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(54) Title: VACCINE FORMULATION

(57) Abstract: Spore coat-associated proteins from members of *Bacillus* genera, and in particular spore-coat associated protein N (CotN), have utilisation as adjuvants in vaccine formulations. The vaccine formulations most likely contain a virulence factor of bacterial origin, which in the case of *Bacillus* genera is the protective antigen.

WO 2006/095176 A2

VACCINE FORMULATION

This invention relates to vaccine formulations comprising spore coat-associated proteins from members of *Bacillus* genera as adjuvants.

The genera *Bacillus*, within the family *Bacillaceae*, is a group of spore forming Gram-positive bacteria, of which *Bacillus anthracis*, the causative agent of anthrax, is a member. Anthrax is primarily a disease of domesticated and wild animals, with humans becoming infected incidentally on contact with infected animals. Forms of the disease include cutaneous anthrax, often acquired through open wounds, and inhalation anthrax, most commonly resulting from inhalation of anthrax spores. Spores are formed intacellularly by vegetative cells in response to environmental signals that indicate a limiting factor for vegetative growth, such as exhaustion of an essential nutrient. They have proven to be the most durable type of cell found in nature and can remain viable, in this state of dormancy, for long periods of time, perhaps millions of years. They germinate and become vegetative cells when the environmental stress is relieved. Hence, spore-formation is a mechanism of survival, rather than a mechanism of reproduction. The core of the spore is surrounded by a cell wall, the cortex, and then the spore coat. Depending on the species, an exosporium may be present. The outer spore coat represents 30-60 percent of the dry weight of the spore. The spore coat proteins have an unusually high content of cysteine and of hydrophobic amino acids.

Bacillus anthracis produces two virulence factors, a poly-D-glutamic acid capsule and a tripartite toxin, composed of protective antigen (PA) lethal factor (LF) and Edema factor. PA is composed of four distinct and functionally independent domains, and is also the key protective component in existing vaccines to protect against anthrax infection. Nasally delivered (recombinant) protective antigen is non-immunogenic unless delivered with a mucosal adjuvant.

Disease causing organisms contain proteins called antigens which stimulate the immune response. The resulting immune response includes the synthesis of proteins called antibodies. These proteins bind to the disease causing organisms leading to eventual destruction.

The first step in making a vaccine is to isolate or create an organism, or part of one, that is unable to cause full blown disease, but that still retains the antigen responsible for inducing a protective immune response. One way is to kill the organism, for example using formalin. Vaccines produced in this way are called "inactivated" or "killed" vaccines. Another way is to use the antigen itself, for example the capsule, the flagella, or part of the protein cell wall.

Vaccines can be made by attenuation or weakening of a live microorganism by ageing or altering the growth conditions. Examples of attenuated vaccines are those that protect against measles, mumps, and rubella.

Some vaccines are made from toxins. In these cases, the toxin is often treated or modified to reduce the harmful effect. The modified/treated toxin is called a toxoid. Examples of toxoids are the diphtheria and tetanus vaccines. Vaccines made from toxoids often induce low level immune responses and are therefore sometimes administered with an adjuvant, an agent used to boost the immune response.

Vaccines may be used therapeutically in response to an exposure, or suspected exposure, to a pathogen or they may be used prophylactically to provide protection to an individual before any exposure or potential exposure occurs. Accordingly, as used herein, the term "vaccine" includes both therapeutic and prophylactic vaccines.

To elicit a strong mucosal immune response, particularly for intranasally administered antigens, an adjuvant is required. One of the most effective mucosal adjuvants is cholera toxin from *Vibrio cholerae*. Cholera toxin is extremely toxic, even at low concentrations, and is unlikely to be licensed for use in human vaccines.

There is a requirement for effective mucosal adjuvants that are not based on toxins.

According to the present invention there is a vaccine formulation comprising a spore-coat associated protein from a member of *Bacillus* genera as adjuvant, preferably a spore-coat associated protein from a strain of *Bacillus cereus* or *Bacillus anthracis*.

The vaccine may be used as a therapeutic or prophylactic vaccine but it is preferred the vaccine is a prophylactic vaccine.

In a preferred embodiment the vaccine formulation is a live attenuated vaccine or an inactivated vaccine, and most preferably a subunit vaccine.

Adjuvants are known to increase the effectiveness of vaccines against a variety of diseases e.g. MPL™ (produced by Corixa™) adjuvant, a lipid A derivative from gram-negative bacteria, has been associated with vaccines against papillomavirus, herpes simplex virus, allergies, tuberculosis and various forms of cancer.

In one aspect of the invention, the vaccine formulation comprises a spore coat-associated protein from a member of Bacillus genera as adjuvant and an antigen.

Preferably, the antigen is from viral, plant or animal origin. More preferably, the antigen is capable of causing one or more diseases selected from papillomavirus, herpes simplex virus, pneumonic plague, allergies, and various forms of cancer including breast cancer and prostate cancer. Furthermore, the vaccine formulation can comprise a virulence factor of bacterial origin, preferably from a member of *Bacillus* genera, and most preferably from a strain of *Bacillus anthracis*. Alternatively, the virulence factor is from a tuberculosis causing bacteria. e.g. *M. tuberculosis*

The term 'virulence factor' denotes either an intact virulence factor, or a part thereof, throughout the entire document.

The virulence factor is preferably the protective antigen from a member of *Bacillus* genera, and most preferably the protective antigen from *Bacillus anthracis*.

In a further embodiment the virulence factor is a recombinant form of protective antigen, a mutant form of protective antigen, or a distinct and functionally independent domain of protective antigen.

The invention also discloses a vaccine formulation wherein the adjuvant and virulence factor are microencapsulated, or the adjuvant is microencapsulated, or preferably wherein the virulence factor is microencapsulated.

Delivery of the vaccine is by any suitable method. Preferably the delivery of the vaccine is by a non-parenteral route. More preferably, the delivery is by the intra-nasal route or an oral route.

Where the vaccine is suitable for oral administration e.g. in the form of a dragee, a tablet, a capsule, a spray, an aerosol, a liquid e.g. a syrup, a tincture (particularly when the pharmaceutical composition is solubilised in alcohol). The vaccine may be vaccine may be suitable for pulmonary administration e.g. in the form of an aerosol, a spray or an inhaler.

The vaccine of the invention may also be prepared in a solid form which is suitable for solubilising or suspending in a liquid. Preferably, the liquid is water or alcohol. The solid form can be a lyophilized composition or a spray freeze-dried composition. The solid form can be solubilised or suspended in liquid immediately prior to administration. Advantages of using lyophilized vaccines include economical savings because of cheaper transportation costs and easier storage conditions because the compositions tend to be more stable in a lyophilized state compared to being in solution. In such cases, the vaccine is preferably supplied as a kit that includes all or some of the components necessary for reconstitution into a form suitable for administration to a host organism. Such a kit may contain a mixture of forms, e.g. the vaccine could be in a lyophilized form and in addition, the kit would provide a liquid for solubilization or suspension of the lyophilised vaccine.

A "vaccine formulation", as used herein, can refer to either a prophylactic or a therapeutic formulation, that is, as well as protecting against disease, it is possible that the vaccine formulation can alleviate a symptom of a disease (i.e. act as a therapeutic).The present invention discloses any spore coat-associated protein from a member of *Bacillus* genera, preferably CotN from a member of *Bacillus* genera, and most preferably CotN from *Bacillus anthracis*.

The present invention also discloses the amino acid sequence for CotN from *Bacillus anthracis* as shown in SEQ ID NO. 1, and the nucleic acid sequence, SEQ ID NO 2, encoding for CotN from *Bacillus anthracis*.

Specific embodiments of the invention will now be described by way of example.

Example 1.

a) Single, intra-nasally delivered 75μg dose of recombinant protective antigen (rPA) microspheres administered plus 10μg 'free' CotN protein provided substantially higher antibody titres than microspheres administered without adjuvant.
b) These antibody titres were comparable to those seen in mice given microspheres administered with 0.2μg Cholera Toxin (CT) as an adjuvant.
c) Following challenge on day 128 post immunisation with 1000 MLD of anthrax strain STI spores given by the intra-peritoneal route, mice immunised with 75μg of microspheres alone, or with CT or CotN were all fully protected.

Example 2.

a) Single, intra-nasally delivered 50μg dose of 'free' rPA administered plus either 0.2μg of CT or 10μg of CotN provided comparable antibody titres.
b) Previous work has shown that a dose of up to 120μg of rPA delivered intra-nasally without adjuvant does not produce a detectable antibody response.
c) Mice were challenged with 100 MLD of anthrax strain STI spores given by the intra-peritoneal route on day 90 post immunisation and were all fully protected.

Example 3.

a) Single, intra-nasally delivered 25μg dose of rPA microspheres administered plus 10μg 'free' CotN protein provided substantially higher antibody titres then microspheres administered with 0.2μg CT as adjuvant or microspheres administered without adjuvant

b) Single, intra-nasally delivered 25μg or 10μg dose of 'free' rPA administered plus either 0.2μg of CT or 10μg of CotN provided comparable antibody titres. 25μg 'free' rPA administered without adjuvant did not produce a detectable antibody response.

c) Mice were challenged with 94 MLD of anthrax strain STI spores delivered by the aerosol route on day 80 post challenge. Mice immunised with 25 μg of rPA microspheres either alone or with CT or CotN were all fully protected against challenge.

d) Mice immunised with either 25μg or 10μg of 'free' rPA plus CotN had an 83% survival rate compared to 100% survival rate of mice immunised with CT as the adjuvant and a 0% survival rate of mice immunised without adjuvant.

A single dose of CotN administered intra-nasally with recombinant protective antigen (rPA) either as a microsphere (microencapsulated) formulation or as free protein enhances the immune response to the rPA antigen.

This immune response is comparable to that seen when the antigen is administered with the potent mucosal adjuvant CT and is substantially better then that seen when the antigen is delivered without adjuvant.

CotN as an adjuvant provided full protection against inject challenge and significantly better protection then control immunised mice against aerosol challenge with anthrax strain STI.

Example 4

The methods of the previous examples can be easily adapted by a skilled artisan for use with antigens other than rPA as may be required for vaccines that are effective against diseases other than anthrax.

The rPA of example 1, therefore, could be replaced by another antigen and the resulting vaccine would particular for that disease rather than anthrax.

A vaccine formulation in accordance with the present invention is one that shows comparable or better results to that demonstrated by the same antigen but with the CT adjuvant, e.g. as demonstrated in example 1, where antibody titres were compared.

CLAIMS

1. A vaccine formulation comprising a spore coat-associated protein from a member of *Bacillus* genera as adjuvant.
2. A vaccine formulation as claimed in Claim 1 wherein a member of *Bacillus* genera is *Bacillus cereus*.
3. A vaccine formulation as claimed in Claim 1 wherein a member of *Bacillus* genera is *Bacillus anthracis*.
4. A vaccine formulation as claimed in any preceding Claim is a live attenuated vaccine, or an inactivated vaccine.
5. A vaccine formulation as claimed in Claims 1-3 is a subunit vaccine.
6. A vaccine formulation as claimed in any preceding Claim incorporating a virulence factor of bacterial origin.
7. A vaccine formulation as claimed in Claims 1-5 incorporating a virulence factor from a member of *Bacillus* genera.
8. A vaccine formulation as claimed in Claims 1-5 incorporating a virulence factor from any strain of *Bacillus* anthracis.
9. A vaccine formulation as claimed in Claims 7-8 wherein the virulence factor is the protective antigen.
10. A vaccine formulation as claimed in Claims 7-8 wherein the virulence factor is a distinct and functionally independent domain of the protective antigen.
11. A vaccine formulation as claimed in Claim 9 wherein the protective antigen is a recombinant form of protective antigen.
12. A vaccine formulation as claimed in Claim 9 wherein the protective antigen is a mutant form of protective antigen.

13. A vaccine formulation as claimed in Claims 6-12 wherein the virulence factor is microencapsulated.

14. A vaccine formulation as claimed in Claims 1-13 wherein the adjuvant is microencapsulated.

15. A vaccine formulation as claimed in any preceding Claim wherein delivery of the vaccine is by any suitable method.

16. A vaccine formulation as claimed in Claims 1 –14 administered by the intra-nasal route.

17. A vaccine formulation as claimed in Claims 1-16 wherein the dose of adjuvant is of from about 1 ng to 100 μg.

18. A vaccine formulation as claimed in Claims 1-16 wherein the dose of adjuvant is of from about 100 ng to 10 μg.

19. A vaccine formulation as claimed in Claims 6-18 wherein the dose of virulence factor is of from about 1 ng to 1 mg.

20. A vaccine formulation as claimed in Claims 6-18 wherein the dose of adjuvant is of from about 100 ng to 100 μg.

21. A vaccine formulation as claimed in any preceding Claim wherein the spore coat-associated protein is spore coat-associated protein N (Cot N).

22. A vaccine formulation as claimed in Claim 21 wherein the spore-coat associated protein N (CotN) is spore-associated protein N (CotN) from *Bacillus anthracis,* the amino acid sequence of which is shown in SEQ ID NO. 1.

23. A vaccine formulation as claimed in Claim 22 wherein spore-associated protein N (CotN) from *Bacillus Anthracis* is encoded by a nucleic acid, the sequence of which is shown in SEQ ID No. 2.

24. Use of the vaccine formulation of any one of claims 1 to 23 for the treatment of anthrax infection.

25. Use of the vaccine formulation of any one of claims 1 to 5 for the treatment of one or more diseases selected from a group consisting of pneumonic plague, tuberculosis, allergies and cancer.

SEQUENCE LISTING

NUMBER OF SEQ ID NOS: 2

SEQ ID NO. 1

LENGTH: 197

TYPE: PROTEIN

ORGANISM: *Bacillus anthracis*

SQUENCE: 1

MSLKKKLGMGVASAALGLSLIGGGTFAYFSDKEVSNNTFAAGTLDLTLDPKT
LVDIKDLKPGDSVKKEFLLKNSGSLTIKDVKLATKYTVKDVKGDNAGEDFGK
HVKVKFLWNWDKQSEPVYETTLADLQKTDPDLLAQDIFAPEWGEKGGLEAG
TEDYLWVQFEFVDDGKDQNIFQGDSLNLEWTFNANQEAGEEK

SEQ ID NO. 2

LENGTH: 594

TYPE: DNA

ORGANISM: *Bacillus anthracis*

SEQUENCE: 2

gtgagtctga aaaagaaatt aggtatggga gttgcatcag cagcattggg gttatcttta attggtggag
gaacatttgc ttactttagc gataaagaag tatcgaacaa tacatttgca gctgggacgt tagatcttac attagaccct
aaaacgcttg tagatattaa agatttaaaa ccaggggatt ctgttaagaa agagttctta ttaaagaata gcggttcatt
aacaattaaa gacgttaaac tagcaacaaa gtatactgtg aaagatgtaa aaggtgataa tgctggtgaa
gactttggta agcacgttaa agtgaaattc ctttggaact gggataaaca aagtgagcct gtatatgaaa caactttagc
agacttacaa aaaactgatc cagatctttt agctcaagac atttttgctc ctgagtgggg ggaaaagggt
ggattagaag ctggtaccga ggattattta tgggtacaat ttgaatttgt agatgatgga aaagaccaaa atatcttcca
aggtgattca ttgaatttag aatggacatt caatgctaac caagaagctg gagaagaaaa ataa