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(71) Applicant and
(72) Inventor: **BLAY, Moshe, Amihod** [IL/IL]; 6, Halperin St., Tel-Aviv 63404 (IL).

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(54) Title: COMPOSITION AND METHOD FOR PREVENTION OF INFLUENZA

(57) Abstract: Novel compositions and methods for vaccinating a subject against influenza.. The compositions and methods are based on the use a vaccine that includes an antigen that is extracted from Streptococcus pneumonia. The methods are particularly useful for vaccination of subjects who do not have an increased risk of developing pneumonia, subjects who have 4×10^9 to 11×10^9 white blood cells in a liter of blood, subjects who are not immunocompromised, subject who have functional spleen, subjects who are allergic to inactivated virus-based influenza vaccine, subjects who are allergic to eggs, subjects who are diagnosed with a Guillain-Barré syndrome, subjects who do not have cancer, leukemia, lymphoma, or multiple myeloma, subjects who do not have HIV infection, subjects who are not diagnosed with cirrhosis of the liver, subjects who are not recipients of organ transplants or bone marrow transplants, subject who do not take medications that lower immunity, and subject that are less than 65 years old.

COMPOSITION AND METHOD FOR PREVENTION OF INFLUENZA

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This invention claims priority to U.S. Provisional Patent Applications Serial Nos. 60/717,742, filed September 19, 2005, and 60/830,184, filed July 12, 2006.

FIELD OF THE INVENTION

[0002] This invention relates generally to compositions and methods for prevention of influenza and influenza-related diseases.

BACKGROUND

[0003] Influenza (flu) is a disease that explodes worldwide every autumn/winter. It is contracted by a significant part of the population. On average, 5% to 20% of the population in the U.S.A. contracts influenza each year, with damage caused by the influenza being both heavy and costly. In 2004, more than 200,000 people were hospitalized because of influenza. In 2004, the respiratory diseases influenza and pneumonia together cost the U.S. economy \$37.5 billion (American Lung Association, *Trends in Pneumonia and Influenza Morbidity and Mortality*, August 2004).

[0004] The typical influenza vaccine (Flu-V) can be effective in preventing and minimizing both the onset of influenza and its side effects. However, only 39.2% of the high-risk population (mainly persons aged 65 years and older) is vaccinated each year. Naturally, improving the compliance rate will reduce both damages and costs resulting from the disease.

[0005] The typical influenza vaccine (Flu-V) is a vaccine that contains inactivated virus. Flu-V suffers from disadvantages. First, the composition of Flu-V is based on the strains of the viruses expected to be active in the coming season. In March-April each year, the World Health Organization (WHO) publishes the types of recommended strains to be included in the Flu-V for prevention of epidemics. Vaccination of the targeted population must be

completed before November-December, the period of the influenza outburst. Consequently, the very short period that is left for the "supply-chain" (i.e., manufacturers - distributors - pharmacists - physicians - nurses) exerts heavy pressure on the whole system. Second, the fact that each season the virus strains in the vaccine are usually modified results in a short shelf-life of the Flu-V of only 3-5 months. Manufacturers produce limited quantities of vaccine in order to avoid financial loss by holding large inventories of dead-stocks/expired Flu-V at the end of the season. These limitations can create shortages of vaccines leading to unvaccinated population, and the activity of the vaccine is of one season duration. Third, the strains recommended by the World Health Organization are not always compatible with the pathogens that cause outburst of the disease in the approaching season.

[0006] Pneumococcal vaccine (PV) can prevent pneumonia that is caused by *Streptococcus pneumoniae* bacteria. The 23-valent vaccine that is currently used can prevent against 23 types of *Streptococcus pneumoniae*, which account for approximately 90% cases of the pneumococcal disease. To prevent pneumococcal infection, the vaccine is recommended for people who have increased risk of developing pneumonia. These are people with certain medical conditions and people 65 years of age and older. About 80% of pneumonia cases occur in these increased-risk groups. The vaccine protects about 50-80% of people against pneumococcal infection. Vaccination also makes the disease milder for those who may catch it.

[0007] For prevention of pneumonia, the best time to get a pneumococcal vaccine is as soon as an increased-risk medical condition is developed, or when a person turns 65. To prevent pneumococcal infection, the pneumococcal vaccine is usually given just once in a lifetime, although in some cases a second dose of the pneumococcal vaccine is given. A second dose is recommended for people aged 65 and older who got their first dose when they were under 65, if at least 5 years have passed since that dose; people who do not have a functional spleen, i.e. have a damaged spleen or people with post-splenectomy, functional or anatomic asplenia; have cancer,

leukemia, lymphoma, multiple myeloma; are taking medication that lowers immunity (such as chemotherapy or long-term steroids); have had an organ or bone marrow transplant.

[0008] The 23-valent adult pneumococcal vaccine is considered to be very safe. Some people have side effects from the vaccine, but these are usually minor and last only a short time. It is quite common to have some swelling and soreness in the arm where the needle was inserted. Occasionally slight fever may occur. Other side effects - such as headache, a higher fever or fatigue may occur, but these are rare.

[0009] Some populations should not receive the 23-valent adult pneumococcal vaccine. For example, it is not recommended for children under two years of age. Nor should the vaccine be given to a subject who has a severe allergy to any component of the vaccine. The pneumococcal vaccine used between 1978 and 1983 protected against only 14 types of the pneumococcus. People who received this vaccine do not usually need to get another shot.

[0010] Cost effectiveness in the prevention of lung diseases, including influenza- and pneumonia-related diseases, is of major concern for health providers. Economic analyses indicate that influenza vaccination is cost beneficial not only in the elderly people, but also in healthy working adults (Lee *et al.*, 2002, *Ann Int Med* 137: 225-231). Pneumococcal vaccination has been effective in reducing hospitalization for chronic lung diseases, while influenza vaccination alone did not possess this effect (Christenson *et al.*, 2002, *Epidemiol Infect* 129: 515-524).

BRIEF SUMMARY

[0011] The invention provides compositions and methods for the prevention of influenza and influenza-related diseases. In one aspect of the invention, a vaccine composition for preventing influenza is provided. The vaccine composition includes an amount of an antigen that causes an anti-influenza response. The antigen is extracted from *Streptococcus pneumoniae*.

[0012] The vaccine composition may include antigen that is extracted from at least one serotype of *Streptococcus pneumoniae*. In one preferred embodiment, the antigen is extracted from at least 7 different serotypes of *Streptococcus pneumoniae*. In another preferred embodiment, the antigen is extracted from 23 different serotypes of *Streptococcus pneumoniae*. The antigen can, for example, be extracted from serotypes 1, 2, 3, 4, 5, 6B (6A), 7F (7C), 8, 9N (9A), 9V, 10A, 11A (11B), 12F, 14, 15B (15A, 15C, 15F), 17F, 18C (18A, 18B), 19F (19B), 19A, 20, 22F (22A), 23 F (23A, 23B), and 33F (33C) of *Streptococcus pneumoniae*.

[0013] In another aspect of the invention, a method of vaccinating a subject against influenza is provided. The method includes administering to the subject a preventatively effective amount of a vaccine composition that includes an antigen that is extracted from *Streptococcus pneumoniae*.

[0014] In preferred embodiments, the method provides for vaccination of subjects who do not have an increased risk of developing pneumonia, subjects that have 4×10^9 to 11×10^9 white blood cells in a liter of blood, subjects who are not immunocompromised, subject who have a functional spleen, subjects who are allergic to inactivated virus-based influenza vaccine, subjects who are allergic to eggs, subjects who do not have cancer, leukemia, lymphoma, or multiple myeloma, subjects who do not have HIV infection, subjects who are not diagnosed with cirrhosis of the liver, subjects who are not recipients of organ transplants or bone marrow transplants, subject who do not take medications that lower immunity, and subject that are less than 65 years old.

[0015] In some aspects of the invention, administration of the vaccine is provided in a single dose. In other aspects, administration of the vaccine is provided in multiple doses, which are administered less frequently than once a year, and may be administered once in two years, three years, four years, or five years.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] Figure 1 is a graph showing phagocytic activity of mouse peritoneal cells following inoculation with PV and Flu-V.

[0017] Figure 2 is a graph showing mitogen response of mouse peripheral blood mononuclear cells following inoculation with PV and Flu-V.

[0018] Figure 3 is a graph showing a mitogen response of splenocytes following inoculation with PV and Flu-V.

DETAILED DESCRIPTION OF THE PRESENTLY PREFERRED EMBODIMENTS

[0019] "Antigen" is a substance that evokes an immune response, especially the production of antibodies. Antigens are usually proteins or polysaccharides foreign to the body, but can also be any type of molecule, including small molecules (haptens) coupled to a carrier-protein. For example, an influenza antigen is a substance that evokes an anti-influenza response in the subject, when the subject is immunized with that antigen.

[0020] "Vaccine" is a preparation of antigenic material for administration to induce in the recipient subject immunity to infection or intoxication by a given infecting agent. Vaccines may be prepared from viruses, rickettsiae, bacteria, protozoa and metazoa or toxins. Vaccines may be sterile suspensions of the killed organisms, of toxoids or other antigenic material derived from the organisms or recombinant sources, which can be administered by injection. Vaccines may be either simple vaccines prepared from one species of organism or a variety of organisms, or they may be mixed vaccines containing two or more simple vaccines. They are prepared in such a manner as not to destroy the antigenic material, although the methods of preparation vary, depending on the vaccine.

[0021] "Vaccine adjuvants" consist of agents that are included in the formulation that are used to enhance the ability of the antigenic material in a vaccine to induce the desired immune response, and with some poorly antigenic materials the success of vaccination may depend on the presence of

a suitable adjuvant in the vaccine. The adjuvant is sometimes conveniently incorporated in the vaccine before the latter is distributed into containers, although it may be provided in a separate container for mixing with the antigenic material when the vaccine is required for use in immunizing the recipient.

[0022] "Vaccination" is the process of administering a vaccine to a subject, with the intent of conferring immunity against a targeted disease agent. Vaccination against influenza refers to the process of administering a vaccine to a subject, with the intent of conferring immunity against influenza in that subject.

[0023] "Immunodeficiency" or "immunosuppression" is a state in which the immune system's ability to fight infectious disease is compromised or entirely absent. Suppression of a person's natural immune responses can be medically-induced, e.g. via radiation therapy or drugs (cortisone, azathioprine, cyclosporine, etc.). This is usually done to prevent the body from rejecting an organ transplant or for the treatment of auto-immune diseases such as rheumatoid arthritis or Crohn's disease. The downside is that with such an inactive immune system, the body is very vulnerable to opportunistic infections, even those usually considered harmless. Also, prolonged use of immunosuppressants increases the risk of cancer.

[0024] "Immunocompromised" person is a person who has an immunodeficiency or immunosuppression, or whose immune system is weak for other reasons (e.g. chemotherapy, HIV, etc.). An immunocompromised person is very vulnerable to opportunistic infections.

[0025] "Preventing" or "prevention" as used herein refers to the preventing or prevention of a disease or medical condition. Examples of a disease or a medical condition are influenza and influenza-like diseases. For the purposes of this invention, "influenza-like diseases" refers to World Health Organization definition of influenza-like syndromes (i.e., a sudden fever of greater than 39°C, myalgia, and respiratory symptoms). The vaccines and methods of the present invention can be used for prevention of both influenza

and influenza-like diseases. Therefore, as used herein, reference to "prevention of influenza" is meant to also include the prevention of influenza-like diseases.

[0026] "Treating" or "treatment" as used herein refers to the treating or treatment of a disease or medical condition. Examples of a disease or a medical condition are influenza and influenza-like diseases.

[0027] "Preventatively effective amount" refers to an amount sufficient to effect prevention when administered to a subject. For example, preventatively effective amount of an anti-influenza vaccine refers to the amount of vaccine that will effectuate a preventive immune response in the vaccinated subject against influenza and influenza-like diseases.

[0028] "Serotype" is a group of microorganisms that is classified based on the cell surface antigens. For example, the hundreds of pneumococcal strains (Vishniakova *et al.*, 1981, *Zh Mikrobiol Epidemiol Immunobiol* 12: 20-24) are divided into several tens of capsular types (serotypes). At least 90 capsular types (serotypes) of pneumococci (*Streptococcus pneumoniae*) have been identified.

[0029] "Pneumococcal vaccine" (PV) or "pneumococcal polysaccharide vaccine" is a vaccine that is typically used to prevent *Streptococcus pneumoniae* (pneumococcus) infections such as pneumonia and septicaemia. Generally, the pneumococcal vaccine can be composed of polysaccharides that are found in the capsular types of pneumococci. Since at least 90 capsular types of pneumococci have been identified, the PV can include one or more of the capsular polysaccharides of *Streptococcus pneumoniae*. The vaccine can include polysaccharides from multiple *Streptococcus pneumoniae* serotypes.

[0030] The present invention provides compositions and methods that can be used for prevention of influenza. The composition for vaccination against influenza includes an antigen that is extracted from *Streptococcus pneumoniae* bacteria. The antigen can be polysaccharide or any other antigen molecule that will effectuate a preventive immune response against

influenza and influenza-like diseases. The methods provide for vaccinating a subject for the prevention of influenza, using an antigen that is extracted from *Streptococcus pneumoniae*. Thus, the methods also provide the use of the existing typical pneumococcal vaccine (PV) for the prevention of influenza.

[0031] The invention is based on the discovery that pneumococcal vaccine is able to prevent not only pneumococcal infections, but also influenza. This discovery is partly a result of the inventor's personal experience: having been splenectomized approximately 45 years ago, but not being vaccinated against pneumococcal infections as recommended for individuals after splenectomy, for about three decades the inventor had experienced episodes of influenza and influenza-like diseases at rates similar to those observed in the general population. Fifteen years ago, the inventor was advised to be vaccinated regularly with PV. The pneumococcal vaccine is claimed to be active for five years. Since the first vaccination with PV, the inventor noticed that during a follow-up period of approximately fifteen years, he did not contract influenza or similar diseases.

[0032] Despite the long-term use of PV for prevention of pneumonia, it has not been recognized that it is possible to use just PV for the prevention of influenza and influenza-related diseases. Because both the flu vaccine and the pneumococcal vaccine are used for prevention of respiratory diseases, the administration and the effects of the flu vaccine and the pneumococcal vaccine have been discussed together. The positive effect of simultaneous administration of both influenza vaccine (Flu-V) and pneumococcal vaccine (PV) vaccines on prevention of influenza and invasive pneumococcal disease has already been shown (Gardner *et al.*, 1982, *J Reticuloendothel Soc* 32: 443-448; Bertz, 2001, *Fortschr Med Orig* 119 Suppl 2: 71-75). However, to the inventor's knowledge, there have been no efforts to administer PV with the specific objective of prevention of influenza and influenza-related diseases.

[0033] The present invention provides for a vaccine that includes an antigen extracted from *Streptococcus pneumoniae*, and is useful for prevention of influenza in different populations of subjects.

[0034] For prevention of pneumonia, it is recommended that pneumococcal vaccine should be given to anyone 65 years of age and older, as well as adults and children 2 years and older who have the following increased-risk medical conditions: chronic heart diseases, kidney, or lung disease; nephrotic syndrome; cirrhosis of the liver; alcoholism; diabetes mellitus; chronic cerebrospinal fluid leak; HIV infection and AIDS; multiple myeloma; Hodgkin's disease; lymphoma, leukemia; other diseases that suppress the immune system; no spleen or a spleen that does not work properly; sickle cell disease; or have an organ transplant.

[0035] In contrast, for prevention of influenza, pneumococcal vaccine could be given to subjects that are not otherwise candidates for receiving pneumococcal vaccine. A nonlimiting list of such subjects includes people who: are not immunocompromised; have 4×10^9 to 11×10^9 white blood cells in a liter of blood; are not over 65 years old; do not have chronic heart, kidney, or lung disease; are not diagnosed with a nephrotic syndrome; are not diagnosed with cirrhosis of the liver; are not diagnosed with diabetes mellitus; do not have a chronic cerebrospinal fluid leak; do not have HIV infection and AIDS; do not have multiple myeloma; do not have Hodgkin's disease; do not suffer from lymphoma, leukemia; do not suffer from other diseases that suppress the immune system; have a functional spleen; do not suffer from sickle cell disease; or have not had an organ transplant.

[0036] An example of a useful pneumococcal vaccine for practicing the present invention is the 23-valent pneumococcal polysaccharide vaccine (PPV23, e.g., Pneumovax II[®], available from Aventis Pasteur MSD; Pneumovax 23[®], available from Merck; Pnu-immune 23[®], available from Lederle Laboratories). In another example, the pneumococcal vaccine that was used between 1978 and 1983 and protected against 14 types of the pneumococcus can be used. In yet another example, the vaccine for prevention of influenza is a pneumococcal conjugate vaccine, which consists of a capsular saccharide from *Streptococcus pneumoniae* covalently bound to a carrier protein. An example of a 7-valent pneumococcal conjugated vaccine

is Prevenar[®], which is available, e.g., from Wyeth-Lederle Vaccines and Pediatrics. In another example, a 9-valent pneumococcal conjugated vaccine includes the *Streptococcus pneumoniae* serotypes 1, 4, 5, 6B, 9V, 14, 18C, 19F, and 23F.

[0037] Administration of the vaccine of the present invention for preventing influenza possesses several important advantages in comparison to Flu-V. The use of the vaccine can be considered as safe and effective as Flu-V for the following reasons: (i) there is a long experience with pneumococcal vaccine's beneficial effects in preventing infections of the respiratory tract in children, adults, and elderly individuals; (ii) the side effects, if any, are not greater than those observed with Flu-V (Socan *et al.*, 2004, *Vaccine* 22: 3087-3091); (iii) both its expiration period ("shelf-life") and activity are longer than one year; (iv) there is no time/season need for its administration.

[0038] In addition to the above examples of vaccines useful for practicing the invention, a skilled artisan will recognize that it would be possible to create new vaccine variations with the inclusion or omission of polysaccharides from a variety of pneumococcal serotypes. For example, an anti-influenza vaccine according to the present invention can be manufactured using polysaccharides from all known serotypes of *Streptococcus pneumoniae*. In general, it is only required to include the polysaccharides necessary to elicit the anti-influenza response.

[0039] A different number of *S. pneumoniae* serotypes could be used to extract the antigens for preparation of the vaccine composition of the present invention. In one preferred embodiment, a minimum number of 7 serotypes (for example, 4, 6B, 9V, 14, 18C, 19F, and 23F) could be used as an anti-influenza vaccine.

[0040] The adult version of the currently used PV for prevention against pneumonia contains 23 serotypes of *S. pneumoniae*. The same vaccine could be used for practicing the present invention. In one preferred embodiment, *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23 F, and 33F could be

used to manufacture the anti-influenza vaccine of the present invention. A skilled artisan will recognize that it is possible to substitute certain serotypes without compromising efficacy of the vaccine. Thus, in one preferred embodiment, *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6B (6A), 7F (7C), 8, 9N (9A), 9V, 10A, 11A (11B), 12F, 14, 15B (15A, 15C, 15F), 17F, 18C (18A, 18B), 19F (19B), 19A, 20, 22F (22A), 23 F (23A, 23B), and 33F (33C) could be used to manufacture the anti-influenza vaccine of the present invention. The numbers in parentheses refer to serotype substitutions that reflect alternative embodiments of the vaccine. For example, as indicated in parentheses above, the *S. pneumoniae* serotype 6B could be substituted with serotype 6A; similarly, serotype 23 F could be substituted with either 23A or 23B. One or more substitutions of serotypes could be performed without compromising efficacy of the vaccine. Alternatively, the number of *S. pneumoniae* serotypes in a vaccine could be changed by one skilled in the art.

[0041] Typically, the vaccine of the present invention is prepared as a suspension for injection. Various preservatives could be added to the vaccine, according to methods known in the art. In one embodiment, a typical 0.5 mL dose of anti-influenza vaccine contains 25 µg of each purified antigen (serotype) with either thimerosal or phenol as a preservative. The amount of each purified antigen can be varied. Generally, between 5 and 500 µg of each antigen could be used in one 0.5 mL dose of vaccine. In some embodiments, in addition to the antigens, the vaccine of the present invention also includes an adjuvant.

[0042] The vaccine is administered by intramuscular or subcutaneous injection. The vaccine should preferably be given by intramuscular injection. The preferred sites of intramuscular injection are the anterolateral aspect of the thigh (*vastus lateralis* muscle) in infants, or the deltoid muscle of the upper arm in children and adults.

[0043] In contrast to the existing typical Flu-V vaccine that is based on inactivated viruses, the present invention provides a flu vaccine that may be valid for a period longer than one season. In comparison to Flu-V, the vaccine

composition of the present invention has an extended expiration period ("shelf life"), and is not dependent on seasonal viral strains. The vaccine composition of the present invention can be administered at different times of the year, and is as effective against influenza as the Flu-V itself. Moreover, the vaccine is deprived of significant side effects.

[0044] The vaccine is useful for vaccinating people who do not have an increased risk of developing pneumonia. Subjects who do not have an increased risk of developing pneumonia would not normally have the need to receive a vaccine that includes antigens from *S. pneumoniae*. When subjects who do not have an increased risk of developing pneumonia are vaccinated with the composition of the present invention, they should typically develop immunity against influenza.

[0045] The vaccine is useful for vaccinating people who have 4×10^9 to 11×10^9 white blood cells in a liter of blood. Normal white blood cell count is considered to be in the range of 4×10^9 to 11×10^9 white blood cells (leukocytes) in a liter of blood from a healthy adult. When subjects who have 4×10^9 to 11×10^9 white blood cells in a liter of blood are vaccinated with the composition of the present invention, they should typically develop immunity against influenza.

[0046] The vaccine is useful for vaccinating people who are not immunocompromised. Subjects who are not immunocompromised would not normally have the need to receive a vaccine that includes antigens from *S. pneumoniae*. When subjects who are not immunocompromised are vaccinated with the composition of the present invention, they should typically develop immunity against influenza.

[0047] The vaccine is useful for vaccinating people who have a functional spleen. Subjects who have functional spleen would not normally have the need to receive a vaccine that includes antigens from *S. pneumoniae*. When subjects who have functional spleen are vaccinated with the composition of the present invention, they should typically develop immunity against influenza.

[0048] The vaccine is useful in vaccinating people with allergies against the typical flu vaccine (Flu-V). Flu-V, based on inactivated virus, is usually grown in fertilized chicken eggs, and is thus contraindicated for those with severe allergies to egg proteins. Therefore, in one aspect of the invention, the target population for the present invention is the group of people with Flu-V allergies. For example, the vaccine is useful in vaccinating people with egg allergies against influenza. Because the vaccine of the present invention can be manufactured without being grown in fertilized chicken eggs, one preferred target population for the present invention is the group of people with egg allergies.

[0049] The vaccine is useful in vaccinating people who cannot be vaccinated with the typical flu vaccine (Flu-V). Flu-V is contraindicated for people with a history of Guillain-Barré syndrome (acquired immune-mediated inflammatory disorder of the peripheral nervous system). Therefore, in one aspect of the invention, the target population for the present invention is the group of people with Guillain-Barré syndrome. When subjects who have Guillain-Barré syndrome are vaccinated with the composition of the present invention, they should typically develop immunity against influenza.

[0050] The vaccine is useful for vaccinating people who do not have cancer, leukemia, lymphoma, or multiple myeloma. Subjects who do not have cancer, leukemia, lymphoma, or multiple myeloma would not normally have the need to receive a vaccine that includes antigens from *S. pneumoniae*. When subjects who do not have cancer, leukemia, lymphoma, or multiple myeloma are vaccinated with the composition of the present invention, they should typically develop immunity against influenza.

[0051] The vaccine is useful for vaccinating people who do not have HIV infection. Subjects who do not have HIV infection would not normally have the need to receive a vaccine that includes antigens from *S. pneumoniae*. When subjects who do not have HIV infection are vaccinated with the composition of the present invention, they should typically develop immunity against influenza.

[0052] The vaccine is useful for vaccinating people who are not diagnosed with cirrhosis of the liver. Subjects who are not diagnosed with cirrhosis of the liver would not normally have the need to receive a vaccine that includes antigens from *S. pneumoniae*. When subjects who are not diagnosed with cirrhosis of the liver are vaccinated with the composition of the present invention, they should typically develop immunity against influenza.

[0053] The vaccine is useful for vaccinating people who are not recipients of organ transplants or bone marrow transplants. Subjects who are not recipients of organ transplants or bone marrow transplants would not normally have the need to receive a vaccine that includes antigens from *S. pneumoniae*. When subjects who are not recipients of organ transplants or bone marrow transplants are vaccinated with the composition of the present invention, they should typically develop immunity against influenza.

[0054] The vaccine is useful for vaccinating people who do not take medications that lower immunity. Subjects who do not take medications that lower immunity would not normally have the need to receive a vaccine that includes antigens from *S. pneumoniae*. When subjects who do not take medications that lower immunity are vaccinated with the composition of the present invention, they should typically develop immunity against influenza.

[0055] The vaccine is useful for vaccinating people who are less than 65 years old. Subjects who are less than 65 years old would not normally have the need to receive a vaccine that includes antigens from *S. pneumoniae*. When subjects who are less than 65 years old are vaccinated with the composition of the present invention, they should typically develop immunity against influenza.

[0056] Based on the observations that for a period of least two years the vaccine of the present invention reduces influenza morbidity in humans by at least 30% in comparison to the efficacy of Flu-V, and in view of the huge financial damage from influenza, administration of the vaccine would save to any nation's economy a lot of expenses.

[0057] The methods of the present invention are illustrated by the following examples, which are illustrative, but not limiting, of the combined preparations and methods of the present invention. Other suitable modifications and adaptations of a variety of conditions and parameters normally encountered in clinical prevention and therapy, obvious to those skilled in the art, are within the scope of this invention. All publications, patents, and patent applications cited herein are incorporated by reference in their entirety for all purposes.

EXAMPLES

[0058] It is to be understood that this invention is not limited to the particular methodology, protocols, subjects, or reagents described, and as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which is limited only by the claims. The following examples are offered to illustrate, but not to limit the claimed invention.

[0059] Two studies were performed. One study was done in animals (mice), to evaluate and compare the effect of PV and Flu-V on the phagocytic activity of mouse peritoneal cells, the mitogen response of mouse peripheral blood mononuclear cells and splenocytes to Con A, as well as to assess the peripheral blood counts before and after vaccination. Example 1 below describes a study with mice that was carried out in two phases: phase 1, involving 24 animals, followed by phase 2, with 60 mice and slight changes in the protocol, as detailed below. Example 2 describes an observational study in humans to verify the potency of PV to prevent influenza and influenza-related diseases.

Example 1. Study in Animals.

[0060] *Vaccines.* Pneumovax (PV), a 23-valent pneumococcal vaccine containing 25 µg of the caps-PS (Danish nomenclature) serotypes 1-5, 6B, 7F, 8, 9N, 10A, 11A, 12F, 14, 15C, 19A, 20, 22F, 23F, and 33F, as well as the influenza vaccine, Inluvac, Flu-V, 2002-2003, were obtained from Aventis Pasteur, Brussels, Belgium.

[0061] *Mice.* The Animal Investigation Committee of the Rabin Medical Center, Petah Tiqva, Israel, approved the study. Precautions were taken to ensure that the animals did not suffer unduly during and after the experimental procedures.

[0062] Phase 1: Twenty-four, two month-old Balb/c female mice were included in the study. The animals were divided in four groups, containing six animals each. Group I served as control. The mice from group II were immunized by intraperitoneal administration with 500 µl PV diluted 1/25 in 0.9% NaCl. The animals from group III were injected IM with 0.1 ml of Flu-V, diluted 1/200 in 0.9% NaCl two weeks after the onset of the study. The mice from group IV received PV at above specified doses and fourteen days later they were further immunized with Flu-V as indicated.

[0063] Phase 2: Sixty mice divided into six groups (10 mice each) were included in this experimental phase. Groups I to IV were treated as described above. The mice in group V were immunized with Flu-V at the onset of the study as indicated and animals in group VI were immunized at the onset of the study with both Flu-V and PV as detailed above. Laboratory examinations were carried out four weeks from the beginning of the study. The animals were kept at room temperature, standard diet and given fluids ad libitum.

[0064] *Cells.* Blood samples were collected from the tail vein and cell counts were done using a Technicon H-2 cell counter (Bayer, Germany). Peripheral blood mononuclear cells (PBMC) were isolated using Histopaque gradient centrifugation (Sigma) and suspended in RPMI-1640 medium containing 1% penicillin, streptomycin, and nystatin, and supplemented with 10% fetal calf serum (designated as complete medium-CM).

[0065] A cell suspension of splenocytes was obtained by mincing the spleens through a fine stainless steel mesh. The cells were suspended in RPMI-1640 containing 1% penicillin, streptomycin and nystatin, and supplemented with 10% fetal calf serum. The cells' viability tested by trypan blue dye exclusion was over 95%.

[0066] *Phagocytosis of latex particles.* To achieve conditions closest to the physiological ones, a method for "in vivo incubation" of the macrophages with latex particles, was applied (Djaldetti *et al.*, 1997, *Acta Haematol* 98: 56-57). In short, one ml of 5% suspension of uniform polystyrene latex particles (0.8 μm in diameter, Difco, Detroit, Mich.) in PBS was injected into the peritoneal cavity of each animal. After 60 min, 4.5 ml of endotoxin free physiological saline were injected into the peritoneal cavity of the animals. After approximately 2 min, 3-4 ml of peritoneal fluid were withdrawn, the cells were sedimented by centrifugation at 250 xg for 10 min, smeared on glass slides and stained using the May Grünwald-Giemsa method.

[0067] In phase 2 of the study, phagocytosis was examined in vitro. Briefly, peritoneal cells were collected as described above, the cells were counted and suspended at $3 \times 10^6/\text{ml}$ CM and incubated with 0.1 ml of 5% latex suspension for 60 min at 37°C in atmosphere containing 5% CO₂. After incubation the cells were sedimented by centrifugation at 250 xg for 10 min, washed twice in PBS and smeared on glass slides and stained using the May-Grünwald-Giemsa method.

[0068] At both stages the cells that engulfed latex particles were counted using a light microscope. At least 200 cells from each animal were counted.

[0069] *Mitogen response.* 0.1 ml aliquots of PBMC suspension or splenocytes ($2 \times 10^6/\text{ml}$ CM) were divided into each well of 96 well plates containing 0.1 ml of Concanavalin A (Con A, 10 $\mu\text{g}/\text{ml}$, Sigma). Cultures, set up in triplicates, were incubated for 3 days. ³H-TdR (methyl-³H-thymidine, 5 Ci/mmol, Amersham, England) (1 $\mu\text{Ci}/\text{well}$) was added 18 hours before harvesting. Radioactivity was measured with LKB liquid scintillation counter model 3380.

[0070] *NK cell cytotoxicity.* Cytotoxicity was evaluated by the standard chromium specific release assay with ^{51}Cr labeled Yac 1 cells being used as target cells whereas splenocytes or PBMC served as effector cells. Final effector to target (E:T) ratios for splenocytes varied from 200:1 to 25:1 and for PBMC 100:1. The supernatants from the ^{51}Cr -labeled target cells incubated with effector cells for 4 h at 37°C were collected, and the radioactivity was detected in a gamma counter (LKB). All reactions were carried out in triplicates and the specific ^{51}Cr release was calculated as described previously (D'Alessandro *et al.*, 2004, *Indian J Med Res* 119, Suppl: 108-114).

[0071] *Statistics.* Statistical analysis was performed using one-way analysis of variance (ANOVA) and the paired or independent t-test. The results are expressed as a mean \pm SEM.

[0072] Following immunization with both vaccines the animals behaved normally during both phases of the study and did not show any visible signs of discomfort. The effect of immunization on the peripheral blood cell counts is shown in Table 1.

Table 1. Effect of immunization on the peripheral blood cell counts (Phase 1 and Phase 2)

Group	Hemoglobin, gr%		WBC x 10 ⁶ /ml		Platelets x 10 ⁹ /ml		Polymorpho. %		Lymphocytes %	
	Ph.1	Ph.2	Ph.1	Ph.2	Ph.1	Ph.2	Ph.1	Ph.2	Ph.1	Ph.2
Control	14.2±0.2	17.6±0.2	12.9±1.6	13.9±0.3	0.92±0.1	1.07±0.02	1.4±0.2	0.93±0.1	92.8±1.0	94.3±0.3
PV	15.7±0.2	17.8±0.3	14.4±0.7	13.2±0.6	1.05±0.1	1.05±0.02	1.0±0.1	1.11±0.1	91.9±0.9	93.1±0.4
Influ-v	14.9±0.4	18.0±0.3	11.1±0.4	16.6±0.9*	1.00±0.1	1.12±0.02	1.1±0.1	0.84±0.1	92.0±0.6	93.7±0.4
PV+ Influ-v	15.0±0.2	17.3±0.3	12.3±0.6	16.6±0.6**	0.97±0.1	1.05±0.04	1.2±0.1	0.91±0.1	91.3±1.1	92.8±0.5*
Influ-v (4w)	17.8±0.4		16.5±0.9*		1.06 ±0.02		1.08±0.1		94.5±0.4	
PV (4w) + Influ-v (4w)	18.5±0.3		14.3±0.7		1.12±0.03		0.9±0.1		93.0±0.4	

PV- Pneumovax vaccine; **Influ-v-** Influenza vaccine; **(w)** - weeks;

*p < 0.05; **p < 0.01

[0073] In phase 1, there was no difference in the hemoglobin values in animals from the four groups. On the other hand, the white blood cell (WBC) counts showed a slight, but significant increase in animals of groups II and IV, i.e. those treated with PV and with both PV followed by Flu-V, as compared with mice treated with Flu-V only ($14.4 \pm 0.7 \times 10^6/\text{ml}$; $12.3 \pm 0.6 \times 10^6/\text{ml}$ and $11.1 \pm 0.4 \times 10^6/\text{ml}$, respectively, $p < 0.002$; $p < 0.05$). The platelet number from animals in all groups did not show any significant changes.

[0074] In phase 2, the hemoglobin level and platelet counts did not differ significantly in animals from all groups. However, the WBC counts were significantly higher in groups II, III and IV (16.6 ± 0.9 , 16.6 ± 0.6 and 16.5 ± 0.9 , respectively), as compared with the controls (13.9 ± 0.3 , $p < 0.015$; $p < 0.005$ and $p < 0.02$ respectively). Four weeks after immunization with PV or with both PV and Flu-V the WBC count was similar to that of control animals (13.2 ± 0.65 and 14.3 ± 0.75 respectively). The percentage of lymphocytes was significantly lower in animals from group IV as compared with control mice (92.8 ± 0.46 vs. 94.3 ± 0.3 and $p < 0.02$).

[0075] *Phagocytosis.* This was monitored in both phase 1 and phase 2.

[0076] Phase 1. Figure 1 shows the phagocytic capacity of peritoneal cells from mice from all groups. A significant decrease in the engulfing ability of peritoneal cells from mice treated with PV was found (8.8 ± 2.3) as compared with cells from animals in the control group (23.9 ± 6) or those injected either with influenza vaccine or with pneumovax followed by influenza vaccine (21.4 ± 2.8 and 27.0 ± 5.9 respectively, $p < 0.05$).

[0077] Phase 2. A significant decrease in the engulfing ability of peritoneal cells was found two weeks after injection of mice with influenza vaccine (15.7 ± 1.7 , $p < 0.03$) compared with cells from animals in the control group (23.8 ± 2.8). The phagocytic capacity of peritoneal cells from mice in all other groups was similar to that of animals from the control group (Figure 1). Four weeks after immunization with PV, Flu-V, with both PV and Flu-V or PV followed by Flu-V the percentage of peritoneal cells that engulfed latex particles was 19.36 ± 1.8 , 20.8 ± 1.1 , 25.3 ± 3.1 and 22.6 ± 3.8 respectively.

[0078] Neutrophils provide the first line of defense against the influenza virus. Following incubation of human polymorphonuclear leukocytes with influenza virus at 0°C, the viral particles bind to the cell membrane, but they disappear rapidly at 37°C, being ingested by cytoplasmic vesicles via endocytosis (Yamamoto *et al.*, 1989, *J Med Microbiol* 28: 191-198). Therefore, it is conceivable that certain macrophage functions, including their phagocytic capacity may be altered. Impaired phagocytic activity of mouse peritoneal granulocytes incubated with antigen obtained from A/Scotland/74 and APR-8 influenza viruses has already been reported (Kowalska *et al.*, 1987, *Arch Immunol Theor Exp Warsz* 35: 453-456; Szydłowska *et al.*, 1987, *Arch Immunol Theor Exp Warsz* 35: 457-461). Incubation with influenza virus caused a decrease in phagocytic activity of human polymorphonuclear leukocytes (Henricks *et al.*, 1985, *Scand J Immunol* 22: 721-725) and monocytes (Gardner *et al.*, 1982, *J Reticuloendothel Soc* 32: 443-448).

[0079] Figure 1 shows the results of phagocytic activity of mouse peritoneal cells following inoculation with PV and Flu-V, separately and jointly during the two phases of the study, where * $p < 0.05$. There was a decrease in the engulfing capacity of the peritoneal macrophages at both phases of the study following immunization with either PV or Flu-V (Figure 1).

[0080] *Mitogen response.* The mitogen response of mouse peripheral blood mononuclear cells following inoculation with PV and Flu-V, separately and jointly during the two phases of the study, is shown in Figure 2, where: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

[0081] Phase 1. The proliferative response of PBMC to Con A was significantly higher in animals injected with PV as compared with the control group (20754±1995 vs. 13468±913 respectively, $p < 0.01$) and with animals immunized with Flu-V (13640±990, $p < 0.01$) (Figure 2). The mitogen response further increased in mice immunized with PV followed by Flu-V (26105±1065, $p < 0.001$ vs. control and Flu-V injected animals and $p = 0.053$ vs. PV only).

[0082] Phase 2. The proliferative response of PBMC to Con A was significantly higher in animals 4 weeks after immunization with PV or Flu-V as

compared with the control group (10156 ± 898 , 9811 ± 979 vs. 6406 ± 424 respectively, $p < 0.015$, Figure 2). In comparison to control animals, the mitogen response was higher in mice immunized with both PV and influenza vaccines (8481 ± 982 , $p = 0.068$). The proliferative response to Con A was higher in mice immunized with PV followed by injection of Flu-V (7335 ± 765 , $p = 0.052$), when compared to that of mice two weeks after injection of Flu-V only (5434 ± 505), but did not differ significantly from the control group.

[0083] The mitogen response of splenocytes following inoculation with PV and Flu-V separately and jointly during phase 2 of the study, is shown in Figure 3, where $*p < 0.05$; $**p < 0.01$.

[0084] The proliferative response of splenocytes to Con A was significantly higher 4 weeks after PV administration (3092 ± 241) and was further increased in mice immunized with PV followed by Flu-V (4292 ± 611) as compared with control animals (2172 ± 533 , $p < 0.02$, $p < 0.005$, respectively), as shown in Figure 3. Four weeks after immunization with both PV and Flu-V, the mitogen response of splenocytes to Con A was significantly elevated (3333 ± 338) as compared with control mice ($p < 0.015$) or with mice injected with Flu-V only (2482 ± 152 , $p < 0.005$). However, it was similar to that of mice immunized with PV only or mice immunized with PV followed by Flu-V.

[0085] *NK cell cytotoxicity.* The NK cell activity of splenocytes and PBMC was similar in all groups of animals at the various effector to target ratios tested. At 100:1 ratio, the percentage of NK cell cytotoxicity of the splenocytes was 19.4 ± 0.8 ; 20.9 ± 1.8 ; 21.4 ± 0.8 and $19.7 \pm 0.7\%$ in mice from groups I, II, III, and IV, respectively, and that of PBMC was 23.9 ± 0.7 ; 24.7 ± 1.0 ; 25.4 ± 0.6 and 25.1 ± 1.3 , respectively. NK cell cytotoxicity was not determined in phase 2 of the study since no difference was found in phase 1.

[0086] While the mitogen response of the PBMC was increased after immunization with PV at both phases of the study, it did not differ from that of the controls in animals inoculated with Flu-V. However, the highest response was obtained after inoculation with both PV and Flu-V. On the other hand, the proliferative response of the splenocytes was increased following inoculation

with both vaccines given either separately or jointly. NK cell cytotoxicity did not show any alteration following injection of either PV or Flu-V, or with both vaccines together. This effect can be explained by the ability of NK cells to recognize virus-infected cells at the early stage of infection.

[0087] Both vaccines, PV and Flu-V, were able to induce a comparable immune response in mice, at least regarding the parameters hereby examined. Moreover, in the first phase of the study the mitogen response of the PBMC to Con A was elevated after inoculation with PV, as well as PV followed by Flu-V, and not after administration of Flu-V only. It has been reported that vaccination against pneumococcal infections exerted an additional effect with influenza vaccination in reducing hospitalization for chronic lung diseases, whereas vaccination with influenza vaccine alone did not achieve this effect (Christenson *et al.*, 2004, *Eur Respir J* 23: 363-368).

Example 2. Study in Humans.

[0088] *Subjects.* A total of 450 individuals, 259 females and 191 males with a mean age of 64.9 ± 7.75 years (range 50-85) were enrolled in the study. Details concerning subjects' condition were extracted from the computerized medical file database of Leumit Health Fund that provides health insurance and services to about 10 percent of the Israeli population whose physicians use disease classification based on ICD-9-CM2004 coding according to the International Classification of Diseases. The participants were allocated to one of the following four groups: group A- individuals who did not receive any vaccination in the course of the study period between 1999-2003; group B included subjects who received PV at year 1999; group C consisted of subjects who during the months September-October, when most influenza cases occur, have been vaccinated with influenza vaccine every year throughout the study period; group D - subjects who received both PV once in 1999 and influenza vaccine each year during the study period. The mean number of chronic diseases per individual was calculated for each of the four groups. These conditions included cardiovascular diseases, hypertension,

diabetes, hyperlipidemia chronic lung diseases, chronic renal failure and malignancies.

[0089] Subjects who received medical help because of diseases relevant to the study were assigned to three groups: "influenza-related", including influenza and flu-like conditions; "pneumonia related", including pneumococcal pneumonia, pneumonia of other bacterial origin and viral pneumonia; and other respiratory tract infections hereby designated as "other diseases".

[0090] *Vaccines.* Pneumovax (PV), a 23-valent pneumococcal vaccine containing 25 µg of the caps-PS (Danish nomenclature) type 1-5, 6B, 7F, 8, 9N, 10A, 11A, 12F, 14, 15C, 19A, 20, 22F, 23F, and 33F was obtained from Aventis Pasteur (Brussels, Belgium). The type of influenza vaccine (Flu-V) administered each study year was the one prepared according to instructions given by WHO.

[0091] *Statistics.* Statistical analysis was carried out using the Student's t-test for continuous variables and chi-square for categorical variables. All tests were two-tailed. To control the rate of subjects' morbidity, i.e. the subject being ill once or more and for potential confounders, such as age, gender and co-morbidity, a logistic regression model was used.

Table 2. Demographic characterization of subjects included in the study

	Group A No vaccination	Group B PV	Group C Flu-V	Group D PV + Flu-V
No. of subjects	99	167	93	91
F/M	51/48	105/62	54/39	49/42
Age	62±9	67±7 ^a	63±8	66±7 ^b
Chronic conditions	1.98±1.4	2.03±1.4	1.78±1.2	2.23±1.4

^a Significantly different from group A and group C (p<0.001)

^b Significantly different from group A and group C (p<0.02 and p<0.004, respectively)

[0092] Table 2 shows that subjects from groups C and D were older than those from the other two groups, the difference being statistically significant. The presence of chronic conditions did not differ in the four groups.

[0093] The number of subjects from the four groups who required medical help because of influenza-related, pneumonia related, or other diseases is detailed in Table 3. In the year 2000, both the number and percentage of non-vaccinated subjects (group A) who developed one of the above-mentioned diseases was significantly higher than those in subjects from the remaining three groups ($p < 0.01$). PV-immunized subjects showed lower morbidity than those of the Flu-V immunized individuals ($p < 0.05$). During the next two years, only individuals immunized with PV showed significantly lower morbidity compared to that of subjects from the other groups ($p < 0.05$). The number of ill subjects in the four groups during the year 2003 was similar.

Table 3. Total number and percentage of subjects from the four groups who contracted influenza related, pneumonia related, or other diseases

	Group A No vaccination		Group B PV		Group C Flu-V		Group D PV + Flu-V	
	No	%	No	%	No	%	No	%
No. of subjects	99		167		93		91	
2000	49	49.5 ^a	29	17.4 ^b	26	28	24	26.4
2001	52	53.0	55	32.9 ^c	65	70	59	64.8
2002	62	62.6	87	52 ^d	64	68.8	69	75.8
2003	51	51.5	115	68.8	71	76.3	63	69.2

^a Significantly higher than groups A, B, and C ($p < 0.01$)

^b Significantly lower than groups A ($p < 0.01$) and C ($p < 0.05$)

^c Significantly lower than groups A, C, and D ($p < 0.01$)

^d Significantly lower than groups A ($p < 0.05$), C, and D ($p < 0.01$)

[0094] Table 4 presents data on influenza-related diseases. During 2000 the morbidity of non-immunized subjects was significantly higher than that of individuals from the other three groups ($p < 0.02$). Throughout 2001 only individuals immunized with PV showed lower morbidity in comparison with individuals from the remaining three groups ($p < 0.05$). The morbidity of subjects from the four groups did not differ in the course of years 2002-2003.

Table 4. Number and percentage of subjects from the four groups who contracted influenza-related, pneumonia-related, or other diseases

	Group A No vaccination		Group B PV		Group C Flu-V		Group D PV + Flu-V	
	No	%	No	%	No	%	No	%
No. of subjects	99		167		93		91	
Influenza-related diseases								
2000	25	25.3	17	10.2 ^a	11	11.8 ^d	11	12.1 ^d
2001	25	25.3	21	12.6 ^{abc}	30	32.3	28	30.8
2002	29	29.3	41	24.6	33	35.5	33	36.3
2003	23	23.2	53	31.7	30	32.3	35	38.5
Pneumonia-related diseases								
2000	2	2	2	1.2	1	1.1	2	2.2
2001	1	1	5	3.0	8	8.6	6	6.6
2002	5	5	9	5.4	3	3.2	4	4.4
2003	3	3	9	5.4	6	6.5	3	3.3
Other								
2000	22	22.2	10	6.0 ^{ab}	14	15.1	11	12.1
2001	26	26.3	29	17.4 ^b	27	29.0	25	27.5
2002	28	28.3	37	22.2 ^c	28	30.1	32	35.2
2003	25	25.3	53	31.7	35	37.6	25	27.5

^a Significantly lower than group A ($p < 0.01$)

^b Significantly lower than groups C ($p < 0.02$)

^c Significantly lower than group D ($p < 0.05$)

^d Significantly lower than group A ($p < 0.02$)

[0095] Using logistic regression to control data concerning subjects' age, gender, and chronic diseases in 2000, it appears that PV vaccination did reduce the rate of influenza related diseases by 52% (OR=0.48; p=0.01). As for Flu-V, it exerted only a limited effect. Male individuals showed lower morbidity than that of females (OR=0.53; p=0.04). All chronic conditions exerted only a small and non-significant effect on the results. While in 2001 PV vaccination showed still a protective effect for the same variables (OR=0.62; p=0.047) this effect was not observed for in year 2002 (OR=0.95). During the same years, Flu-V-receiving individuals showed increased morbidity of influenza-related diseases (OR=2.1; p=0.001 and OR=1.6; p=0.004 respectively). As for 2003, the morbidity of subjects from the four groups did not show any difference.

[0096] Other diseases were also analyzed. During the year 2000 the morbidity of PV-vaccinated participants was lower than that of groups A and C (p<0.001 and p<0.02, respectively) and in year 2001 lower from that in group C (p<0.02). As for the next year the morbidity of PV-immunized subjects was lower in comparison with that of subjects from group D (p<0.05).

[0097] Applying logistic regression analysis to control the above-mentioned parameters, i.e. subjects' age, gender and chronic diseases, it was found that PV vaccination reduced the morbidity rate by 61% (OR=0.39; p=0.002) during the year 2000 only. No effect on morbidity was observed in Flu-V-immunized subjects.

[0098] The results of the study indicate that individuals who were vaccinated once with PV only, or with PV and Flu-V concomitantly in 1999, showed lower morbidity of influenza-related diseases during 2000 compared with non-vaccinated subjects. During the following years only individuals immunized with PV (group B) showed the same pattern, whereas the morbidity in individuals from group C and D did not differ from that of non-vaccinated subjects (group A). Although during the year 2002 people who received PV still showed morbidity lower than that of individuals from the other

three groups, the difference was not statistically significant. During the year 2003 both vaccines did not show any protective effect. Similar pattern was observed when the effect of both vaccines was analyzed in relation to other respiratory tract infections defined in the study as "other diseases".

[0099] It is to be understood that this invention is not limited to the particular devices, methodology, protocols, subjects, or reagents described, and as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which is limited only by the claims. Other suitable modifications and adaptations of a variety of conditions and parameters normally encountered in clinical prevention and therapy, obvious to those skilled in the art, are within the scope of this invention. All publications, patents, and patent applications cited herein are incorporated by reference in their entirety for all purposes.

CLAIMS

What is claimed is:

1. A vaccine composition for preventing influenza, comprising an amount of an antigen that causes an anti-influenza response, wherein said antigen is extracted from *Streptococcus pneumoniae*.
2. The vaccine composition of claim 1, wherein the antigen is extracted from at least 7 different serotypes of *Streptococcus pneumoniae*.
3. The vaccine composition of claim 1, wherein the antigen is extracted from 23 different serotypes of *Streptococcus pneumoniae*.
4. The vaccine composition of claim 1, wherein the antigen is extracted from serotypes 1, 2, 3, 4, 5, 6B (6A), 7F (7C), 8, 9N (9A), 9V, 10A, 11A (11B), 12F, 14, 15B (15A, 15C, 15F), 17F, 18C (18A, 18B), 19F (19B), 19A, 20, 22F (22A), 23 F (23A, 23B), and 33F (33C) of *Streptococcus pneumoniae*.
5. A method of vaccinating a subject against influenza comprising administering to the subject a preventatively effective amount of a vaccine composition that comprises an antigen extracted from *Streptococcus pneumoniae*.
6. The method of claim 5, wherein the subject is not at an increased risk of developing pneumonia.
7. The method of claim 5, wherein the subject has 4×10^9 to 11×10^9 white blood cells in a liter of blood.
8. The method of claim 5, wherein the subject is not immunocompromised.

9. The method of claim 5, wherein the subject has a functional spleen.
10. The method of claim 5, wherein the subject is allergic to an inactivated virus-based influenza vaccine.
11. The method of claim 5, wherein the subject is allergic to eggs.
12. The method of claim 5, wherein the subject does not have an autoimmune disease.
13. The method of claim 5, wherein the subject does not have cancer, leukemia, lymphoma, or multiple myeloma.
14. The method of claim 5, wherein the subject does not have an HIV infection.
15. The method of claim 5, wherein the subject is not diagnosed with cirrhosis of the liver.
16. The method of claim 5, wherein the subject is not a recipient of an organ transplant or a bone marrow transplant.
17. The method of claim 5, wherein the subject does not take a medication that lowers immunity.
18. The method of claim 5, wherein the subject is less than 65 years old.
19. The method of claim 5, wherein said administering is of a single dose.
20. The method of claim 5, wherein said administering is of doses administered less frequently than once a year.

21. The method of claim 5, wherein said administering is of doses administered once in two years, three years, four years, or five years.

22. A method of vaccinating a subject against influenza comprising administering to the subject a preventatively effective amount of a vaccine composition that comprises an antigen extracted from serotypes 1, 2, 3, 4, 5, 6B (6A), 7F (7C), 8, 9N (9A), 9V, 10A, 11A (11B), 12F, 14, 15B (15A, 15C, 15F), 17F, 18C (18A, 18B), 19F (19B), 19A, 20, 22F (22A), 23 F (23A, 23B), and 33F (33C) of *Streptococcus pneumoniae*.

FIGURE 1.

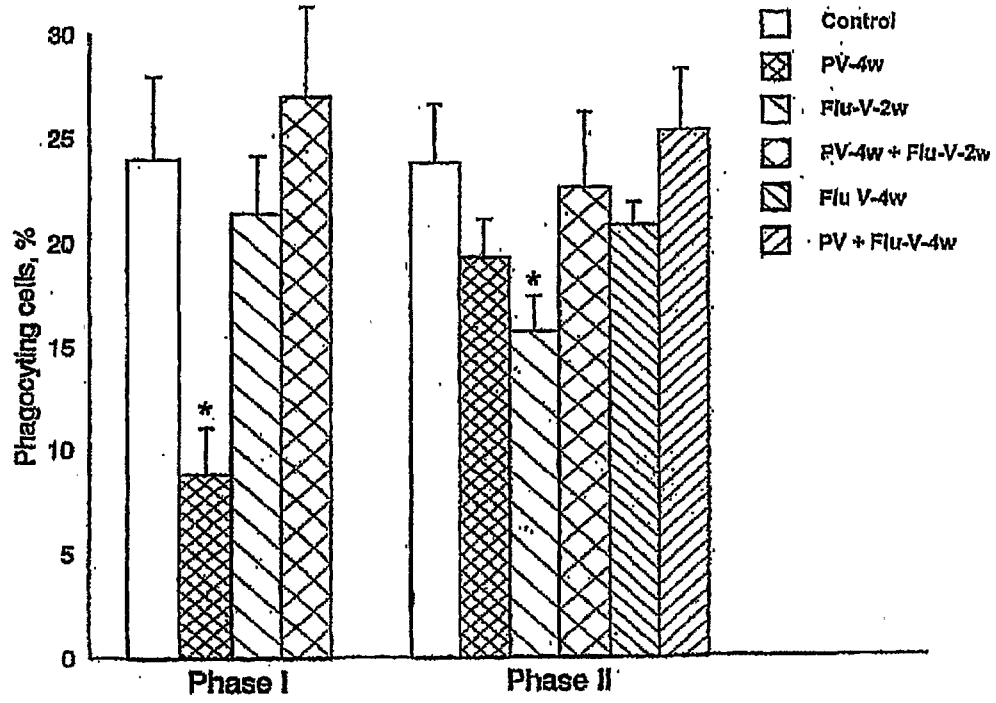


FIGURE 2.

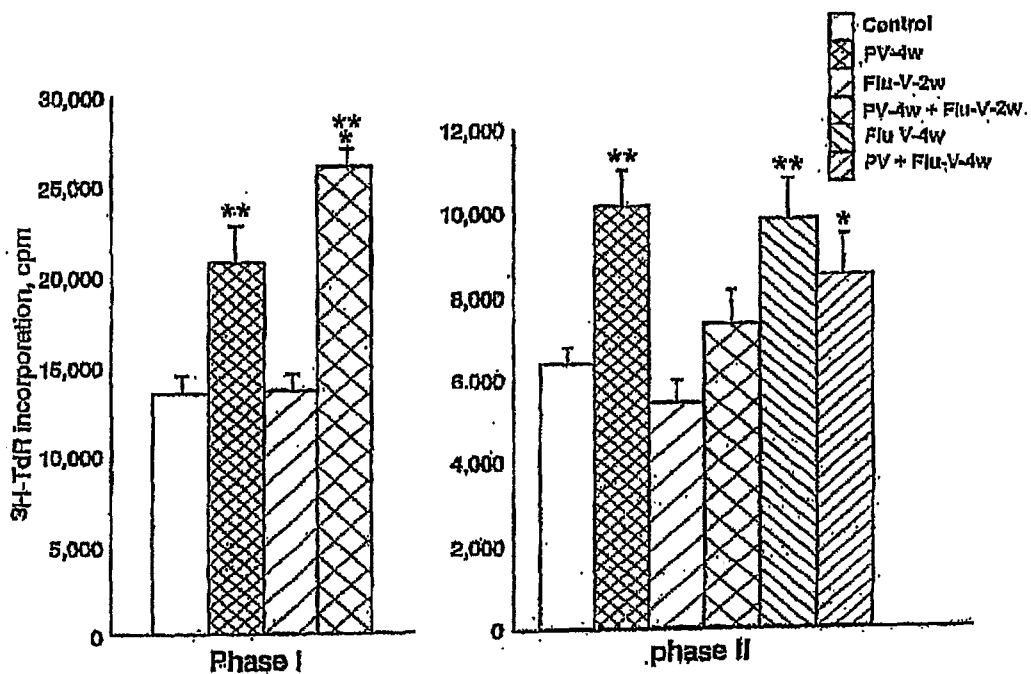


FIGURE 3.

