1938)" A simple method of estimating fifty percent endpoints".The American Journal of Hygiene 27:493-497)所述方法计算VN滴度。

[0225] 上呼吸道和下呼吸道中病毒的复制

[0226] 在第0、1、2、3和4天攻击之后,在麻醉下从动物中取出鼻和咽喉拭子。攻击之后四天,雪貂在其进行全身大体病理学之后在麻醉下通过放血进行安乐死并且收集组织。收集右鼻甲的样品和右肺所有叶和附加叶的样品并在-80℃下储存直到进一步处理。对鼻甲和肺样品进行称重并随后在包含0.5%乳白蛋白、10%甘油、200U/ml青霉素、200μg/ml链霉素、100U/ml多黏菌素B硫酸盐、250μg ml庆大霉素和50U/ml制霉菌素(ICN Pharmaceuticals,Zoetermeer,Netherlands)的Hank平衡盐溶液中用FastPrep-24(MP Biomedicals,Eindhoven,Netherlands)匀浆并简单地离心,然后稀释。

[0227] 在收集之后,将鼻和咽喉拭子在用作组织样品的处理的相同介质中于-80℃下储存。一式四份将10倍连续稀释的肺和拭子上清液用于确定前述MDCK细胞汇合层中的病毒滴度(Rimmelzwaan GF等,J Virol Methods 1998Sep;74(1)57-66)。

[0228] 抗体滴度结果

[0229] 在第0、21、42、64和70天每次免疫之前,确定抗体的血清水平。针对H1N1 A/The Netherlands/602/2009和2个不同病毒(H1N1 A/猪/Ned/25/80和H1N1 A/猪/

Italy/14432/76 的滴度通过血细胞凝集抑制测定 (HI) 和病毒中和测定 (VNT) 进行确定。针对不同病毒 H1N1 A/New Jersey/08/76 的抗体滴度通过血细胞凝集抑制测定 (HI) 进行确定。

[0230] HI 抗体滴度 - 同源 :H1N1 A/The Netherlands/602/2009

[0231] 几何平均数 HI 滴度在图 1 中描述。≤ 5 值用相应绝对值 5 来代替用于计算几何平均数。所有前血清 (第 0 天) 是 HI 抗体阴性 (滴度 :≤ 5)。

[0232] 通过组的 HI 滴度分析揭示以下结果 :

[0233] 第 1 组 (盐水 ;感染对照)

[0234] 所有血清样品为 HI 抗体阴性

[0235] 第 2 组 (**Fluarix®;** 肠胃外对照)

[0236] 在第一次免疫之后 (第 42 天) 收集的一个血清样品是低 HI 抗体阳性 (滴度 :13)。在第二次免疫之后,在六只动物中的五只的血清中检测为低 HI 滴度 (范围 13 至 70)。

[0237] 第 3 组 (疫苗 A,5μg HA ;鼻内)

[0238] 在第一次免疫之后收集的所有样品是 HI 抗体阳性 (第 21 天 ;GMT :477,范围 160 至 1120)。第二次免疫之后 (第 42 天 ;GMT :1669,范围 1120 至 2560) 显著提高 HI 抗体滴度并且在第三次免疫之后 (第 64 天 ;GMT :2158,范围 1280 至 3840) 也提高了六只动物中的四只的 HI 抗体滴度。在第 70 天 (攻击当天) 收集的样品显示出 HI 滴度可比得上在第 64 天测量的那些 (第 70 天 ;GMT :2103,范围 1120 至 3840)。

[0239] 第 4 组 (疫苗 A,15μg HA ;鼻内)

[0240] 第一次免疫之后所收集的六个样品中的五个为 HI 抗体阳性 (第 21 天 ;GMT :1130,范围 5-5760)。第二次免疫之后收集的所有样品为 HI 抗体阳性 ;在五只动物中 HI 抗体滴度显著提高 (第 42 天 ;GMT :3673,范围 1120 至 5760)。第三次免疫不导致 HI 抗体滴度的提高 (第 64 天 ;GMT :2386,范围 1920 至 4480)。在第 70 天 (攻击当天) 收集的样品显示 HI 滴度可比得上第 64 天测量的那些 (第 70 天 ;GMT :2281,范围 1280 至 2560)。

[0241] 第 5 组 (疫苗 A,30μg HA ;鼻内)

[0242] 在第一次免疫之后收集的样品为 HI 抗体阳性 (第 21 天 ;GMT :1249,范围 400 至 3200)。在第二次免疫之后六只动物中的五只中提高了 HI 抗体滴度 (第 42 天 ;GMT :1874,范围 640 至 3840) 并且在第三次免疫之后两只动物中也提高了 HI 抗体滴度 (第 64 天 ;GMT :1837,范围 1280 至 3200)。第 70 天 (攻击当天) 收集的样品显示 HI 滴度可比得上第 64 天测量的那些 (第 70 天 ;GMT :1699,范围 640 至 3200)。

[0243] 第 6 组 (疫苗 B,15μg HA ;鼻内)

[0244] 在第一次免疫之后所收集的六个样品中的五个为 HI 抗体阳性 (第 21 天 ;GMT :87,范围 5-1280)。第二次免疫之后所有动物中 HI 抗体滴度显著提高 (第 42 天 ;GMT :577 ;范围 100 至 2880) 并且在第三次免疫之后两只动物中的 HI 抗体滴度也显著提高 (第 64 天 ;GMT :626,范围 160 至 2560)。第 70 天 (攻击当天) 收集的样品显示 HI 滴度可比得上第 64 天测量的那些 (第 70 天 ;GMT :583,范围 160 至 2240)。

[0245] 异 源 :H1N1 A/ 猪 /Ned/25/80, H1N1 A/ 猪 /Italy/14432/76 和 H1N1 A/New Jersey/08/76

[0246] 检测针对不同病毒 H1N1 A/ 猪 /Ned/25/80、H1N1 A/ 猪 /Italy/14432/76 和 H1N1

A/New Jersey/08/76 的 HI 抗体滴度。针对不同病毒 HI 滴度的几何平均数在图 2 中描述。≤5的值用相应的绝对值5进行代替用于计算几何平均数。所有前血清（第0天）为HI抗体阴性（滴度：≤5)。交叉反应性HI抗体滴度显著地低于同源H1N1 A/Netherlands/602/2009 HI 抗体滴度。

[0247] 通过组的 HI 滴度的分析揭示了以下结果：

[0248] 第 1 组（盐水；感染对照）

[0249] 除了一个以外，所有血清样品为 HI 抗体阴性。在第 64 天收集的一个样品显示出非常低的针对 H1N1 A/ 猪 /Italy/14432/76 的 HI 抗体滴度（为 7.5)。

[0250] 第 2 组（**Fluarix®;** 肠胃外对照）

[0251] 所有样品是 H1N1 A/ 猪 /Ned/25/80 和 H1N1 A/ 猪 /Italy/14432/76 HI 抗体阴性。在第 42 天收集的血清中第一次免疫之后六只动物中的三只中检测到针对 H1N1 A/New Jersey/08/76 的低 HI 滴度。

[0252] 第 3 组（疫苗 A，5μg HA；鼻内）

[0253] 所有动物发展出针对三种不同病毒的交叉反应性 HI 抗体。在第二次免疫和 / 或第三次免疫之后测量最高滴度。第 21、42、64 和 70 天 H1N1 A/ 猪 /Ned/25/80 HI 抗体滴度 (GMT) 分别为 6（范围 5 至 7.5)、24（范围 5 至 60)、32（范围 20 至 80) 和 19（范围 5 至 70)。第 21、42、64 和 70 天 H1N1 A/ 猪 /Italy/14432/76 HI 抗体滴度 (GMT) 分别为 16（范围 5 至 50)、38（范围 10 至 80)、63（范围 40 至 160) 和 42（范围 20 至 120)。第 21、42、64 和 70 天 H1N1 A/New Jersey/08/76 HI 抗体滴度 (GMT) 分别为 5、26（范围 7.5 至 70)、39（范围 5 至 80) 和 29（范围 20 至 50)。

[0254] 第 4 组（疫苗 A，15μg HA；鼻内）

[0255] 在第二次免疫之后所有动物发展出针对三种不同病毒的交叉反应性 HI 抗体。第三次免疫不会导致 HI 滴度的提高。第 21、42、64 和 70 天 H1N1 A/ 猪 /Ned/25/80 HI 抗体滴度 (GMT) 分别为 42（范围 5 至 90)、239（范围 20 至 1120)、88（范围 50 至 160) 和 75（范围 40 至 160)。第 21、42、64 和 70 天 H1N1 A/ 猪 /Italy/14432/76 HI 抗体滴度 (GMT) 分别为 78（范围 5 至 280)、327（范围 35 至 1280)、153（范围 80 至 320) 和 105（范围 70 至 160)。第 21、42、64 和 70 天 H1N1 A/New Jersey/08/76 HI 抗体滴度 (GMT) 分别为 25（范围 5 至 80)、176（范围 60 至 400)、64（范围 40 至 140) 和 63（范围 40 至 160)。

[0256] 第 5 组（疫苗 A，30μg HA；鼻内）

[0257] 除了一只以外，所有动物发展出针对 H1N1 A/ 猪 /Ned/25/80 的交叉反应性 HI 抗体。所有动物发展出针对 H1N1 A/ 猪 /Italy/14432/76 和 H1N1 A/New Jersey/08/76 的交叉反应性 HI 抗体。在第二次免疫和 / 或第三次免疫之后测量最高滴度。在第 21、42、64 和 70 天 H1N1 A/ 猪 /Ned/25/80 HI 抗体滴度 (GMT) 分别为 23（范围 5 至 80)、41（范围 5 至 320)、42（范围 5 至 320) 和 34（范围 5 至 320)。在第 21、42、64 和 70 天 H1N1 A/ 猪 /Italy/14432/76 HI 抗体滴度 (GMT) 分别为 39（范围 5 至 160)、54（范围 5 至 640)、78（范围 20 至 720)、50（范围 5 至 480)。第 21、42、64 和 70 天 H1N1 A/New Jersey/08/76 HI 抗体滴度 (GMT) 分别为 9（范围 5 至 30)、40（范围 5 至 400)、35（范围 5 至 160) 和 27（范围 5 至 160)。

[0258] 第 6 组（疫苗 B，15μg HA；鼻内）

[0259] 所有动物发展出针对 H1N1 A/ 猪 /Italy/14432/76 的交叉反应性 HI 抗体。除了一只以外，所有动物发展出针对 H1N1 A/ 猪 /Ned/25/80 的交叉反应性 HI 抗体并且除了一只以外，所有动物发展出针对 H1N1 A/New Jersey/08/76 的交叉反应性 HI 抗体。在第二次免疫和 / 或第三次免疫之后测量最高滴度。第 21、42、64 和 70 天 H1N1 A/ 猪 /Ned/25/80 HI 抗体滴度 (GMT) 分别为 7(范围 5 至 40)、19(范围 5 至 80)、15(范围 5 至 80) 和 9(范围 5 至 40)。第 21、42、64 和 70 天 H1N1 A/ 猪 /Italy/14432/76 HI 抗体滴度 (GMT) 分别为 9(范围 5 至 160)、32(范围 5 至 160)、27(范围 5 至 160)、15(范围 5 至 80)。第 21、42、64 和 70 天 H1N1 A/New Jersey/08/76 HI 抗体滴度 (GMT) 分别为 8(范围 5 至 80)、47(范围 10 至 240)、19(范围 5 至 140) 和 13(范围 5 至 80)。

[0260] VN 抗体滴度 :

[0261] 同源 :H1N1 A/Netherlands/602/2009

[0262] 在所有实验动物的血清样品中测量 VN 抗体滴度。几何平均数 VN 滴度在图 3 中描述。所有前血清 (第 0 天) 为 VN 抗体阴性 (滴度 :≤ 8)。

[0263] 通过组的 VN 滴度的分析揭示了以下结果 :

[0264] 第 1 组 (盐水 ;感染对照)

[0265] 除了一只以外，第 42 天收集的所有血清样品为 VN 抗体阴性，测量值≤ 64。

[0266] 第 2 组 (**Fluarix®;** 肠胃外对照)

[0267] 所有血清样品为 VN 抗体阴性。

[0268] 第 3 组 (疫苗 A，5 μg HA ;鼻内)

[0269] 第一次免疫之后收集的六个样品中的四个为低 VN 抗体阳性 (第 21 天 ;GMT :19，范围 8 至 64)。第二次免疫之后收集的所有样品为 VN 抗体阳性。在第二次免疫之后 (第 42 天 ;GMT :242，范围 64 至 859) 和第三次免疫之后 (第 64 天 ;GMT :995，范围 362 至 2436) 五只动物中 VN 抗体滴度显著提高。第 70 天 (攻击当天) 收集的样品显示出可比得上或比第 64 天 (第 70 天 ;GMT :535，范围 304 至 859) 测量的那些低的 VN 滴度。

[0270] 第 4 组 (疫苗 A，15 μg HA ;鼻内)

[0271] 第一次免疫之后收集的六个样品中的五个为 VN 抗体阳性 (第 21 天 ;GMT :147，范围 8 至 724)。第二次免疫之后收集的所有样品为 VN 抗体阳性。在第二次免疫之后 (第 42 天 ;GMT :2376，范围 64 至 8192) 五只动物中和第三次免疫之后 (第 64 天 ;GMT :1688，范围 662 至 4871) 两只动物中 VN 抗体滴度显著提高。在第 70 天 (攻击当天) 收集的样品显示出 VN 滴度可比得上第 64 天 (第 70 天 ;GMT :1581，范围 351 至 3444) 测量的那些。

[0272] 第 5 组 (疫苗 A，30 μg HA ;鼻内)

[0273] 在第一次免疫之后收集的样品为 VN 抗体阳性 (第 21 天 ;GMT :74，范围 11 至 627)。在第二次免疫之后 (第 42 天 ;GMT :504，范围 41 至 3435) 六只动物中的五只中和第三次免疫之后 (第 64 天 ;GMT :1673，范围 724 至 4884) 六只动物中的三只中 VN 抗体滴度显著提高。在第 70 天 (攻击当天) 收集的样品显示出 VN 滴度可比得上第 64 天 (第 70 天 ;GMT :1699，范围 304 至 5793) 测量的那些。

[0274] 第 6 组 (疫苗 B，15 μg HA ;鼻内)

[0275] 第一次免疫之后收集的六个样品中的两个为低 VN 抗体阳性 (第 21 天 ;GMT :12，范围 8 至 64)。第二次免疫之后收集的所有样品为 VN 抗体阳性 (第 42 天 ;GMT :78，范围 32 至

304)。第三次免疫之后(第64天;GMT:242,范围113至747)VN抗体滴度提高。在第70天(攻击当天)收集的样品显示出比得上或比第64天(第70天;GMT:177,范围91至362)测量的那些低的VN滴度。

[0276] 异源:H1N1 A/猪/Ned/25/80、H1N1 A/猪/Italy/14432/76。测定针对不同病毒H1N1 A/猪/Ned/25/80和H1N1 A/猪/Italy/14432/76的VN抗体滴度(数据未示出)。在第42、64和70天,第3、4、5和6组都优于第1组和第2组。

[0277] 实施例2

[0278] 确定所有实验动物某些临床和病理学参数,即,死亡率、体温、体重、充气的肺容积、鼻甲和肺中病毒载量、上呼吸道中病毒脱落、宏观的病理学检测死后的肺重量、影响肺组织的损伤的平均百分比。微观检测鼻甲和肺的炎症参数。在所测试的所有宏观和大多数微观参数(数据未示出)中,第3、4和5组的动物优于第1组和第2组。

[0279] 上呼吸道和下呼吸道中病毒的复制

[0280] 第0、1、2、3和4天在攻击之后,在麻醉下从动物中取出鼻和咽喉拭子。四天攻击之后,雪貂在其进行全身大体病理学之后在麻醉下通过放血来进行安乐死并且收集组织。收集右鼻甲的样品和右肺所有叶和附加叶的样品并在-80℃下储存直到进一步处理。对鼻甲和肺样品称重并随后在包含0.5%乳白蛋白、10%甘油、200U/ml青霉素、200μg/ml链霉素、100U/ml多黏菌素B硫酸盐、250μg ml庆大霉素和50U/ml制霉菌素(ICN Pharmaceuticals,Zoetermeer,Netherlands)的Hank平衡盐溶液中用FastPrep-24(MP Biomedicals,Eindhoven,Netherlands)匀浆并简单地离心,然后稀释。

[0281] 在收集之后,将鼻和咽喉拭子于-80℃下在用于组织样品处理的相同培养基中储存。一式四份将10倍连续稀释的肺和拭子上清液用于确定前述MDCK细胞汇合层中的病毒滴度(Rimmelzwaan GF等,J Virol Methods 1998 Sep;74(1)57-66)。

[0282] 大体病理学和组织病理学

[0283] 动物根据标准方案进行尸检,如先前所述(van den Brand JM等,PLoS One 2012;7(8)e42343)。简言之,夹紧气管使得肺在打开胸膜腔时不会放气,为了准确视觉定量受影响的肺实质的面积。取左肺组织学检查的样品并储存在10%中性缓冲的福尔马林中(缓慢注入福尔马林之后),石蜡包埋,切成4μm的片,并且用苏木精和曙红(HE)染色用于通过光学显微镜检查。以标准化方式取样,不是由大体病理所观察的变化进行引导。肺中流感病毒相关炎症的半定量评估如先前所述(表4)(Munster VJ等,Science 2009Jul 24;325(5939):481-3)进行。在没有识别信息或动物处理的情况下,检查所有切片。

[0284] 肺和上呼吸道中病毒载量结果

[0285] 对照组1(鼻内,盐水)和2(肠胃外,TIV)的所有雪貂在肺(平均滴度;分别为5.7和5.5log10TCID50/克组织)和鼻甲(平均滴度:分别为7.2和6.9log10TCID50/克组织)中显示出复制能力病毒的高滴度(表3)。第3、4和5组的雪貂(鼻内,Endocine™佐剂化裂解抗原pH1N1/09疫苗)在其肺和鼻甲中具有不可检出的感染性病毒。第6组的雪貂(鼻内,15μg HA的Endocine™佐剂化完整病毒)在其肺中具有不可检出的感染性病毒并且平均滴度为4.1log10TCID50/克组织,与对照组1相比鼻甲中具有显著较低的病毒滴度($p = 0.02$)。

[0286] 与盐水或TIV施用相比,Endocine™佐剂化pH1N1/09疫苗的鼻内免疫降低了取自

鼻和咽喉的拭子中的病毒滴度。在鼻和咽喉的拭子中，在 1 至 4dpi 的时间间隔中，表示为曲线下面积 (AUC) 的病毒载量示于表 3 中。第 3、4 和 5 组鼻拭子中病毒载量（鼻内，分别为 5、15 和 30 μg HA 的 Endocine™佐剂化裂解抗原），但不是第 2 组和第 6 组显著低于第 1 组（第 1 组与第 3 至 5 组对比 ;$p \leq 0.03$）。第 1 组和第 2 组的咽喉拭子病毒载量可比于并显著高于第 3、4、5 和 6 组 ($p \leq 0.03$)。

[0287] 大体病理学和组织病理学结果

[0288] 在用 Endocine™佐剂化 pH1N1/09 疫苗鼻内免疫组中降低的病毒复制与肺大体病理学改变的降低相一致（表 3）。

[0289] 宏观死后肺损伤由局灶或多局灶肺实变组成，由很好描绘的实质的红化来表征。对照组 1（鼻内，盐水）和组 2（肠胃外，TIV）中所有的雪貂显示出影响的肺组织的平均百分率分别为 50％和 37％并且相应的平均相对肺重量 (relative lung weight,RLW) 分别为 1.5 和 1.3（表 3）。相反，第 3、4、5 和 6 组（鼻内，Endocine™佐剂化 pH1N1/09 疫苗）的肺很少受影响，受影响的肺组织的平均百分率为 7％至 8％。这四个 Endocine™接种疫苗的组中的 RLW 与这些观察一致（接近范围为 0.8 至 0.9）。

[0290] 肺实变在显微镜检查下与急性支气管间质性肺炎一致。其由肺泡的腔和壁内炎症细胞（大多数为巨噬细胞和嗜中性粒细胞）的存在以及肺细胞衬里的膨胀和损失来表征。此外，肺泡间隙中的富含蛋白质的水肿液、纤维蛋白链和渗出的红细胞以及 II 型肺细胞增生通常在肺泡炎的更严重情况中观察到。经评分的组织学参数概括于表 4 中。在对照组 1（鼻内，盐水）和 2（肠胃外 TIV）中发现最严重的肺泡损伤。肺泡损伤的所有参数在第 5 组中得分最低，但是实际上第 3、4、5 和 6 组之间的差异不是显著的。

[0291] 最后，在肺中 - 在该雪貂模型中具有 H1N1 流感 Netherlands/602/2009 病毒的气管内攻击导致轻度至重度肺炎。然而，一些动物（均来自接种疫苗组）根本不受宏观可辨别的肺损伤的影响。基于肺损伤的宏观死后评价（估计的受影响肺的％），接种疫苗的（疫苗 -A 15 μg HA）第 4 组和接种疫苗的（疫苗 -A 30 μg HA）第 5 组同样经受了最少的肺损伤，两者均具有非常低的 7％的得分，随后直接接种疫苗的（疫苗 -A 5 μg HA）第 3 组和接种疫苗的（疫苗 -B 15 μg HA）第 6 组为 8％。安慰剂 -PBS- 处理的第 1 组动物经受了最多的肺损伤，显著的平均得分为 50％。肠胃外疫苗接种的对照组 2 经受稍微少但仍然突出的肺损伤，平均为 37％。平均相对肺重量 (RLW) 显然是根据受影响的肺组织的这些估计的百分率，证实受影响的肺组织的这些估计的百分率的有效性。

[0292] 所证实的肺的微观检查的结果对于大多数肺损伤的评估参数而言，最好评分为最高剂量免疫接种（疫苗 -A 30 μg HA）的第 5 组，并且呼吸道损伤中逐渐进展与疫苗 -A 的 HA 剂量的降低相关（分别为第 3 组和第 2 组）。具有疫苗 -B 15 μg HA 疫苗接种几乎等于最低剂量疫苗 -A 5 μg HA（第 3 组）的结果。在所有评估的组织病理学参数中，安慰剂 -PBS- 处理（第 1 组）目前得分最差，接近之后肠胃外疫苗接种的对照组 2。显著地，所有鼻内疫苗接种的动物（第 3、4、5 和 6 组）均免受肺泡出血。

[0293] 总结论 - 因此，在结论中，基于该雪貂病毒攻击模型中平均的病理学得分，疫苗 -A 30 μg HA 的疫苗接种（第 5 组）进行的最好并且导致最少的呼吸道损伤，而安慰剂 -PBS- 处理进行的最差并且导致最多的呼吸道损伤。疫苗 -A 15 μg HA 的疫苗接种（第 4 组）与第 5 组相比仅稍微少一点，随后疫苗 -A 5 μg HA 的疫苗接种（第 3 组）与疫苗 -B 15 μg HA

的疫苗接种（第6组）相比实际上相似。不管疫苗的剂量和类型，所有鼻内接种疫苗的动物均免受肺泡出血。肠胃外的对照疫苗接种（第2组）进行得差，具有显著的呼吸道损伤并且与安慰剂-PBS-处理（第1组）相比仅略微更好。

[0294] 实施例3：

[0295] 以下表2和图4在首次免疫雪貂中将本发明疫苗与其他产品FluMist和可注射疫苗进行了比较。

[0296] 表2

[0297]

疫苗来源	雪貂（首次免疫）	剂量	途径	疫苗株 (H1N1)	评价株 (H1N1)	NT滴度评价
GSK * (GSK H1N1)	N=6	15ug HA, 非佐剂化的	IM	A/California/7/09	A/The Netherlands/602/09	攻击之前（两次疫苗接种之后）
GSK *	N=6	15ug HA, $AS03_A$	IM			
Novartis # (Novartis TIV)	N=3	15ug HA, 非佐剂化的	IM	A/Brisbane/59/07	A/California/7/09	攻击之前（两次疫苗接种之后）
Medimmune # （大流行LAIV）	N=3	$1x10^7$ $TCID_{50}$	IN	A/California/7/09 (*ca*)		
GSK ¤ (GSK TIV)	N=6	15ug HA, 非佐剂化的	SC	A/California/7/09	A/The Netherlands/602/09	第42天（两次疫苗接种之后）
Eurocine 疫苗 ¤ (Immunose™ FLU)	N=6	15ug HA, Endocine 20mg/ml	IN			

[0298] *Baras et al.Vaccine 29(201 1)2120-2126

[0299] #Chen et al.JID 2011:203

[0300] ¤ Eurocine疫苗：本研究

[0301] GSK单价大流行疫苗（GSK H1N1），Novartis三价灭活疫苗（Novartis TIV）、GSK三价灭活的疫苗（GSK TIV）组具有中和滴度（NT），滴度低于15。

[0302] 结果显示出本发明的疫苗组合物，这里包含具有20mg/ml(2％)Endocine™的15μg HA裂解流感抗原的Immunose™ FLU，显示出与Medimmune的大流行LAIV疫苗FluMist相似的中和滴度（参见图5）并且相对于注射的疫苗具有较优的滴度，而非佐剂化TIV得到差的响应。

[0303] 实施例中所使用的缩写：

[0304] HA 流感病毒血凝素蛋白

[0305] TCID50 组织培养感染剂量50％

[0306] PBMC 外周血单核细胞

[0307] HI 流感血细胞凝集抑制测定

[0308] SOP 标准操作规程

[0309] PBS 磷酸缓冲盐水

[0310] EDTA 乙二胺四乙酸

[0311] GMT 几何平均数滴度（用于表示血清学数据）

[0312] FCS 胎牛血清（培养基补充物）

[0313] VN 病毒中和测定

[0314] DMSO 二甲基亚砜

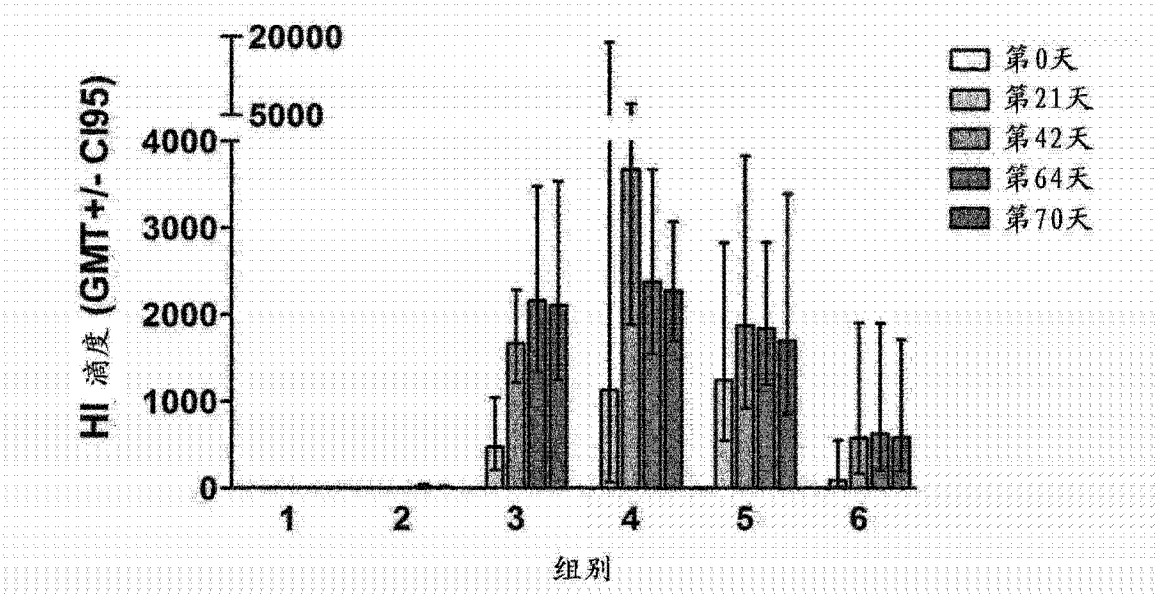

20000
5000
4000
3000
2000
1000
0
HI 滴度 (GMT+/- CI95)
1
2
3
4
5
6
组别
第0天
第21天
第42天
第64天
第70天

图 1

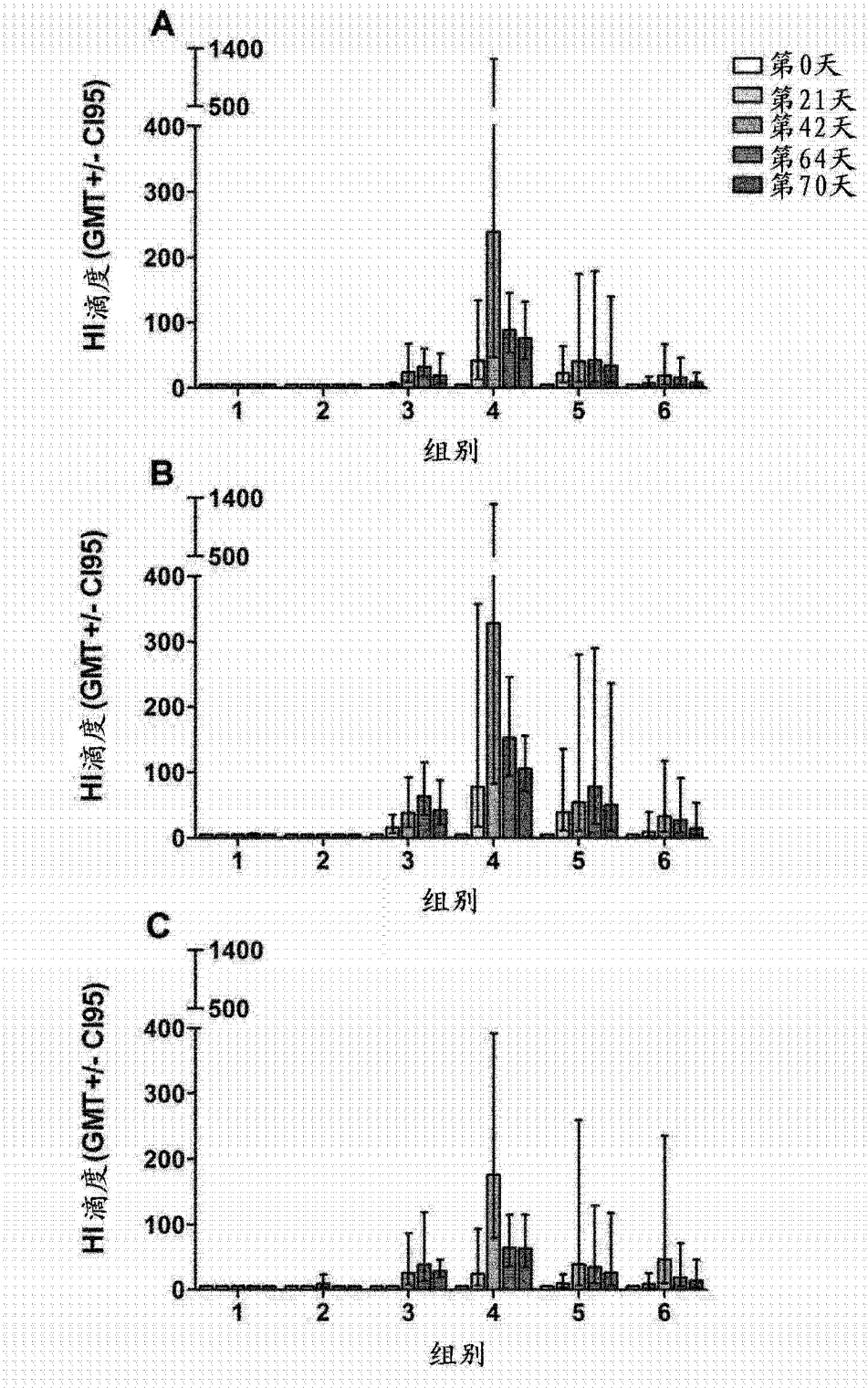

A
B
C
HI滴度(GMT+/- CI95)
1400
500
400
300
200
100
0
1
2
3
4
5
6
组别
第0天
第21天
第42天
第64天
第70天

图 2

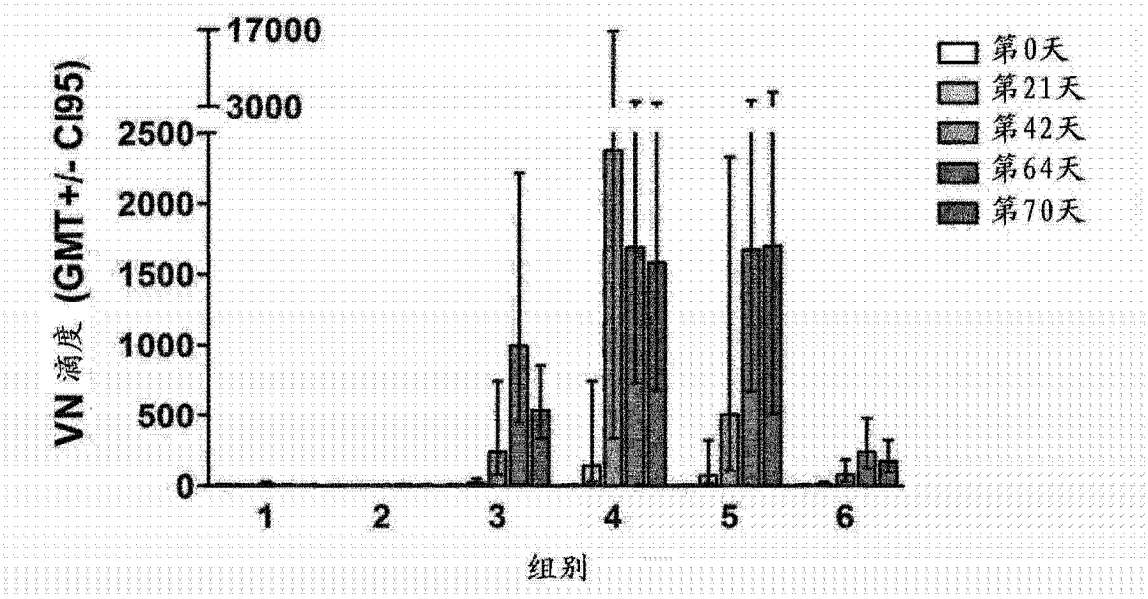

17000
3000
2500
2000
1500
1000
500
0
VN 滴度 (GMT+/- CI95)
1
2
3
4
5
6
组别
第0天
第21天
第42天
第64天
第70天

图 3

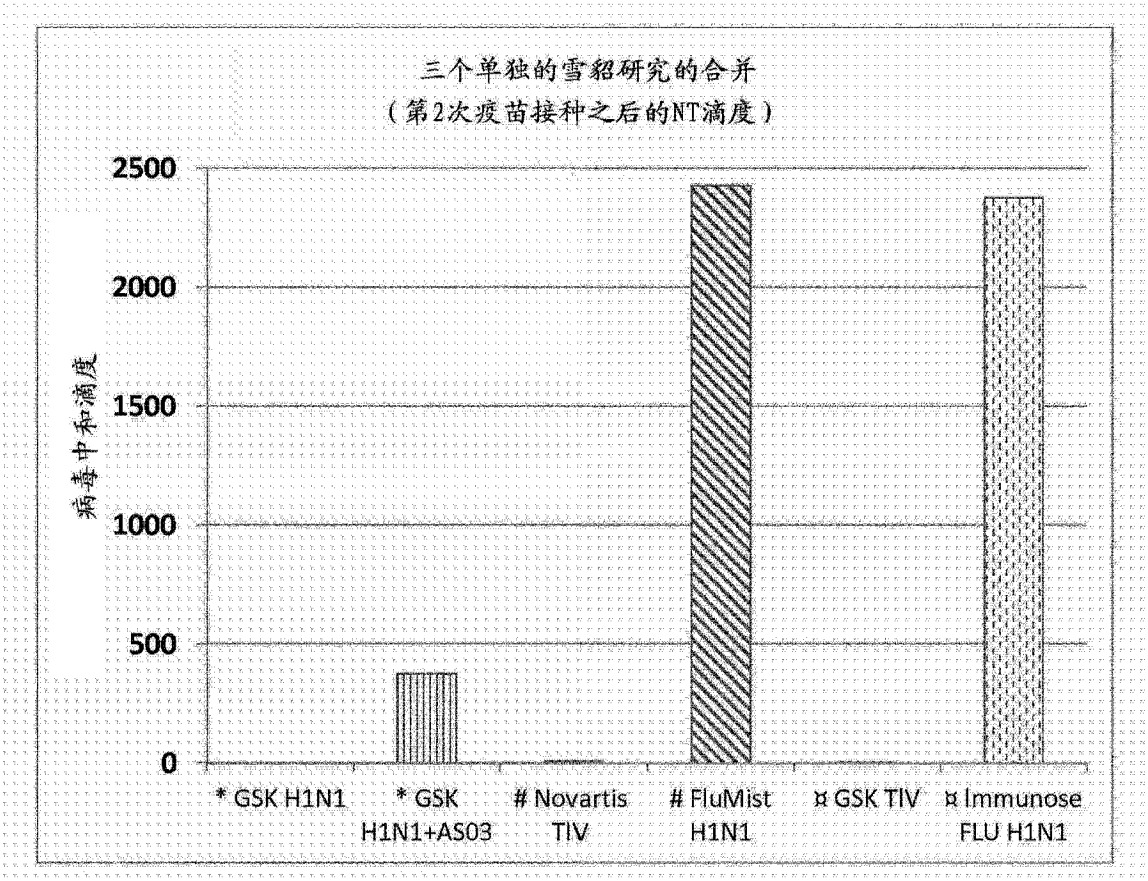

三个单独的雪貂研究的合并
（第2次疫苗接种之后的NT滴度）
病毒中和滴度
2500
2000
1500
1000
500
0
* GSK H1N1
* GSK H1N1+AS03
# Novartis TIV
# FluMist H1N1
¤ GSK TIV
¤ Immunose FLU H1N1

图 4

表3

		组别*					
		1	2	3	4	5	6
临床评分	存活	6/6	5/6	6/6	5/6	6/6	6/6
	发热	1.7±0.6 (6/6)	1.1±0.4 (6/6)	1.3±0.3(6/6)	1.2±0.6(4/5^)	1.1±0.6(6/6)	1.3±0.2(6/6)
	体重减轻	18.0±4.6 (6/6)	11.5±2.1 (6/6)	2.2±2.6 (1/6)	1.7±1.5 (4/6)	2.7±3.3 (4/6)	4.7±3.1 (5/6)
病毒学	肺病毒载量[$log_{10}TCID_{50}$/g]	5.7±0.5 (6/6)	5.5±0.9 (6/6)	≤1.5 (0/6)	≤1.4 (0/6)	≤1.3 (0/6)	≤1.3 (0/6)
	鼻甲病毒载量[$log_{10}TCID_{50}$/g]	7.2±2.4 (6/6)	6.9±1.5 (6/6)	≤1.9 (0/6)	≤1.7 (0/6)	≤1.7 (0/6)	4.1±2.7 (3/6)
	鼻拭子中的病毒脱落	2.6 (5/6)	1.2 (4/6)	0.058 (1/6)	0.0 (0/6)	0.0 (0/6)	1.4 (3/6)
	咽喉拭子中的病毒脱落	10 (6/6)	10 (6/6)	0.0 (1/6)	0.14 (1/6)	0.0 (1/6)	4.2 (5/6)
大体病理学	受影响的肺组织[%]	50±25 (6/6)	37±21 (6/6)	8±4 (5/6)	7±5 (4/6)	7±5 (4/6)	8±4 (5/6)
	相对肺重量	1.5±0.5	1.3±0.1	0.8±0.1	0.8±0.1	0.8±0.2	0.9±0.1

表4

		组别[a]					
		1	2	3	4	5	6
组织病理学	肺泡炎/胚泡损伤的程度（评分0至3）	2.08±0.74 (6/6)	1.88±0.54 (6/6)	0.42±0.52 (3/6)	0.08±0.20 (1/6)	0.04±0.10 (1/6)	0.42±0.41 (4/6)
	肺泡炎的严重程度（评分0至3）	2.04±0.68 (6/6)	1.63±0.31 (6/6)	0.50±0.69 (3/6	0.08±0.20 (1/6)	0.04±0.10 (1/6)	0.46±0.46 (4/6)
	肺泡水肿（阳性载玻片%）	29±29 (4/6)	21±19 (4/6)	4±10 (1/6)	0±0 (0/6)	0±0 (0/6)	8±13 (2/6)
	肺泡出血（阳性载玻片%）	21±40 (2/6)	17±26 (2/6)	0±0 (0/6)	0±0 (0/6)	0±0 (0/6)	0±0 (0/6)
	II型肺细胞增生（阳性载玻片%）	42±34 (4/6)	46±37 (4/6)	8±20 (1/6)	4±10 (1/6)	0±0 (0/6)	4±10 (1/6)

Abstract

The invention relates to nasally-administered vaccine compositions effective in naive subjects such as children. Further, the vaccine composition is suitable for vaccinating the general population during a pandemic. One aspect of the invention is directed to the paediatric use of the vaccine of the invention including a vaccine effective in children against seasonal influenza virus strains. A further aspect of the invention is directed to subjects of all age groups when the composition is for pandemic use.