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Prolactin as a vaccine adjuvant

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(54) Title: PROLACTIN AS A VACCINE ADJUVANT

(57) Abstract

The present invention relates to a composition for enhancing the immune response of an animal to an infectious disease vaccine wherein the composition comprises prolactin. Preferably, the composition is human prolactin and the animal to be vaccinated is, as well, human. The present invention further relates to a composition for enhancing the immune response of an animal to an infectious disease vaccine wherein the composition comprises prolactin cDNA. Human prolactin cDNA is preferred.

PROLACTIN AS A VACCINE ADJUVANT

Background of the Invention

The use of vaccines to prevent diseases in humans, farm livestock, sports animals and household pets is a common practice, and considerable effort has been, and is being, made to extend this practice to cover a more extensive array of diseases to which these patients are subject. For example, the use of rabies vaccine in animals is by now commonplace, and efforts are being made to obtain suitable vaccines to immunize animals against other diseases.

One problem that frequently is encountered in the course of active immunization is that the antigens used in the vaccine are not sufficiently immunogenic to raise the antibody titer to sufficient levels to provide protection against subsequent challenge or to maintain the potential for mounting these levels over extended time periods. Another problem is that the vaccine may be deficient in inducing cell-mediated immunity which is a primary immune defense against bacterial and viral infection.

In order to obtain a stronger humoral and/or cellular response, it is common to administer the vaccine in a formulation containing an adjuvant, a material which enhances the immune response of the patient to the vaccine. The most commonly used adjuvants for vaccines are oil preparations and alum. The mechanisms by which such adjuvants function are not understood, and whether or not a particular adjuvant preparation will be sufficiently effective in a given instance is not predictable.

In addition, with the advent of gene therapy it has been reported that some success has been accomplished with using genes or "naked DNA" as vaccines. However, as with some of the conventional vaccines, the immune response obtained was insufficient to afford immunization.

Accordingly, there is a need for additional effective adjuvant preparations which are suitable for potentiating vaccines for animals in general, and particularly in humans.

Summary of the Invention

The present invention relates to a composition for enhancing the immune response of an animal to an infectious disease vaccine wherein the composition comprises prolactin. Preferably, the composition is human prolactin and the animal to be vaccinated is, as well, human.

The present invention further relates to a composition for enhancing the immune response of an animal to an infectious disease vaccine wherein the composition comprises prolactin cDNA. Human prolactin cDNA is preferred.

In another aspect, the invention relates to a method of enhancing the immune response of a subject animal to an infectious disease vaccine comprising co-administering an effective amount of prolactin or prolactin cDNA along with a vaccine.

Detailed Description of the Invention

Definitions

As used herein, "prolactin" refers to a polypeptide obtained from tissue cultures or by recombinant techniques and other techniques known to those of skill in the art, exhibiting the spectrum of activities characterizing this protein. The word includes not only human prolactin (hPRL), but also other mammalian prolactin such as, e.g., mouse, rat, rabbit, primate, pig and bovine prolactin. The amino acid sequence of a recombinant hPRL is shown below as SEQ ID NO:1. The recombinant PRL (r-PRL) is preferred herein.

The term "recombinant prolactin", designated as r-PRL preferably human prolactin, refers to prolactin having comparable biological activity to native prolactin prepared by recombinant DNA techniques known by those of skill in the art. In general, the

gene coding for prolactin is excised from its native plasmid and inserted into a cloning vector to be cloned and then inserted into an expression vector, which is used to transform a host organism. The host organism expresses the foreign gene to produce prolactin under expression conditions.

As used herein, the term "adjuvant" has its conventional meaning, i.e., the ability to enhance the immune response to a particular antigen. Such ability is manifested by a significant increase in immune-mediated protection. Furthermore, the term "genetic adjuvant" refers to prolactin cDNA which comprises the complement to the DNA sequence encoding the prolactin protein as defined above. The sequence for prolactin cDNA is shown below as SEQ ID NO:2.

As used herein, the term "vaccine" refer to a composition of matter that comprises an antigen and at least capable of conferring an immune response, cell-mediated immunity against said antigen or a protective immune response to said antigen when administered to a human or animal subject.

The term "infectious disease vaccine" shall be taken to mean a vaccine as hereinbefore defined wherein the antigen component thereof comprises a live or killed infectious disease agent of the human or animal subject, such as a bacterial or viral pathogen or alternatively, an antigenic component of said infectious disease agent and wherein the immune response, protective immune response or cell-mediated immunity confers protection against said infectious disease agent. Accordingly, persons skilled in the art will be aware than an infectious disease vaccine is distinct from other vaccines which may confer immunity against any antigen.

General Method

Formulations containing prolactin for adjuvant purposes are most conveniently administered by intramuscular or subcutaneous injections or intraperitoneal although other methods of administration are possible.

Standard formulations are either liquid injectables or solids which can be taken up in suitable liquids as suspensions or solutions for injection. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, and so forth. Nontoxic auxiliary substances, such as wetting agents, buffers, or emulsifiers may also be added.

Sustained and continuous release formulations are of considerable variety and could be used in the method of the present invention, as is understood by those skilled in the art.

Prolactin can be administered separately from the vaccine or in combination with the vaccine. When prolactin is combined with the vaccine, the composition administered contains an immunogen that is effective in eliciting a specific response to a given pathogen or antigen, a pharmaceutically acceptable vaccine carrier and an immunopotentiating amount of prolactin. The vaccine will normally be administered per manufacturer's instructions. Other adjuvants may be administered either with the vaccine or together with the prolactin.

Prolactin will typically be used to enhance the protection afforded by animal or human vaccines that are considered "weak" (i.e., provide diminished protection in terms of level, extent, and/or duration). Examples of such vaccines are bacterins such as Pseudomonas Staphylococcal, Enterotoxin Streptococci, cytomegalovirus, HIV, Bordetella bacterin, Escherichia coli bacterins, Haemophilus bacterins, Leptospirosis vaccines, Moraxella bovis bacterin, Pasteurella bacterin and Vibrio fetus bacterin and attenuated live or killed virus products such as bovine respiratory disease vaccine (infectious bovine rhinotracheitis, parainfluenza-3, respiratory syncytial virus), bovine virus diarrhea vaccine, equine influenza vaccine, feline leukemia vaccine, feline respiratory disease vaccine (rhinotracheitis-calicipneumonitis viruses), canine parvovirus vaccine,transmissible gastroenteritis vaccine, pseudorabies vaccine, and rabies vaccine.

In addition, because we have demonstrated *in vitro* and *in vivo* data that indicate that prolactin can enhance the immune response to an immunogen and thereby function as a vaccine adjuvant, it is believed that the exogenous administration of the prolactin gene would result in the expression of prolactin *in vivo* which would be available to function as an adjuvant to any immunogen whether administered through conventional means or via gene inoculation. The "genetic adjuvant" could be produced by inserting prolactin cDNA into a DNA delivery vehicle (e.g., plasmid vectors, liposomes, viral vectors). This could be accomplished as described by *Pellegrini I., et al., Molec. Endocrinolgy, 6*, 1023 (1992), *Maniatis T., et al., Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Press* (1989) and *Felger P., et al., Proc. Natl. Acad. Sci., 84*, 7413, (1991). The "genetic adjuvant" is then administered along with either cDNA encoding the immunogen in an appropriate delivery vehicle or "naked" (i.e., solely the cDNA). In addition, the "genetic adjuvant" could be administered along with the immunogen itself. The injection sequence would be optimized per immunogen, i.e., the prolactin cDNA could be co-administered with the immunogen or immunogen cDNA, or administered in advance or subsequent to their administration. It is believed that the prolactin cDNA could be inserted into the same DNA delivery vehicle. Various routes of administration could be used.

EXAMPLE 1

Co-mitogenicity of recombinant human prolactin (r-hPRL)

Peripheral blood lymphocytes (PBL) were isolated from the blood of normal human volunteers by density gradient centrifugation on Ficoll Paque (Pharmacia). Heparinized blood was diluted 3 fold in phosphate-buffered saline (PBS) and centrifuged at 2000 rpm for 20 minutes. The buffy coat, located on the surface of the red blood cell pellet and consisting of white blood cells, was collected and diluted with an equal volume of PBS. The diluted buffy coat was layered on

Ficoll Paque (6 mls of buffy coat on 4 mls of Ficoll Paque in a 15 ml tube) and centrifuged for 30 minutes at 1400 rpm. The PBL layer, found at the Ficoll-plasma interface, was collected and the cells were washed three times in PBS. PBL were then resuspended at $2x10^6$/ml in serum-free AIM-V medium from Gibco and added to the wells of round bottom 96 well microtiter plates in a 100 μl volume ($2x10^5$ PBL/well).

A suboptimal dose of the T cell mitogen concanavalin A (Con A; 0.2 μg/ml) was added in a 50 μl volume together with 50 μl of varying concentrations of r-hPRL (0-1000 ng/ml final). Cultures were done in triplicate. The cells were incubated at 37°C/5% CO_2 for 72 hours and the amount of proliferation measured by tritiated thymidine incorporation. Tritiated thymidine (0.5μ Ci/well) was added for the last 18 hours of incubation and cell-associated radioactivity was measured by scintillation counting after harvesting the cells onto glass fiber filters using a Skatron 96 well cell harvester.

Results, obtained with cells from different individuals, shown in Table 1 below, indicated that r-hPRL was able to enhance the proliferative response of T lymphocytes to a suboptimal concentration of Con A. This co-mitogenic activity was best observed with r-hPRL concentrations of 1-10 ng/ml.

Table 1

Co-mitogenic activity of recombinant human prolactin (cpm +/- SEM)

Con A + r-hPRL (ng/ml)	Donor 1	Donor 2	Donor 3
No prolactin	22323±4585	35942±810	16549±1618
0.1	22949±2003	34040±1446	17083±1895
1	35882±3665	45839±2137	27590±3151
10	32832±1972	37658±150	22991±2358
100	25963±4855	35009±2105	22674±1662
1000	23990±1534	35921±1690	26646±2574

EXAMPLE 2

Enhancement of antigen-specific proliferation by r-hPRL

To test the ability of r-hPRL to enhance the proliferative response of human T cells to a specific antigen, PBL were incubated with various concentrations of r-hPRL and streptokinase, a common antigen to which most individuals are exposed. Cultures were performed in triplicate in the wells of 96 well round bottom microtiter plates and consisted of 100 µl PBL ($2x10^5$/well), 50 µl streptokinase (25 µg/ml final) and 50 µl of r-hPRL at varying concentrations (0-1000 ng/ml final). Proliferation was measured by tritiated thymidine incorporation after 6 days of culture at 37°C/5%CO_2.

The results, shown in Table 2 below, indicated that r-hPRL, at a concentration of 1 ng/ml, significantly enhanced streptokinase-induced proliferation.

Table 2

Effect of recombinant human prolactin on streptokinase-specific proliferation

Streptokinase + r-hPRL (ng/ml)	Proliferation (cpm +/- SEM)
No prolactin	31807±4235
0.1	30220±5448
1	50964±6469
10	35620±11318
100	36713±2230
1000	33494±7990

EXAMPLE 3

Effect of prolactin in enhancing the immune response to an immunogen

Twenty-four 150 gram male Sprague-Dawley rats were divided into 4 groups. The control group received an intraperitoneal injection of 10 µg BSA mixed with alum. The other 3 groups received intraperitoneal injections of 10 µg BSA mixed with alum along with either 180 µg prolactin, 375 µg prolactin or 750 µg prolactin. Tail vein bleeds were taken weekly for 4 weeks and the serum evaluated for antibody to BSA by a Radioimmunosorbent Assay (RIA). The animals were boosted after the 4th bleed with 10µg BSA mixed with alum. Tail vein bleeds were taken over a 7 week period to obtain serum which was evaluated for the development of antibody to BSA by RIA.

Bovine serum albumin (BSA)-specific proliferation of peripheral blood lymphocytes from rats immunized with BSA +/- r-hPRL

To measure the effect of r-hPRL on the cellular response of rats immunized with BSA, blood was collected from individual animals sacrificed 101 days after boosting. To isolate peripheral blood lymphocytes (PBL), blood samples were diluted 4 fold in the phosphate-buffered saline (PBS) and centrifuged at 2000 rpm for 20 minutes. The buffy coat was collected and contaminating red blood cells were removed by the addition of Tris-ammonium chloride lysis buffer followed by a 10 minute incubation at 37°C. PBL were then washed twice in PBS and resuspended at $5x10^6$/ml in RPMI-1640 medium supplemented with 100 u/ml penicillin, 100 µg/ml streptomycin, 20 mM Hepes buffer, 2 mM L-glutamine, $5x10^{-5}$ M 2-mercaptoethanol and 5% heat-inactivated fetal calf serum. PBL were added to the wells of flat bottom 96 well microtiter plates in a 100 µl volume ($5x10^5$ cells/well) and cultured in the presence of medium alone (background control) or 1000 µg/ml BSA added in a 100 µl volume. Cultures were done in

triplicate. Proliferation was measured by tritiated thymidine incorporation after 5 days of culture at 37°C/5% CO_2.

The results indicated that, overall, PBL rats immunized with BSA + 180 μg rhPRL displayed higher levels of BSA-specific proliferation than PBL from rats immunized with antigen alone. This observation suggests that r-hPRL may act to enhance the cellular component of the immune response to an immunizing antigen. Results are compiled in Table 3 below.

Table 3

BSA-specific proliferation of rat PBL (cpm +/- SEM)

101 days after boosting

Group	Background	BSA-specific response
BSA alone		
Rat 1	918 ± 35	1236 ± 100
Rat 2	559 ± 169	1392 ± 185
Rat 3	614 ± 51	930 ± 265
Rat 4	242 ± 21	2122 ± 257
BSA + 180 μg PRL		
Rat 1	426 ± 99	2552 ± 30
Rat 2	269 ± 18	756 ± 37
Rat 3	723 ± 185	4328 ± 77
Rat 4	676 ± 29	2023 ± 397

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Richards, Susan
Kaplan, Johanne
Moscicki, Richard

(ii) TITLE OF INVENTION: PROLACTIN AS ADJUVANT

(iii) NUMBER OF SEQUENCES: 2

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(v) COMPUTER READABLE FORM:
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(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Gosz, William G
(B) REGISTRATION NUMBER: 27,787
(C) REFERENCE/DOCKET NUMBER: GEN 4-2.0

(ix) TELECOMMUNICATION INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 351 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: human prolactin

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Thr Ile Gly Phe His Met Pro Arg Leu Cys His Glu Cys Lys Phe Arg
1 5 10 15

Met Thr Thr Arg Ala Asn Ser Leu Ala Thr Glu Phe His Met Pro Arg
20 25 30

Leu Ser Glu Gln Cys His Glu Cys Lys Phe Arg Met Thr Gly Glu Asn
35 40 45

Glu Arg Ala Thr Glu Asp Ser Tyr Met Asx Leu Ser Thr His Met Pro
50 55 60

Arg Leu Leu Cys Ser His Met Pro Arg Leu Asx Pro Met Arg Asn Ala
65 70 75 80

Glu Asn Thr Glu Arg Glu Asp Asp Glu Phe Ile Asn Ile Thr Ile Asn
85 90 95

His Met Ala Asn Pro Arg Glu Pro Arg Leu Ala Cys Thr Ile Asn Pro
100 105 110

Arg Leu Met Arg Asn Ala Ala Cys Cys Glu Ser Ser Ile Asn His Met
115 120 125

Pro Arg Leu Pro Glu Pro Leu Glu Asn Gly Thr His Leu Tyr Cys His
130 135 140

Glu Cys Lys His Met Pro Arg Leu Leu Pro Ile Cys Pro Gly Gly Ala
145 150 155 160

Ala Arg Cys Gln Val Thr Leu Arg Asp Leu Phe Asp Arg Ala Val Val
165 170 175

Leu Ser His Tyr Ile His Asn Leu Ser Ser Glu Met Phe Ser Glu Phe
180 185 190

Asp Lys Arg Tyr Thr His Gly Arg Gly Phe Ile Thr Lys Ala Ile Asn
195 200 205

Ser Cys His Thr Ser Ser Leu Ala Thr Pro Glu Asp Lys Glu Gln Ala
210 215 220

Gln Gln Met Asn Gln Lys Asp Phe Leu Ser Leu Ile Val Ser Ile Leu
225 230 235 240

Arg Ser Trp Asn Glu Pro Leu Tyr His Leu Val Thr Glu Val Arg Gly
245 250 255

Met Gln Glu Ala Pro Glu Ala Ile Leu Ser Lys Ala Val Glu Ile Glu
260 265 270

Glu Gln Thr Lys Arg Leu Leu Glu Gly Met Glu Leu Ile Val Ser Gln
275 280 285

Val His Pro Glu Thr Lys Glu Asn Glu Ile Tyr Pro Val Trp Ser Gly
290 295 300

Leu Pro Ser Leu Gln Met Ala Asp Glu Glu Ser Arg Leu Ser Ala Tyr
305 310 315 320

Tyr Asn Leu Leu His Cys Leu Arg Arg Asp Ser His Lys Ile Asp Asn
325 330 335

Tyr Leu Lys Leu Leu Lys Cys Arg Ile Ile His Asn Asn Asn Cys
340 345 350

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1100 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TGCCTCATTA ACTAACCACT CACATTAAAA GAAATATAAC ATATATATTA AAAATAATCA 60

TATCCTATAA TAATTAACTC ATCTAAAATA CAACCTACTG TACCATATAC TAACTGAATA 120

AGACTAGCAT TATTATTCAG GATAACTAAG TCCATAAGAT ATGTACCATA TTATACACAT 180

TTATAGCACG GATATTACTT ACTGGATATA CTTTGATCTA TCTTGATATT TATTATTCAA 240

AATACTACGT GATATATCGC ATGTCCCAAA CATGAACATC AAAGGATCGC CATGGAAAGG 300

GTCCCTCCTG CTGCTGCTGG TGTCAAACCT GCTGCTGTGC CAGAGCGTGG CCCCCTTGCC 360

CATCTGTCCC GGCGGGGCTG CCCGATGCCA GGTGACCCTT CGAGACCTGT TTGACCGCGC 420

CGTCGTCCTG TCCCACTACA TCCATAACCT CTCCTCAGAA ATGTTCAGCG AATTCGATAA 480

ACGGTATACC CATGGCCGGG GGTTCATTAC CAAGGCCATC AACAGCTGCC ACACTTCTTC 540

CCTTGCCACC CCCGAAGACA AGGAGCAAGC CCAACAGATG AATCAAAAAG ACTTTCTGAG 600

CCTGATAGTC AGCATATTGC GATCCTGGAA TGAGCCTCTG TATCATCTGG TCACGGAAGT 660

ACGTGGTATG CAAGAAGCCC CGGAGGCTAT CCTATCCAAA GCTGTAGAGA TTGAGGAGCA 720

AACCAAACGG CTTCTAGAGG GCATGGAGCT GATAGTCAGC CAGGTTCATC CTGAAACCAA 780

AGAAAATGAG ATCTACCCTG TCTGGTCGGG ACTTCCATCC CTGCAGATGG CTGATGAAGA 840

GTCTCGCCTT TCTGCTTATT ATAACCTGCT CCACTGCCTA CGCAGGGATT CACATAAAAT 900

CGACAATTAT CTCAAGCTCC TGAAGTGCCG AATCATCCAC AACAACAACT GCTAAGCCCA 960

CATCCATTTC ATCTATTTCT GAGAAGGTCC TTAATGATCC GTTCCATTGC AAGCTTCTTT 1020

TAGTTGTATC TCTTTTGAAT CCATGCTTGG GTGTAACAGG TCTCCTCTTA AAAAATAAAA 1080

ACTGACTCGT TAGAGACATC 1100

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A composition that enhances the immune response of an animal to an infectious disease vaccine, wherein the composition comprises prolactin and the infectious disease vaccine.

2. The composition of claim 1 wherein the prolactin is human prolactin.

3. The composition of claims 1 or 2 wherein the animal is a human.

4. The composition according to any one of claims 1 to 3 wherein the prolactin comprises an amino acid sequence selected from all or a portion of the amino acid sequence of SEQ ID NO:1.

5. A method of enhancing the immune response of a subject animal to an infectious disease vaccine comprising co-administering an effective amount of prolactin along with a vaccine.

6. The method of claim 5 wherein the prolactin is human prolactin.

7. A method for enhancing the immune response in accordance with claim 5 wherein the animal is a human.

8. The method of claim 5 wherein the prolactin comprises an amino acid sequence selected from all or a portion of the amino acid sequence of SEQ ID NO:1.

9. The composition according to any one of claims 1 to 4 when used to enhance the immune response of an animal to an infectious disease vaccine.

10. The composition according to any one of claims 1 to 4 or claim 9 substantially as hereinbefore described with reference to the Examples.

11. The method according to any one of claims 5 to 8 substantially as hereinbefore described with reference to the Examples.

DATED this FIFTEENTH day of OCTOBER, 1998

GENZYME CORPORATION
by DAVIES COLLISON CAVE
Patent Attorneys for the Applicants