(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 November 2014 (27.11.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/189654 A1

(51) International Patent Classification:
A61K 39/205 (2006.01) *A01N 63/00* (2006.01)
A61K 39/12 (2006.01) *C12N 7/04* (2006.01)

(21) International Application Number:
PCT/US2014/036168

(22) International Filing Date:
30 April 2014 (30.04.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/826,624 23 May 2013 (23.05.2013) US

(71) Applicant: **THOMAS JEFFERSON UNIVERSITY** [US/US]; 1020 Walnut Street, Philadelphia, PA 19107 (US).

(72) Inventors: **DIETZSCHOLD, Bernhard**; 3430 Goshen Road, Newtown Square, PA 19073 (US). **HOOPER, Douglas, Craig**; 2 Oregon Trail, Medford, NJ 08055 (US). **FABER, Milosz**; 258 W. Marshall Road, Lansdowne, PA 19050 (US).

(74) Agents: **MONACO, Daniel A.** et al.; Drinker Biddle & Reath LLP, One Logan Square, Ste. 2000, Philladelphia, PA 19103-6996 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WO 2014/189654 A1

(54) Title: CO-IMMUNIZATION WITH ATTENUATED RABIES VIRUS AND NON-RABIES ANTIGEN

(57) Abstract: Provided is a method of enhancing an antigen-induced long-lasting immune response in a host comprising administering to a host an effective amount of: (a) a nonpathogenic recombinant rabies virus comprising at least three copies of a mutated G gene, wherein said mutated G gene encodes a rabies virus glycoprotein wherein the glycoprotein amino acid 194 is serine and the glycoprotein amino acid 333 is glutamic acid, wherein said recombinant rabies virus does not express a foreign protein antigen; and (b) a non-rabies antigen that is not expressed by the rabies virus. Also provided are related compositions, kits and vaccines.

CO-IMMUNIZATION WITH ATTENUATED RABIES VIRUS AND NON-RABIES ANTIGEN

Cross Reference to Related Application

[0001] This application claims the benefit of U.S. Provisional Application No. 61/826,624, filed May 23, 2013, the entire disclosure of which is incorporated herein by reference.

Reference to Government Grant

[0002] The invention described herein was made with government support under grant nos. R21AI068837 and R01AI093666 awarded by the National Institutes of Health. The government has certain rights in this invention.

Field of the Invention

[0003] The invention relates to the field of biotechnology and immunology, and in particular to the utilization of recombinant attenuated rabies virus to immunize against rabies and to promote the development of immunity to co-administered non-rabies antigens.

Background of the Invention

[0004] Rabies virus (RV) is a non-segmented negative-strand RNA virus within the Rhabdoviridae family and lyssavirus genera. The RV genome is about 12-kb in size and encodes five monocistronic RNAs encoding the nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), the transmembrane glycoprotein (G), and the viral polymerase (L). The RV N protein encapsidates the viral RNA to form the ribonucleoprotein (RNP), which is the template for RNA transcription and replication by the viral polymerase-complex composed of the P and L proteins. The RV M bridges the RNP with the cytoplasmic domain (CD) of RV G in the host cell-derived viral membrane. The RV G mediates infection of the host cell. The main feature of rabies virus is neuroinvasiveness, which refers to its unique ability to invade the central nervous system (CNS) from peripheral sites.

[0005] Rabies virus is a promising vaccine vector able to induce humoral and cellular immune responses efficiently to foreign antigens. Recombinant live-viral vectors expressing foreign antigens efficiently induce potent cellular and humoral immune responses against the

expressed antigens. Because of low seroprevalence in the human population, RV is an excellent viral vector candidate. Methods for engineering the virus are well established, up to two foreign genes totaling 6.5 kb have been incorporated thus far, and foreign sequences are stably maintained. RV grows to high titers in cell lines approved for human vaccine production and manufacture is economical. *See*, Smith *et al.*, 2006, *Virology*, 353(2): 344–356. For example, replication-competent RV comprising heterologous nucleic acids sequences encoding the HIV-1 gp160 is described in WO 01/55330. Immunization with RV encoding bacterial, viral or cancer antigens, fused to at least a portion of the RV N protein or G protein is described in US Pat. Pub. 2008/0311147. Expression of HIV-1 Env or Gag results in potent immune responses directed against HIV-1 (Schnell *et al*,. 2000, *Proc. Natl. Acad. Sci USA* 97(7): 3544–3549).

[0006] The availability of reverse genetics technology, has allowed the modification of RV viral elements that account for pathogenicity and immunogenicity, and has made the systematic development of safer and more potent modified-live rabies vector feasible. For example, the pathogenicity of fixed RV strains (i.e., ERA, SAD) can be completely abolished for immunocompetent mice by introducing single amino acid exchanges in their G protein (Faber et al., 2005, *J Virol* 79:14141–14148). RVs containing a SADB19 G with an Arg_{333} → Glu_{333} mutation are nonpathogenic for adult mice after intracranial/intracerebral inoculation; an Asn_{194} → Ser_{194} mutation in the same gene prevents the reversion to pathogenic phenotype (Faber et al., 2005, *J Virol* 79:14141-14148; Dietzschold et al., 2004, *Vaccine* 23:518-524; US Pat. 7,695,724). The G gene containing both mutations has been designated as "GAS". The nucleotide sequence of the GAS gene is provided in US Pat. 7,695,724 as SEQ ID NO: 5, incorporated herein by reference. Using the GAS gene, the single and double GAS RV variants, SPBNGAS and SPBNGAS-GAS, respectively, were constructed (Faber et al., 2005, *J Virol* 79:14141-14148; Li et al., 2008, *Vaccine* 26:419-426). The introduction of a second G gene significantly improves the efficacy of the vaccine by enhancing its immunogenicity through higher expression of G (Faber et al., 2002, *J Virol* 76:3374-3381). Elevated G expression is associated with the strong up-regulation of genes related to the NFκB signaling pathway, including IFN-α/β and IFN-γ (Li et al., 2008, *Vaccine* 26:419-426) and increased cell death (Faber et al., 2002, *J Virol* 76:3374-3381). Furthermore, the presence of two G genes also decreases substantially the probability of reversion to pathogenicity because the nonpathogenic phenotype determined by GAS is

dominant over a pathogenic G that could emerge during virus growth in vivo or in vitro (Faber et al., 2007, *J Virol* 81:7041-7047).

[0007] A further improvement in recombinant RV safety is the highly attenuated triple RV G variant, SPBAANGAS-GAS-GAS (Faber et al., 2009, *Proc. Natl. Acad. Sci USA* 206(27):11300-11305). The SPBAANGAS-GAS-GAS, also referred to herein as "TriGAS," variant is completely nonpathogenic after intracranial infection of mice that are either developmentally immunocompromised (e.g., 5-day-old mice) or mice that have inherited deficits in immune function. The nucleotide sequence of the GAS gene is provided in US Pat. 7,695,724 as SEQ ID NO: 5.

[0008] Attenuated rabies viruses have been engineered to express foreign antigens in the context of the virus particle, generally with limited success in terms of inducing immunity to the foreign antigen.

[0009] The control of rabies in free-ranging animals, such as feral dogs, can only be achieved with rabies immunization and population control (contraceptive immunization for example). Bender *et al., Vaccine* 27(51):7210-7213 (2009) discloses administration to dogs of a commercial rabies vaccine and the GnRH immunocontraceptive vaccine GonaCon™. Notably, immune responses to GnRH in dogs receiving both vaccines were lower than in dogs receiving only GonaCon™. *See id.* at Fig. 2.

[0010] What are needed are recombinant RV-based compositions and immunization methods that provide for a vigorous immune response to non-rabies antigens that are not expressed by the recombinant RVs but are instead co-administered. In particular, what are needed are compositions and immunization methods for concurrent immunization against rabies virus and against non-rabies antigens, e.g., mammalian reproductive hormones, that provides for high levels of immunity to both immunogens.

Summary of the Invention

[0011] Provided is a method of enhancing an antigen-induced immune response in a host comprising administering to a host an effective amount of: (a) a nonpathogenic recombinant rabies virus comprising at least three copies of a mutated G gene, wherein said mutated G gene encodes a rabies virus glycoprotein wherein the glycoprotein amino acid 194 is serine and the glycoprotein amino acid 333 is glutamic acid, wherein said recombinant rabies virus

does not express a foreign protein antigen; and (b) a non-rabies antigen that is not expressed by the rabies virus.

[0012] In some embodiments the nonpathogenic recombinant rabies virus and the non-rabies antigen are administered simultaneously. In further embodiments, the nonpathogenic recombinant rabies virus is administered before the non-rabies antigen. In yet further embodiments the nonpathogenic recombinant rabies virus and the non-rabies antigen are administered in the same composition. In some embodiments the nonpathogenic recombinant rabies virus and the non-rabies antigen are administered in a different composition.

[0013] In some embodiments the antigen-induced immune response is long-lasting. In further embodiments the antigen-induced response lasts up to 21 days or up to 2 months. In yet further embodiments, the antigen-induced response lasts 21 days or more, or 2 months or more.

[0014] In some embodiments, the non-rabies antigen comprises at least one antigen selected from the group consisting of protein reproductive hormone antigens, cancer-associated antigens, viral antigens, bacterial antigens, and protozoal antigens. In some embodiments, the protein reproductive hormone antigen is selected from the group consisting of gonadotropin releasing hormone and zona pelucida protein 3 (ZP3). In yet further embodiments, the cancer-associated antigen is selected from the group consisting of AIM-2, alphafetoprotein, bladder cancer-associated protein, breast carcinoma associated MAR-binding proteins p90 and p70, breast cancer–associated protein 3, carcinoembryonic antigen, CA-125, epithelial tumor antigen, c-erb/B2, gp100, HER-2, IL13Rα2, MAGE-1, MART-1, melanoma-associated antigens, MUC-1, MCU-2, prostate-associated antigen, abnormal products of p53, abnormal products of ras, survivin, tyrosinase, TRP-2 and tyrosine kinase 2.

[0015] In some embodiments, the non-rabies antigen is a viral antigen. In further embodiments, the viral antigen is an antigen of a virus selected from the group consisting of cytomegalovirus, Dengue, Ebola virus, equine encephalitis virus, hepatitis virus, HIV, Hendra virus, herpes simplex virus, human papilloma virus, influenza, Japanese Encephalitis virus, neurotropic viruses, Nipah virus, Rift Valley Fever virus and West Nile Virus. In yet further embodiments, the viral antigen is an antigen of Nipah virus G gene. In some embodiments, the non-rabies antigen is an antigen of a pathogen which is associated with chlamydia, diphtheria, pertussis, tetanus, tuberculosis, nontuberculous mycobacteria-

associated disease, bacterial and fungal pneumonias, babesiosis, cholera, typhoid, plague, shigellosis, salmonellosis, Legionnaire's Disease, Lyme disease, malaria, hookworm, onchocerciasis, schistosomiasis, trypanosomiasis, leshmaniasis, giardiasis, amoebiasis, filariasis, borreliosis, or trichinosis.

[0016] In some embodiments the nonpathogenic recombinant rabies virus and the non-rabies antigen are administered simultaneously. In further embodiments the nonpathogenic recombinant rabies virus and the non-rabies antigen are administered in the same composition. In some embodiments the nonpathogenic recombinant rabies virus and the non-rabies antigen are administered in a different composition.

[0017] In some embodiments, the nonpathogenic recombinant rabies virus and the non-rabies antigen are administered simultaneously once. In further embodiments the nonpathogenic recombinant rabies virus and the non-rabies antigen are administered simultaneously more than once.

[0018] In some embodiments, the non-rabies antigen is administered as a booster once after the first co-administration of the nonpathogenic recombinant rabies virus and the non-rabies antigen. In further embodiments the non-rabies antigen is administered as a booster more than once after the first co-administration of the nonpathogenic recombinant rabies virus and the non-rabies antigen. In yet further embodiments, the non-rabies antigen is administered as a booster at 3 weeks, 3 months and 9 months after the first co-administration of the nonpathogenic recombinant rabies virus and the non-rabies antigen.

[0019] In some embodiments the nonpathogenic recombinant rabies virus and the non-rabies antigen are administered intranasally, subcutaneously, epidermaly, intradermally, intrathecally, intraorbitally, intramucosally, intraperitoneally, intravenously, orally, or intramuscularly. In preferred embodiments, the nonpathogenic recombinant rabies virus and the non-rabies antigen are administered intramuscularly.

[0020] Also provided is a composition comprising: (a) a nonpathogenic recombinant rabies virus comprising at least three copies of a mutated G gene, wherein said mutated G gene encodes a rabies virus glycoprotein wherein the glycoprotein amino acid 194 is serine and the glycoprotein amino acid 333 is glutamic acid, wherein said recombinant rabies virus

does not express a foreign protein antigen; and (b) a non-rabies antigen that is not expressed by the rabies virus.

[0021] Also provided is a vaccine comprising the a composition comprising: (a) a nonpathogenic recombinant rabies virus comprising at least three copies of a mutated G gene, wherein said mutated G gene encodes a rabies virus glycoprotein wherein the glycoprotein amino acid 194 is serine and the glycoprotein amino acid 333 is glutamic acid, wherein said recombinant rabies virus does not express a foreign protein antigen; and (b) a non-rabies antigen that is not expressed by the rabies virus. In some embodiments the composition is adjuvant-free.

[0022] Provided is a kit for immunization of a subject comprising: (a) a first composition comprising a nonpathogenic recombinant rabies virus comprising at least three copies of a mutated G gene, wherein said mutated G gene encodes a rabies virus glycoprotein wherein the glycoprotein amino acid 194 is serine and the glycoprotein amino acid 333 is glutamic acid, wherein said recombinant rabies virus does not express a foreign protein antigen; and (b) a second composition comprising a non-rabies antigen that is not expressed by the rabies virus. In some embodiments the first composition is adjuvant-free.

[0023] Provided is a method of immunizing a subject against rabies virus and against a non-rabies virus antigen comprising the steps of: (a) administering a first composition comprising a nonpathogenic recombinant rabies virus comprising at least three copies of a mutated G gene, wherein said mutated G gene encodes a rabies virus glycoprotein wherein the glycoprotein amino acid 194 is serine and the glycoprotein amino acid 333 is glutamic acid, wherein said recombinant rabies virus does not express a foreign protein antigen; and (b) administering a second composition comprising a non-rabies antigen that is not expressed by the rabies virus. In some embodiments the first composition is adjuvant-free.

[0024] In some embodiments, the non-rabies protein antigen comprises a protein reproductive hormone antigen, a prion protein antigen, a cancer-associated antigen, a viral antigen, a bacterial antigen, or a protozoal antigen.

[0025] In some embodiments, the protein reproductive hormone antigen is selected from the group consisting of gonadotropin releasing hormone and ZP3.

[0026] In some embodiments, the non-rabies antigen is administered with a carrier such as keyhole limpet hemocyanin (KLH).

[0027] In some embodiments the cancer-associated antigen is selected from the group consisting of AIM-2, alphafetoprotein, bladder cancer-associated protein, breast carcinoma associated MAR-binding proteins p90 and p70, breast cancer–associated protein 3, carcinoembryonic antigen, CA-125, epithelial tumor antigen, c-erb/B2, gp100, HER-2, IL13Rα2, MAGE-1, MART-1, melanoma-associated antigens, MUC-1, MCU-2, prostate-associated antigen, abnormal products of p53, abnormal products of ras, survivin, tyrosinase, TRP-2 and tyrosine kinase 2.

[0028] In some embodiments the viral antigen is an antigen of a virus selected from the group consisting of cytomegalovirus, Dengue, Ebola virus, equine encephalitis virus, hepatitis virus, HIV, Hendra virus, herpes simplex virus, human papilloma virus, influenza, Japanese Encephalitis virus, neurotropic viruses, Nipah virus, Rift Valley Fever virus and West Nile Virus. In yet further embodiments the non-rabies antigen is an antigen of a pathogen which is associated with chlamydia, diphtheria, pertussis, tetanus, tuberculosis, nontuberculous mycobacteria-associated disease, bacterial and fungal pneumonias, babesiosis, cholera, typhoid, plague, shigellosis, salmonellosis, Legionnaire's Disease, Lyme disease, malaria, hookworm, onchocerciasis, schistosomiasis, trypanosomiasis, leshmaniasis, giardiasis, amoebiasis, filariasis, borreliosis, or trichinosis.

[0029] Also provided is a method of simultaneously immunizing against rabies virus and providing contraception in a host animal comprising administering to the host an effective amount of: (a) a nonpathogenic recombinant rabies virus comprising at least three copies of a mutated G gene, wherein said mutated G gene encodes a rabies virus glycoprotein wherein the glycoprotein amino acid 194 is serine and the glycoprotein amino acid 333 is glutamic acid, wherein said recombinant rabies virus does not express a foreign protein antigen; and (b) a protein reproductive hormone antigen. In some embodiments, the protein reproductive hormone antigen is selected from the group consisting of gonadotropin releasing hormone and ZP3.

[0030] As envisioned in the present invention with respect to the disclosed compositions of matter and methods, in one aspect the embodiments of the invention comprise the components and/or steps disclosed therein. In another aspect, the embodiments

of the invention consist essentially of the components and/or steps disclosed therein. In yet another aspect, the embodiments of the invention consist of the components and/or steps disclosed therein.

Description of the Figures

[0031] Fig. 1A illustrates the production of ovalbumin (OVA)-specific antibody after immunization with a single dose of a mixture of TriGAS and OVA or OVA alone. Groups of 10 Swiss Webster mice were immunized intramuscularly (i.m.) with 100 μl PBS containing 10^6 FFU TriGAS and 50 μg OVA (open bars) or 100 μl PBS containing only 50 μg OVA (closed bars). The mice were bled 21 days after immunization and the isotypes of OVA-specific antibody were determined by ELISA using alkaline-phosphatase conjugated anti-mouse whole IgG or biotinylated anti-mouse IgG1, IgG2a or IgG2b. The titers are presented as mean titers (+/- Standard Error) calculated from 10 mice per group. Fig. 1B illustrates the same experiments, the only difference being that the mice were bled 2 months after immunization.

[0032] Fig. 2A illustrates the production of Nipah virus glycoprotein (NiVG)-specific antibody produced after immunization with a single dose of a mixture of TriGAS and NiVG or NiVG alone. Groups of 10 Swiss Webster mice were immunized i.m. with 100 μl PBS containing 10^6 FFU TriGAS and 12.5 μg NiVG (open bars), or 100 μl PBS containing only 12.5 μg NiVG (closed bars). The mice were bled 21 days (Fig. 2A) after immunization and the isotypes of NiVG-specific antibody were determined by ELISA using alkaline-phosphatase conjugated anti-mouse whole IgG or biotinylated anti-mouse IgG1, IgG2a or IgG2b. The titers are presented as mean titers (+/- Standard Error) calculated from 10 mice per group. Fig. 2B illustrates the same experiments, the only difference being that the mice were bled 2 months after immunization.

[0033] Fig. 3 illustrates the production of Gonadotropin releasing hormone (GnRH)-specific antibody produced after immunization with a single dose of a mixture of TriGAS and GnRH-keyhole limpet hemocyanin (KLH), or with GnRH-KLH or TriGAS alone. Groups of 10 Swiss Webster mice were immunized intramuscularly (i.m.) with 100 μl PBS containing 10^6 FFu TriGAS and 50 μg GnRH (as GnRH-KLH), or 100 ml PBS containing only GnRH-KLH (50 μg GnRH). The mice were bled 21 days, 3 months, and 6 months after immunization and GnRH-specific antibody were determined by ELISA using alkaline

phosphatase-labelled anti-mouse whole IgG. The titers are presented as mean titers (+/- Standard Error) calculated from 10 mice per group.

Definitions

[0034] The definitions used in this application are for illustrative purposes and do not limit the scope of the invention.

[0035] The articles "a" and "an" are used herein to refer to one or to more than one (e.g., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[0036] The term "about" will be understood by persons of ordinary skill in the art and will vary to some extent depending on the context in which it is used. As used herein, "about" is meant to encompass variations of ±20% or ±10%, more preferably ±5%, even more preferably ±1%, and still more preferably ±0.1%.

[0037] The term "antibody," as used herein, refers to an immunoglobulin molecule which is able to specifically bind to a specific epitope on an antigen. Antibodies can be intact immunoglobulins derived from natural sources or from recombinant sources and can be immunoreactive portions of intact immunoglobulins. Antibodies are typically tetramers of immunoglobulin molecules. The antibodies that may be used in the practice of the present invention may exist in a variety of forms including, for example, polyclonal antibodies, monoclonal antibodies, Fv, Fab and $F(ab)_2$, as well as single chain antibodies and humanized antibodies (Harlow *et al.*, 1999, Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York; Harlow *et al.*, 1989, Antibodies: A Laboratory Manual, Cold Spring Harbor, New York; Houston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; Bird et al., 1988, Science 242:423-426).

[0038] As used herein, each "amino acid" is represented by the full name thereof, by the three letter code corresponding thereto, or by the one-letter code corresponding thereto, as indicated in the following table:

Full Name	Three-Letter Code	One-Letter Code
Aspartic Acid	Asp	D
Glutamic Acid	Glu	E
Lysine	Lys	K
Arginine	Arg	R

Histidine	His	H
Tyrosine	Tyr	Y
Cysteine	Cys	C
Asparagine	Asn	N
Glutamine	Gln	Q
Serine	Ser	S
Threonine	Thr	T
Glycine	Gly	G
Alanine	Ala	A
Valine	Val	V
Leucine	Leu	L
Isoleucine	Ile	I
Methionine	Met	M
Proline	Pro	P
Phenylalanine	Phe	F
Tryptophan	Trp	W

[0039] The expression "amino acid" as used herein is meant to include both natural and synthetic amino acids, and both D and L amino acids.

[0040] The term "animal" has its ordinary meaning, and is meant to include human beings.

[0041] "Attenuated" as used herein in the context of a live virus, such as a rabies virus, means that the ability for the virus to infect a cell or subject and/or its ability to produce disease is reduced (for example, eliminated). Attenuation may arise from either a genetic mechanism involving premature termination of transcription from the RV genome, or immunologically as a process whereby a pathogenic RV loses its virulence.

[0042] "Encoding" refers to the inherent property of specific sequences of nucleotides in a polynucleotide, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (i.e., rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein in a cell or other biological system. Both the coding strand, the nucleotide sequence of which is identical to the mRNA sequence and is usually provided in sequence listings, and the non-coding strand, used as the template for transcription of a gene or cDNA, can be referred to as encoding the protein or other product of that gene or cDNA.

[0043] "Gene expression" or "expression" as used herein refers to the process by which information from a gene is made into a functional gene product, such as RNA or protein. Thus, the "level of expression" of a gene product of a marker gene of the, in a sample of interest, refers to the level of RNA, particularly the level of mRNA, or the level of the encoded protein, and is not intended to be limited to either.

[0044] As used herein, an "expression vector" is a genetic element that functions as an autonomous unit of DNA replication under its own control sequence, to which another DNA segment may be attached or inserted so as to bring about replication of the attached or inserted segment. Expression vectors include plasmids, phages or cosmids. In general, expression vectors contain promoter sequences which facilitate the efficient transcription and translation of the attached or inserted DNA segment in a particular host cell. The expression vector also typically contains an origin of replication and transcription terminator(s), as well as specific genes which are capable of providing phenotypic selection in transfected host cells

[0045] By a "foreign protein antigen" with respect to a recombinant rabies virus is meant an antigen of a protein that is not native to the rabies virus expressing the antigen. In certain embodiments where the foreign antigen is an antigen of a virus, the virus is other than a rabies virus.

[0046] By a "non-rabies antigen" is meant an antigen of a protein that is other than a rabies virus protein. In some embodiments, the non-rabies antigen is conjugated to a carrier such as keyhole limpet hemocyanin (KLH).

[0047] As used herein, the term "gene" refers to an element or combination of elements that are capable of being expressed in a cell, either alone or in combination with other elements. In general, a gene comprises (from the 5' to the 3' end): (1) a promoter region, which includes a 5' nontranslated leader sequence capable of functioning in any cell such as a prokaryotic cell, a virus, or a eukaryotic cell (including transgenic animals); (2) a structural gene or polynucleotide sequence, which codes for the desired protein; and (3) a 3' nontranslated region, which typically causes the termination of transcription and the polyadenylation of the 3' region of the RNA sequence. Each of these elements is operably linked by sequential attachment to the adjacent element.

[0048] As used herein, “gene products” include any product that is produced in the course of the transcription, reverse-transcription, polymerization, translation, post-translation and/or expression of a gene. Gene products include, but are not limited to, proteins, polypeptides, peptides, peptide fragments, or polynucleotide molecules.

[0049] As used herein, "RV G protein gene" or "G protein gene" or “G gene” means the nucleic acid sequences which, when present in an RV genome, are sufficient to encode an RV glycoprotein in an infected cell. The G protein gene thus includes a coding sequence which is flanked on the 3' end with a short transcriptional start sequence, and on the 5' end with a short transcriptional stop/polyadenylation sequence. The G gene can include an intergenic region of 1-59 untranslated nucleotides, which is located between the 5' stop/polyadenylation sequence and the 3' transcriptional start sequence of the next viral gene. See, e.g., pgs. 134-136 of Conzelman K-K (1998), *Ann. Rev. Genet.* 32: 123-62,

[0050] The term “immunization” refers to a process to induce partial or complete protection against a disease. Alternatively, the term refers to a process to induce or amplify an immune system response to an antigen.

[0051] An "immune response" to an antigen or vaccine composition is the development in a subject of a humoral and/or a cell-mediated immune response to molecules present in the antigen or vaccine composition of interest. For purposes of the present invention, a "humoral immune response" is an antibody-mediated immune response and involves the generation of antibodies with affinity for the antigen/vaccine of the invention, while a "cell-mediated immune response" is one mediated by T-lymphocytes and/or other white blood cells. A "cell-mediated immune response" is elicited by the presentation of antigenic epitopes in association with Class I or Class II molecules of the major histocompatability complex (MHC).

[0052] A “mutation,” as used herein, refers to a change in nucleic acid or polypeptide sequence relative to a reference sequence (which is preferably a naturally-occurring normal or “wild-type” sequence), and includes translocations, deletions, insertions, and substitutions/point mutations. A “mutant,” as used herein, refers to either a nucleic acid or protein comprising a mutation.

[0053] A “nucleic acid” refers to a polynucleotide and includes poly-ribonucleotides and poly-deoxyribonucleotides.

[0054] A coding sequence is "operably linked" to a control sequence such as transcriptional and translational control sequence in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then trans-RNA spliced and translated into the protein encoded by the coding sequence.

[0055] As used herein, the terms "peptide," "polypeptide," and "protein" are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids which can comprise a protein's or peptide's sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. "Polypeptides" include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptide, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins, among others. The polypeptides include natural peptides, recombinant peptides, synthetic peptides, or a combination thereof.

[0056] As used herein, "polynucleotide" includes cDNA, RNA, DNA/RNA hybrid, anti-sense RNA, ribozyme, genomic DNA, synthetic forms, and mixed polymers, both sense and antisense strands, and may be chemically or biochemically modified to contain non-natural or derivatized, synthetic, or semi-synthetic nucleotide bases. Also, included within the scope of the invention are alterations of a wild type or synthetic gene, including but not limited to deletion, insertion, substitution of one or more nucleotides, or fusion to other polynucleotide sequences, provided that such changes in the primary sequence of the gene do not alter the expressed peptide ability to elicit passive immunity.

[0057] "Pharmaceutically acceptable" means physiologically tolerable, for either human or veterinary applications.

[0058] As used herein, "pharmaceutical compositions" include formulations for human and veterinary use.

[0059] As used herein, "promoter" refers to a region of a DNA sequence active in the initiation and regulation of the expression of a structural gene. This sequence of DNA, usually upstream to the coding sequence of a structural gene, controls the expression of the coding region by providing the recognition for RNA polymerase and/or other elements required for transcription to start at the correct site.

[0060] As used herein, the term "subject" refers to any vertebrate animal, including, but not limited to humans and other primates, rodents (e.g., mice, rats, and guinea pigs), lagamorphs (e.g., rabbits), bovines (e.g., cattle), ovines (e.g., sheep), caprines (e.g., goats), porcines (e.g., swine), equines (e.g., horses), canines (e.g., dogs), felines (e.g., cats), domestic fowl (e.g., chickens, turkeys, ducks, geese, other gallinaceous birds, etc.), as well as feral or wild animals, including, but not limited to, such animals as ungulates (e.g., deer), bear, fish, lagamorphs, rodents, birds, etc.

[0061] As used herein, a "transfected" cell is one into which an exogenous or heterologous nucleic acid sequence has been introduced. The nucleic acid sequence which has been introduced can be integrated into the genome of the transfected cell, or can be maintained episomally. A stably transfected cell is one in which the introduced DNA has integrated into a chromosome so that it is inherited by daughter cells through chromosome replication.

[0062] The term "vaccine" as used herein is defined as a material used to provoke an immune response after administration of the material to a vertebrate, typically a mammal. In preferred embodiments a vaccine can be an immunogenic composition providing or aiding in prevention of disease. In other embodiments, a vaccine is a composition that can provide or aid in a cure of a disease. In others, a vaccine composition can provide or aid in amelioration of a disease. Further embodiments of a vaccine immunogenic composition can be used as therapeutic and/or prophylactic agents.

[0063] A "vector," as used herein, refers to a replicon, such as plasmid, phage or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment.

[0064] The term "virus" as used herein is defined as a particle consisting of nucleic acid (RNA or DNA) enclosed in a protein coat, with or without an outer lipid envelope, which is capable of replicating within a whole cell.

[0065] As envisioned in the present invention with respect to the disclosed compositions of matter and methods, in one aspect the embodiments of the invention comprise the components and/or steps disclosed herein. In another aspect, the embodiments of the invention consist essentially of the components and/or steps disclosed herein. In yet another aspect, the embodiments of the invention consist of the components and/or steps disclosed herein.

Detailed Description of the Invention

[0066] Provided are methods for enhancing an antigen-induced immune response in a host comprising administering to a host an effective amount of: (a) a nonpathogenic recombinant rabies virus comprising at least three (preferably three) copies of a mutated G gene, wherein said mutated G gene encodes a rabies virus glycoprotein wherein the glycoprotein amino acid 194 is serine and the glycoprotein amino acid 333 is glutamic acid, wherein said recombinant rabies virus does not express a foreign protein antigen; and (b) a non-rabies antigen that is not expressed by the rabies virus. The corresponding compositions, vaccines and kits are also provided.

[0067] The RV for use in the practice of the present invention is attenuated. The modified G gene provides for a non-pathogenic live rabies virus that eliminates or resists subsequent mutation resulting in a change of amino acids in the expressed glycoprotein from occurring. Thus, reversion to a pathogenic form is eliminated.

[0068] The recombinant nonpathogenic TriGAS virus is a live rabies virus expressing three copies of a mutated G gene, wherein said mutated G gene encodes a rabies virus glycoprotein wherein the glycoprotein amino acid 194 is serine and the glycoprotein amino acid 333 is glutamic acid, wherein said recombinant rabies virus does not express a foreign protein antigen. The TriGAS virus has been described in U.S. Pat. No. 8,282,939, which is incorporated herein by reference in its entirety.

[0069] The pathogenicity of RV strains can be completely abolished by mutating the glycoprotein so that the glycoprotein amino acid 194 is serine and the glycoprotein amino acid 333 is glutamic acid completely abolishes pathogenicity of rabies virus strains for immunocompetent mice. Rabies viruses containing a G having a mutation of Arg333→Glu333 are non-pathogenic for adult mice after intracranial/intracerebral (i.c.) inoculation, and a mutation of Asn194→Ser194 in the same gene prevents the reversion to pathogenic phenotype. The G gene containing both mutations is designated as GAS. GAS has been described in U.S. Pat. No. 7,695,724, which is incorporated herein by reference in its entirety. The nucleotide sequence of the GAS gene is provided in Pat. No. 7,695,724 as SEQ ID NO:5 therein.

[0070] Because the attenuated TriGAS of the invention is not modified to express a foreign antigen, its tropism is unaffected and its exceptional safety profile is unchanged. Attenuated rabies viruses have been engineered to express foreign antigens in the context of the virus particle, generally with limited success in terms of inducing immunity to the foreign antigen. Recombinant approaches to alter the rabies virus genome and virus structure possess added risks in potentially altering tropism as well as causing cytopathic effects on infected cells that are not seen with TriGAS.

[0071] According to the present invention, the TriGAS virus functions to immunize a subject against rabies virus, and also to stimulate the immune system such that the immune response to a co-administered or separately administered non-rabies antigen is enhanced. Thus, the TriGAS virus is both an immunogen, capable of safely immunizing a host against rabies virus, and also an immunostimulant/adjuvant for augmenting and enhancing the host immune response to a non-rabies virus antigen.

[0072] The subject may comprise a vertebrate, preferably a mammal, most preferably a human being. Subjects for immunization according to the present invention include, for example, humans and other primates, rodents (e.g., mice, rats, and guinea pigs), lagamorphs (e.g., rabbits), bovines (e.g, cattle), ovines (e.g., sheep), caprines (e.g., goats), porcines (e.g., swine), equines (e.g., horses), canines (e.g., dogs), felines (e.g., cats), domestic fowl (e.g., chickens, turkeys, ducks, geese, other gallinaceous birds, etc.).

[0073] In one embodiment, the non-rabies antigen is an antigen that may be a target for immune response that will result in treating or preventing disease caused by disease causing agents. In these embodiments, the non-rabies antigen may comprise an antigen of for which immunity is sought or against which antibodies can be raised.

[0074] The disease causing agent or disease state has associated with it an antigen which triggers immune recognition and ultimate elimination or control of the disease causing agent or disease state in a host, by initiating a humoral and/or cellular immune response against the associated disease causing agent. Disease causing agents include but are not limited to cancer and pathogenic microorganisms.

[0075] Cancers which may be treated using the compositions and methods of the present invention include but are not limited to primary or metastatic melanoma, brain cancers, thymoma, lymphoma, sarcoma, lung cancer, liver cancer, non-Hodgkins lymphoma, Hodgkins lymphoma, leukemias, uterine cancer, and adenocarcinomas such as breast cancer, prostate cancer, ovarian cancer, pancreatic cancer and the like. The antigen associated with the cancer may be expressed on the surface of a cancer cell or may be an internal antigen. Preferred antigens are cell surface molecules.

[0076] Cancer-associated antigens which may in whole or in part may comprise the non-rabies antigen include, for example, Tyrosine kinase 2, Melanoma-associated antigens (MAGE, e.g., MAGE-1 and MAGE-3), MART-1 (melanoma); survivin, AIM-2, MAGE-1, TRP-2, gp100, HER-2 and IL13Rα2 (glioblastoma); prostate-associated antigen (PSA) (prostate cancer), alphafetoprotein (AFP); carcinoembryonic antigen (CEA); CA-125 (ovarian cancer); MUC-1; MCU-2; epithelial tumor antigen (ETA); breast cancer–associated protein 3 (BCA3); c-erb/B2 (breast cancer); breast carcinoma associated MAR-binding Proteins p90 and p70; tyrosinase; abnormal products of ras and abnormal products of p53 (pancreatic cancer); and bladder cancer-associated protein (BLCAP).

[0077] In one embodiment the antigen associated with the cancer is a tumor associated antigen (TAA) or portion thereof. Cancer-associated antigens comprising TAAs may be identified, isolated and cloned by methods known in the art such as those disclosed in U.S. Pat. No. 4,514,506.

[0078] In another embodiment, the compositions and methods of the present invention are for vaccination against a disease causing agent in which the agent is a pathogenic microorganism. The pathogenic microorganisms may comprise a prion, virus, bacterium, protozoan, or yeast. The antigen thus comprises a prion antigen, a viral antigen, a bacterial antigen, a protozoal antigen or a yeast antigen.

[0079] Disease causing virus agents include, for example, the following. wherein antigens provided are intended to be exemplary, not limiting: HIV (GP-120, p17, GP-160 antigens); cytomegalovirus; influenza (NP, HA antigen); Hendra virus; herpes simplex (HSVdD antigen); human papilloma virus; equine encephalitis virus; hepatitis virus (Hep B Surface Antigen); neurotropic viruses; Japanese Encephalitis virus; and the like. The virus may also comprise an emerging or re-emerging virus such as Dengue, West Nile Virus, Ebola virus, Nipah virus or Rift Valley Fever virus. The viral antigen may comprise, for example, virus surface proteins or other virus protein antigens.

[0080] In one embodiment, the non-rabies antigen is an antigen of the Nipah virus (NiV). NiV is a zoonotic virus belonging to the family *Paramyxovirus* genus *Henipaviruses*. The biological properties of NiV, including a broad species tropism, high transmission rate, high mortality in humans, and significant economic impacts on live stock industry have made it a major public and veterinary health concern. Although immune responses necessary for protection against NiV infection have not been completely defined, neutralizing antibodies which are induced by the Nipah virus glycoprotein (NiV-G), are the major effectors against this viral infection. Thus, in one embodiment, the non-rabies antigen is NiV-G or a fragment thereof.

[0081] Pathogenic yeast include, for example, Aspergillus, invasive Candida, and the like.

[0082] Protein antigens of prions include proteins such as BSE prion protein and scrapie prion protein.

[0083] Pathogenic bacteria include but are not limited to, Mycobacteria, Legioniella and the like.

[0084] Further known causative agents responsible for diseases from which suitable antigens may be derived include, but are not limited to, chlamydia, diphtheria, pertussis, tetanus, tuberculosis, nontuberculous mycobacteria-associated diseases, bacterial and fungal pneumonias, babesiosis, cholera, typhoid, plague, shigellosis, salmonellosis, Legionnaire's Disease, Lyme disease, malaria, hookworm, onchocerciasis, schistosomiasis, trypanosomiasis, leshmaniasis, giardiasis, amoebiasis, filariasis, borreliosis, and trichinosis. The non-rabies antigen for use in the practice of the invention is an antigen associated with the disease, more particularly an antigen of the pathogen causing the disease.

[0085] The non-rabies antigen may also comprise a protein reproductive hormone, for example gonadotropin releasing hormone or zona pelucida protein 3 (ZP3). Immunization with nonpathogenic recombinant mutated TriGAS RV and a protein reproductive hormone, such as gonadotropin releasing hormone, induces a protective response to rabies as well as a contraceptive effect in the host. In some embodiments, the contraceptive effect is permanent and may result in sterilization of the host.

[0086] Direct delivery of pharmaceutical compositions containing the nonpathogenic recombinant mutated TriGAS RV and/or non-rabies antigen *in vivo* may be accomplished via any route that provides immunity. In this regard, the compositions can be injected either subcutaneously, epidermaly, intradermally, intrathecally, intraorbitally, intramucosally (e.g., nasally, rectally and vaginally), intraperitoneally, intravenously, orally, or intramuscularly. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal applications. In one embodiment, administration is by means of injection using a conventional syringe, or in the case of free-roaming wildlife, by hyperdermic darting.

[0087] The nonpathogenic recombinant mutated TriGAS RV and non-rabies antigen may be formulated in various pharmaceutical compositions, either separately or in combination. The compositions may include a pharmaceutically acceptable carrier and, optionally, can include other medicinal agents, pharmaceutical agents, carriers, adjuvants, diluents, and excipients. "Pharmaceutically acceptable" means a material that is not biologically or otherwise undesirable, i.e., the material can be administered to an individual along with the non-rabies antigen and/or recombinant RV without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. Examples of physiologically acceptable carriers

include saline solutions such as normal saline, Ringer's solution, PBS (phosphate-buffered saline), and generally mixtures of various salts including potassium and phosphate salts with or without sugar additives such as glucose. Suitable excipients are, for example, water, saline, dextrose, glycerol, and ethanol. Nontoxic auxiliary substances, such as wetting agents, buffers, or emulsifiers may also be added to the composition. In one embodiment, adjuvants are not utilized for immunizations with the compositions of the invention.

[0088] Parenteral administration, if used, is generally characterized by injection. Sterile injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions.

[0089] In preferred embodiments, the nonpathogenic recombinant mutated TriGAS RV and the non-rabies antigen are administered simultaneously.

[0090] The nonpathogenic recombinant mutated TriGAS RV and the non-rabies antigen may be administered in the same composition or in different compositions. In preferred embodiments, the nonpathogenic recombinant mutated TriGAS RV and the non-rabies antigen are administered in the same composition.

[0091] According to the invention, an immunologically effective amount of the compositions of the invention is administered to the subject in need of protection against or treatment of cancer or pathogen-induced disease in order to induce a protective immune response to the foreign protein antigen. An effective immunizing amount given to the subject is one in which a sufficient immunological response to the antigen is attained to provide a medically effective long-lasting immune response to the cancer or pathogen. For each recipient, the total vaccine amount to be administered can be deduced from protocols for immunization with other vaccines. The exact amount of nonpathogenic recombinant mutated TriGAS RV and non-rabies antigen will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the particular strain of RV and the nature of the non-rabies antigen, mode of administration, and the like. The immunologically effective dosage or the effective immunizing amount that inoculates the animal and elicits satisfactory immune response can be easily determined or readily titrated by routine testing such as, for example, by standard dose titration studies. Generally, dosage will approximate that which is typical for the administration of other vaccines. In certain embodiments, a

single dose of nonpathogenic recombinant mutated TriGAS RV administered to the subject is from about 10^4 to about 10^6 tissue culture infectious units (TCIU), more preferably from about 10^5 to about 10^6 TCIU. Multiple dosing is also contemplated.

[0092] In some embodiments, following a prime immunization with the nonpathogenic recombinant mutated TriGAS RV and non-rabies antigen, the subject is inoculated at least once with a booster composition containing the non-rabies antigen. The booster composition comprises the non-rabies antigen in a pharmaceutically acceptable carrier. The booster composition may utilize any vehicle suitable for parenteral administration of the non-rabies antigen. The booster composition may contain the carrier substances noted above for formulation of the nonpathogenic recombinant mutated TriGAS RV. In one embodiment, the booster composition may be adjuvant-free.

[0093] The booster composition may comprise, in preferred embodiments, phosphate-buffered saline, aqueous sodium chloride, e.g., a 0.9% sodium chloride solution. In one embodiment, the booster composition is dispersed or dissolved in the vehicle providing the booster composition. In one embodiment, the non-rabies antigen is water soluble and is dissolved in an aqueous-based adjuvant-free vehicle, such as saline solution or phosphate-buffered saline. The booster composition may comprise additional ingredients such as stabilizers or other formulation aids, provided that it does not contain adjuvant.

[0094] The booster immunization may be administered following initiation of an immune response to the non-rabies antigen by the compositions of the invention. Generally, at least about 5 days, more preferably at least about 10 days, should be permitted to lapse following the primary immunization with the composition of the invention before boosting. In some embodiments, booster immunization occurs within 1 to 300 days, 5 to 250, 10 to 200, 15 to 150, 20 to 100, or 30 to 60 days following primary immunization with recombinant RV and the foreign protein. In specific embodiments, a booster immunization is administered 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 52, 53, 54, 55, 56, 57, 58, 59 or 60 days following primary immunization.

[0095] Multiple booster immunizations may be administered to augment the host immune response to the foreign protein antigen. For example, 2, 3, 4, 5, or 6 booster immunizations

may be administered in intervals. The intervals between booster immunizations may be uniform, e.g., booster immunizations are spaced apart by 5, 10, 20, 25, or 30 day intervals, or the booster immunizations may be scheduled at irregular time intervals.

[0096] The progress of the subject's immune response to the non-rabies antigen may be monitored by conventional assay methods. For example, the appearance of antibodies to the antigen in the blood or serum of the inoculated subject may be monitored by standard antibody assay methods including, but not limited to, radioimmunoassay, ELISA (enzyme-linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitin reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), Western Blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays, etc.), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays.

[0097] In one embodiment, a kit is provided comprising two vaccine preparations suitable for parenteral administration. The kit comprises a first composition comprising nonpathogenic recombinant mutated TriGAS RV and at least one non-rabies antigen of interest, for immunizing a subject. The kit further comprises a second composition for boosting the immune response of the subject to the non-rabies antigen. The first composition may optionally contain adjuvant. It preferably does not contain adjuvant. The second composition is adjuvant-free. The respective compositions may be in liquid or solid (lyophilized) form.

[0098] The kits of the present invention may optionally comprise different containers (e.g., vial, ampoule, test tube, flask or bottle) for each individual composition. The kit may contain additional reagents, such as buffers, diluents and the like, for formulation the individual components. Each component will generally be suitable as aliquoted in its respective container or provided in a concentrated form.

[0099] Instructions for using the kit according to the immunization methods described above may be included. The instructional material may comprise a publication, a recording, a diagram, or any other medium of expression which can be used to communicate directions for use of the kit. A package insert may comprise text housed in any physical medium, e.g.,

paper, cardboard, film, or may be housed in an electronic medium such as a diskette, chip, memory stick or other electronic storage form. The instructional material of the kit of the invention may, for example, be affixed to a container which contains other contents of the kit, or be shipped together with a container which contains the kit. Alternatively, the instructional material may be shipped separately from the container with the intention that the instructional material and the contents of the kit be used cooperatively by the recipient.

[00100] In certain embodiments of the invention, the immunization regimen constitutes a contraceptive treatment, and the foreign non-rabies antigen administered with the nonpathogenic recombinant mutated TriGAS RV comprises a sex hormone such as GnRH or ZP3, or an immunogenic fragment thereof. In further embodiments, the non-rabies antigen is administered conjugated to a carrier, such as keyhole limpet hemocyanin (KLH).

[00101] It has been shown that KLH is safe to administer as a carrier protein (Helling, F. *et al.*, 1995, Cancer Res. 5S:2783-2788, U.S. Pat. No. 5,102,663). KLH or a derivative thereof may be conjugated to an antigen directly, or via a linker. An example of a linker that may be used to conjugate KLH to an antigen is maleimidobenzoyl-N-hydroxy-succinimide ester (MBS).

[00102] The practice of the invention is illustrated by the following non-limiting examples. The invention should not be construed to be limited solely to the compositions and methods described herein, but should be construed to include other compositions and methods as well. One of skill in the art will know that other compositions and methods are available to perform the procedures described herein.

Examples

Example 1: Immunization with TriGAS and OVA

[00103] Production of ovalbumin (OVA)-specific antibody after immunization with a single dose of a mixture of nonpathogenic recombinant mutated TriGAS ("TriGAS") and OVA or OVA alone was studied. Groups of 10 Swiss Webster mice were immunized intramuscularly (i.m.) with 100 µl PBS containing 10^6 FFU TriGAS and 50 µg OVA (open bars) or 100 µl PBS containing only 50 µg OVA (closed bars). The mice were bled 21 days (Fig. 1A) and 2 months (Fig. 1B) after immunization and the isotypes of OVA-specific

antibody were determined by ELISA using alkaline-phosphatase conjugated anti-mouse whole IgG or biotinylated anti-mouse IgG1, IgG2a or IgG2b. The results are illustrated in Figures 1A and 1B. The antibody titers are presented as mean titers (+/- Standard Error) calculated from 10 mice per group.

[00104] Figures 1A and 1B illustrate that while an administration of a single dose of OVA in the absence of TriGAS was poorly or not immunogenic, the mixture of OVA with TriGAS induced a strong immune response to the OVA, as measured by antibody production. Most importantly, the anti-OVA immune response induced by the OVA-TriGAS mixture is long-lasting (Fig. 1B).

Example 2: Immunization with TriGAS and NiVG

[00105] Production of NiVG-specific antibody after immunization with a single dose of a mixture of TriGAS and NiVG or NiVG alone was studied. Groups of 10 Swiss Webster mice were immunized intramuscularly (i.m.) with 100 µl PBS containing 10^6 FFU TriGAS and 12.5 µg NiVG (open bars) or 100 µl PBS containing only 12.5 µg NiVG (closed bars). The mice were bled 21 days (Fig. 2A) and 2 months (Fig. 2B) after immunization and the isotypes of NiVG-specific antibody were determined by ELISA using alkaline-phosphatase conjugated anti-mouse whole IgG or biotinylated anti-mouse IgG1, IgG2a or IgG2b. The results are illustrated in Figures 2A and 2B. The antibody titers are presented as mean titers (+/- Standard Error) calculated from 10 mice per group.

[00106] Figures 2A and 2B illustrate that while an administration of a single dose of NiVG in the absence of TriGAS was poorly or not immunogenic, the mixture of NiVG with TriGAS induced a strong, long-lasting (Fig. 2B) immune response to the NiVG, as measured by antibody production.

Example 3: Immunization with TriGAS and GnRH-KLH or with GnRH-KLH alone

[00107] Production of GnRH-specific antibody after immunization with a single dose of a mixture of TriGAS and GnRH-keyhole limpet hemocyanin (KLH) or with a single dose of GnRH-KLH alone was studied. Groups of 10 Swiss Webster mice were immunized intramuscularly (i.m.) with 100 µl PBS containing 10^6 FFu TriGAS and 50 µg GnRH (as GnRH-KLH), or 100 ml PBS containing only GnRH-KLH (50 µg GnRH). The mice were

bled 21 days, 3 months, and 6 months after immunization and GnRH-specific antibody were determined by ELISA using alkaline phosphatase-labelled anti-mouse whole IgG. The antibody titers are presented as mean titers (+/- Standard Error) calculated from 10 mice per group.

[00108] Figure 3 illustrates that while an administration of a single dose of GnRH-KLH in the absence of TriGAS was poorly or not immunogenic, the mixture of TriGAS and GnRH-KLH induced a strong, long-lasting antibody response to GnRH (whole IgG) which actually increased over time.

[00109] All references discussed herein are incorporated by reference. One skilled in the art will readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The present invention may be embodied in other specific forms without departing from the spirit or essential attributes thereof and, accordingly, reference should be made to the appended claims, rather than to the foregoing specification, as indicating the scope of the invention.

Claims

1. A method of enhancing an antigen-induced immune response in a host to a non-rabies antigen comprising administering to a host an effective amount of:

(a) a nonpathogenic recombinant rabies virus comprising at least three copies of a mutated G gene, wherein said mutated G gene encodes a rabies virus glycoprotein wherein the glycoprotein amino acid 194 is serine and the glycoprotein amino acid 333 is glutamic acid, wherein said recombinant rabies virus does not express a foreign protein antigen; and

(b) a non-rabies antigen that is not expressed by the rabies virus.

2. The method according to claim 1 wherein said non-rabies antigen comprises at least one antigen selected from the group consisting of protein reproductive hormone antigens, cancer-associated antigens, viral antigens, bacterial antigens, and protozoal antigens.

3. The method according to claim 2, wherein the protein reproductive hormone antigen is selected from the group consisting of gonadotropin releasing hormone and ZP3.

4. The method according to claim 2, wherein the cancer-associated antigen is selected from the group consisting of AIM-2, alphafetoprotein, bladder cancer-associated protein, breast carcinoma associated MAR-binding proteins p90 and p70, breast cancer–associated protein 3, carcinoembryonic antigen, CA-125, epithelial tumor antigen, c-erb/B2, gp100, HER-2, IL13Rα2, MAGE-1, MART-1, melanoma-associated antigens, MUC-1, MCU-2, prostate-associated antigen, abnormal products of p53, abnormal products of ras, survivin, tyrosinase, TRP-2 and tyrosine kinase 2.

5. The method according to claim 2 wherein the non-rabies antigen is a viral antigen.

6. The method according to claim 5 where the viral antigen is an antigen of a virus selected from the group consisting of cytomegalovirus, Dengue, Ebola virus, equine encephalitis virus, hepatitis virus, HIV, Hendra virus, herpes simplex virus, human papilloma virus, influenza, Japanese Encephalitis virus, neurotropic viruses, Nipah virus, Rift Valley Fever virus and West Nile Virus.

7. The method according to claim 5, wherein the viral antigen is an antigen of Nipah virus G gene.

8. The method according to claim 2 wherein the non-rabies antigen is an antigen of a pathogen which is associated with chlamydia, diphtheria, pertussis, tetanus, tuberculosis, nontuberculous mycobacteria-associated disease, bacterial and fungal pneumonias, babesiosis, cholera, typhoid, plague, shigellosis, salmonellosis, Legionnaire's Disease, Lyme disease, malaria, hookworm, onchocerciasis, schistosomiasis, trypanosomiasis, leshmaniasis, giardiasis, amoebiasis, filariasis, borreliosis, or trichinosis.

9. The method according to claim 1 wherein the nonpathogenic recombinant rabies virus and the non-rabies antigen are administered simultaneously.

10. The method according to claim 1 wherein the nonpathogenic recombinant rabies virus is administered before the non-rabies antigen.

11. The method according to claim 1 wherein the nonpathogenic recombinant rabies virus and the non-rabies antigen are administered in the same composition.

12. The method according to claim 1 wherein the nonpathogenic recombinant rabies virus and the non-rabies antigen are administered in a different composition.

13. The method of claim 1 wherein the nonpathogenic recombinant rabies virus and the non-rabies antigen are administered intranasally, subcutaneously, epidermaly, intradermally, intrathecally, intraorbitally, intramucosally, intraperitoneally, intravenously, orally, or intramuscularly.

14. A composition comprising:

(a) a nonpathogenic recombinant rabies virus comprising at least three copies of a mutated G gene, wherein said mutated G gene encodes a rabies virus glycoprotein wherein the glycoprotein amino acid 194 is serine and the glycoprotein amino acid 333 is glutamic acid, wherein said recombinant rabies virus does not express a foreign protein antigen; and

(b) a non-rabies antigen that is not expressed by the rabies virus.

15. The composition of claim 14, wherein the non-rabies antigen comprises a protein reproductive hormone antigen, a prion protein antigen, a cancer-associated antigen, a viral antigen, a bacterial antigen, or a protozoal antigen.

16. The composition of claim 15, wherein the protein reproductive hormone antigen is selected from the group consisting of gonadotropin releasing hormone and ZP3.

17. The composition of claim 15, wherein the cancer-associated antigen is selected from the group consisting of AIM-2, alphafetoprotein, bladder cancer-associated protein, breast carcinoma associated MAR-binding proteins p90 and p70, breast cancer–associated protein 3, carcinoembryonic antigen, CA-125, epithelial tumor antigen, c-erb/B2, gp100, HER-2, IL13Rα2, MAGE-1, MART-1, melanoma-associated antigens, MUC-1, MCU-2, prostate-associated antigen, abnormal products of p53, abnormal products of ras, survivin, tyrosinase, TRP-2 and tyrosine kinase 2.

18. The composition of claim 15, wherein the viral antigen is an antigen of a virus selected from the group consisting of cytomegalovirus, Dengue, Ebola virus, equine encephalitis virus, hepatitis virus, HIV, Hendra virus, herpes simplex virus, human papilloma virus, influenza, Japanese Encephalitis virus, neurotropic viruses, Nipah virus, Rift Valley Fever virus and West Nile Virus.

19. The composition of claim 15, wherein the non-rabies protein antigen is an antigen of a pathogen which is associated with chlamydia, diphtheria, pertussis, tetanus, tuberculosis, nontuberculous mycobacteria-associated disease, bacterial and fungal pneumonias, babesiosis, cholera, typhoid, plague, shigellosis, salmonellosis, Legionnaire's Disease, Lyme disease, malaria, hookworm, onchocerciasis, schistosomiasis, trypanosomiasis, leshmaniasis, giardiasis, amoebiasis, filariasis, borreliosis, or trichinosis.

20. A vaccine comprising the composition of claim 14.

21. A kit for immunization of a subject comprising:

(a) a first composition comprising a nonpathogenic recombinant rabies virus comprising at least three copies of a mutated G gene, wherein said mutated G gene encodes a rabies virus glycoprotein wherein the glycoprotein amino acid 194 is serine and the

glycoprotein amino acid 333 is glutamic acid, wherein said recombinant rabies virus does not express a foreign protein antigen; and

(b) a second composition comprising a non-rabies antigen that is not expressed by the rabies virus.

22. The kit according to claim 21, wherein the non-rabies protein antigen comprises a protein reproductive hormone antigen, a prion protein antigen, a cancer-associated antigen, a viral antigen, a bacterial antigen, or a protozoal antigen.

23. The kit according to claim 22, wherein the protein reproductive hormone antigen is selected from the group consisting of gonadotropin releasing hormone and ZP3.

24. The kit according to claim 22, wherein the cancer-associated antigen is selected from the group consisting of AIM-2, alphafetoprotein, bladder cancer-associated protein, breast carcinoma associated MAR-binding proteins p90 and p70, breast cancer–associated protein 3, carcinoembryonic antigen, CA-125, epithelial tumor antigen, c-erb/B2, gp100, HER-2, IL13Rα2, MAGE-1, MART-1, melanoma-associated antigens, MUC-1, MCU-2, prostate-associated antigen, abnormal products of p53, abnormal products of ras, survivin, tyrosinase, TRP-2 and tyrosine kinase 2.

25. The kit according to claim 22, wherein the viral antigen is an antigen of a virus selected from the group consisting of cytomegalovirus, Dengue, Ebola virus, equine encephalitis virus, hepatitis virus, HIV, Hendra virus, herpes simplex virus, human papilloma virus, influenza, Japanese Encephalitis virus, neurotropic viruses, Nipah virus, Rift Valley Fever virus and West Nile Virus.

26. The kit according to claim 22, wherein the non-rabies protein antigen is an antigen of a pathogen which is associated with chlamydia, diphtheria, pertussis, tetanus, tuberculosis, nontuberculous mycobacteria-associated disease, bacterial and fungal pneumonias, babesiosis, cholera, typhoid, plague, shigellosis, salmonellosis, Legionnaire's Disease, Lyme disease, malaria, hookworm, onchocerciasis, schistosomiasis, trypanosomiasis, leshmaniasis, giardiasis, amoebiasis, filariasis, borreliosis, or trichinosis.

27. A method of immunizing a subject against rabies virus and against a non-rabies virus antigen comprising the steps of:

(a) administering a first composition comprising a nonpathogenic recombinant rabies virus comprising at least three copies of a mutated G gene, wherein said mutated G gene encodes a rabies virus glycoprotein wherein the glycoprotein amino acid 194 is serine and the glycoprotein amino acid 333 is glutamic acid, wherein said recombinant rabies virus does not express a foreign protein antigen; and

(b) administering a second composition comprising a non-rabies antigen that is not expressed by the rabies virus.

28. The method according to according to claim 27, wherein the non-rabies protein antigen comprises a protein reproductive hormone antigen, a prion protein antigen, a cancer-associated antigen, a viral antigen, a bacterial antigen, or a protozoal antigen.

29. The method according to according to claim 28, wherein the protein reproductive hormone antigen is selected from the group consisting of gonadotropin releasing hormone and ZP3.

30. The method according to according to claim 28, wherein the cancer-associated antigen is selected from the group consisting of AIM-2, alphafetoprotein, bladder cancer-associated protein, breast carcinoma associated MAR-binding proteins p90 and p70, breast cancer–associated protein 3, carcinoembryonic antigen, CA-125, epithelial tumor antigen, c-erb/B2, gp100, HER-2, IL13Rα2, MAGE-1, MART-1, melanoma-associated antigens, MUC-1, MCU-2, prostate-associated antigen, abnormal products of p53, abnormal products of ras, survivin, tyrosinase, TRP-2 and tyrosine kinase 2.

31. The method according to according to claim 28, wherein the viral antigen is an antigen of a virus selected from the group consisting of cytomegalovirus, Dengue, Ebola virus, equine encephalitis virus, hepatitis virus, HIV, Hendra virus, herpes simplex virus, human papilloma virus, influenza, Japanese Encephalitis virus, neurotropic viruses, Nipah virus, Rift Valley Fever virus and West Nile Virus.

32. The method according to according to claim 28, wherein the non-rabies antigen is an antigen of a pathogen which is associated with chlamydia, diphtheria, pertussis, tetanus, tuberculosis, nontuberculous mycobacteria-associated disease, bacterial and fungal pneumonias, babesiosis, cholera, typhoid, plague, shigellosis, salmonellosis, Legionnaire's Disease, Lyme disease, malaria, hookworm, onchocerciasis, schistosomiasis, trypanosomiasis, leshmaniasis, giardiasis, amoebiasis, filariasis, borreliosis, or trichinosis.

33. A method of simultaneously immunizing against rabies virus and providing contraception in a host animal comprising administering to said host an effective amount of:

(a) a nonpathogenic recombinant rabies virus comprising at least three copies of a mutated G gene, wherein said mutated G gene encodes a rabies virus glycoprotein wherein the glycoprotein amino acid 194 is serine and the glycoprotein amino acid 333 is glutamic acid, wherein said recombinant rabies virus does not express a foreign protein antigen; and

(b) a protein reproductive hormone antigen.

34. The method of claim 33 wherein said protein reproductive hormone antigen is selected from the group consisting of gonadotropin releasing hormone and ZP3

Figure 1A

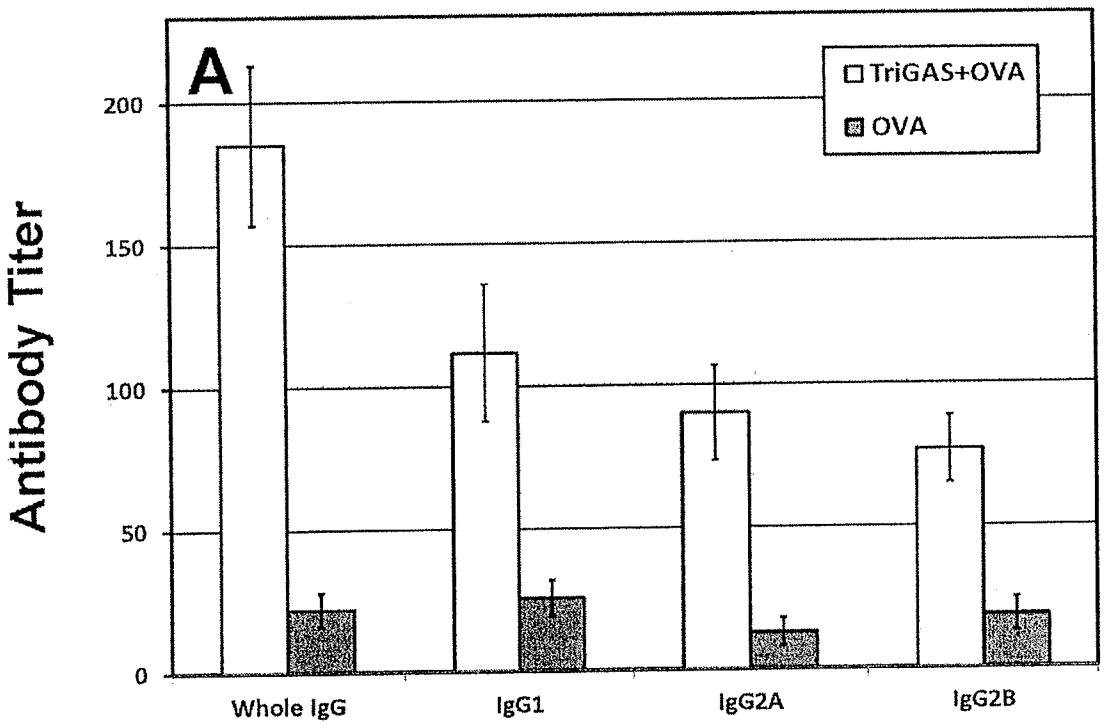


Figure 1B

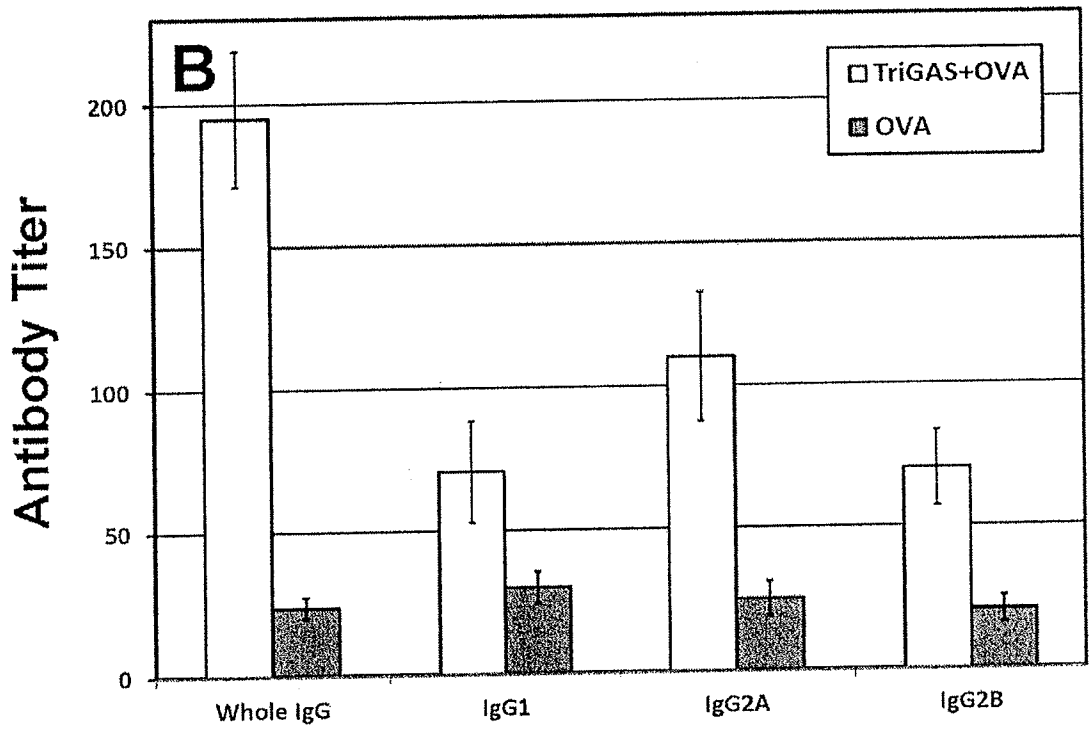


Figure 2A

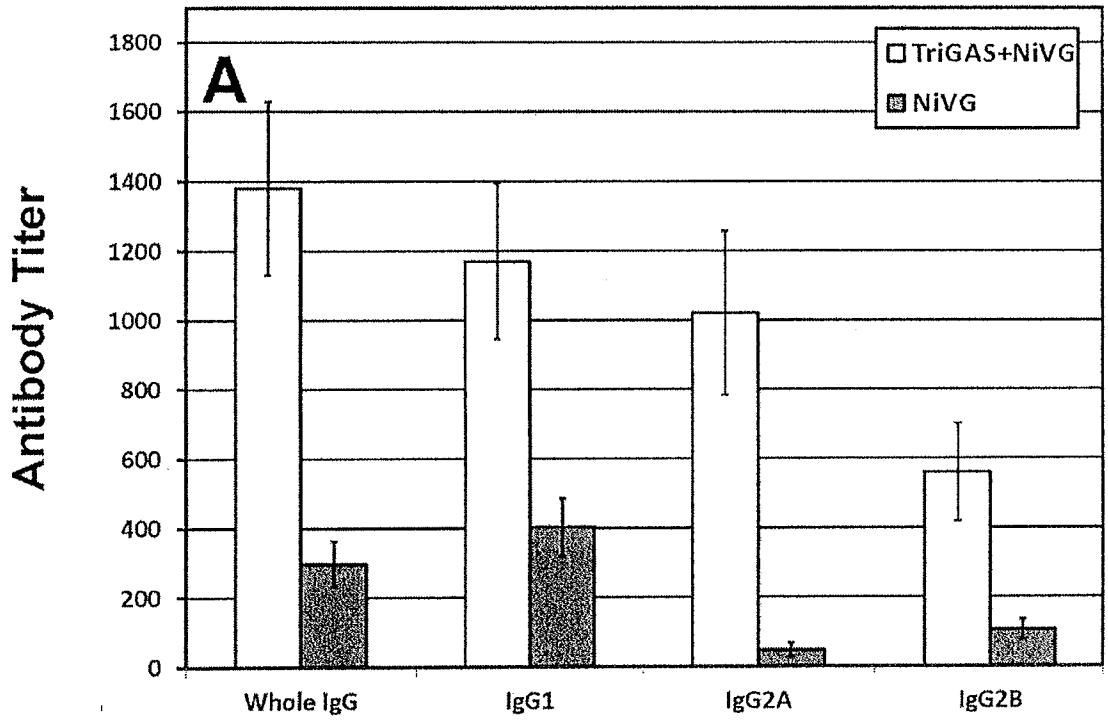


Figure 2B

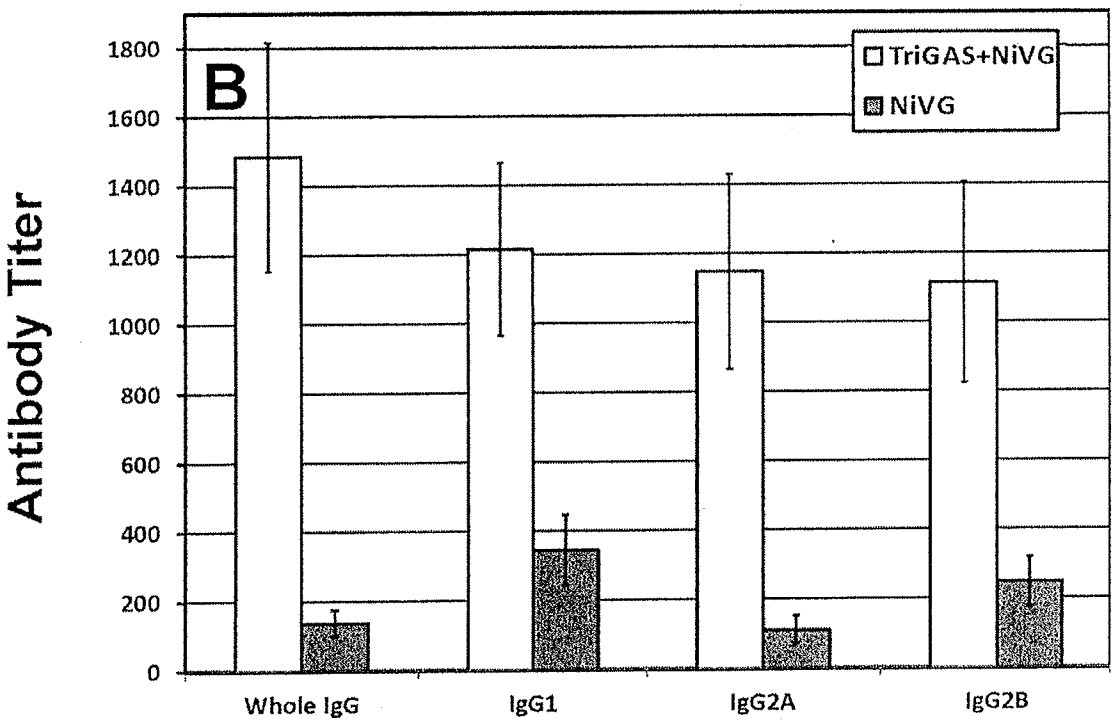
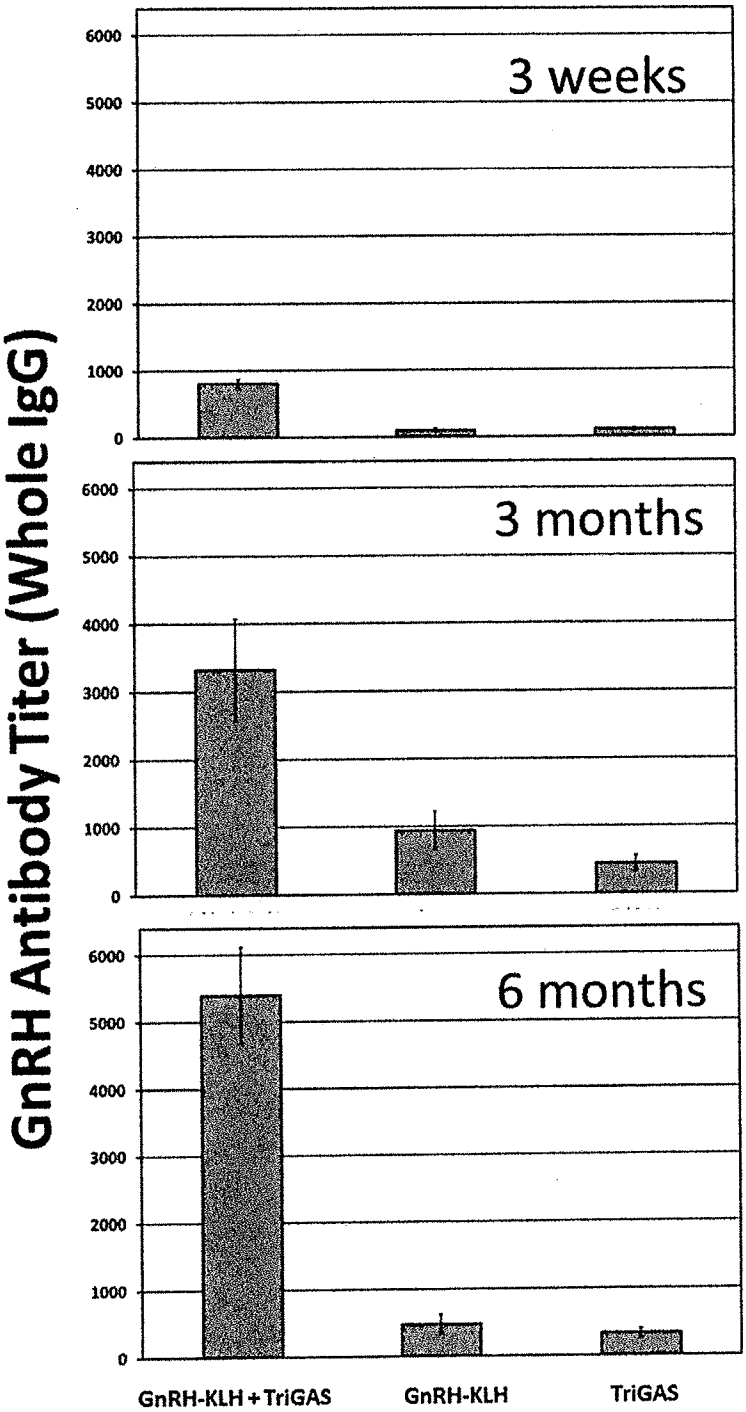


Figure 3


GnRH Antibody Titer (Whole IgG)
3 weeks
3 months
6 months
6000
5000
4000
3000
2000
1000
0
GnRH-KLH + TriGAS
GnRH-KLH
TriGAS

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 14/36168

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/205, A61K 39/12, A01N 63/00, C12N 7/04 (2014.01)
CPC - A61K 39/205, C07K 14/005, C12N 7/00, A61K 35/13
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A61K 39/205, A61K 39/12, A01N 63/00, C12N 7/04 (2014.01)
CPC: A61K 39/205, C07K 14/005, C12N 7/00, A61K 35/13

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 424/224.1, 424/199.1; 424/93.6, 424/204.1, 435/236

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, PubWEST, Google Scholar, Google Patetns: Antigen*, immune response, vaccin*, rabies, co-administration, mutat*, antigen$2 with (non-rabies or protein reproductive hormone or cancer-associated or viral or bacterial or protozoa or gonadotropin releasing hormone or ZP3 or AIM-2 or alphafetoprotein or bladder cancer-associated protein or breas

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2011/0064764 A1 (FABER et al.) 17 March 2011 (17.03.2011); abstract, para [0095], [0104], [0105]	1-34
Y	US 6,080,725 A (MARCIANI) 27 June 2000 (06.27.2000); abstract; col 9, ln 22-25, 44-54; col 10, ln 1-7, claims 1 and 29	1-34
Y	US 2011/0165189 A1 (WU et al.) 07 July 2011 (07.07.2011); abstract	3, 16, 23, 29, 33-34
Y	US 7,803,612 B2 (AUDONNET) 28 September 2010 (28.09.2010); abstract	7

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 August 2014 (14.08.2014)	18 SEP 2014

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)