

Nanocarriers for Systemic and Mucosal Vaccine Delivery

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Abstract: Over the past several years, immunization and treatment of infectious diseases has undergone a paradigm shift. Stemming from the vaccine research and development, not only a large number of disease-specific vaccines have been developed, but also enormous efforts have been made to improve the effectiveness of vaccines in order to provide optimal immunization. Introduction of nanotechnology and the development of nanocarrier-based vaccines have started to receive a lot of attention in order to provide effective immunization through better targeting and by triggering antibody response at the cellular level. Also, in the past several years, attention is placed on routes of vaccine administration in order to induce both mucosal and systemic immunity against the pathogen. Through judicious selection of the nanocarrier systems and the vaccine antigen, an optimal immunization and protection can be induced. This review article focuses on the patented applications of nanocarrier-based vaccine formulations and delivery. We have examined the United States patent literature to select inventions that specifically address this strategic approach for prevention of infectious diseases.

Keywords: Mucosal and systemic vaccination, nanocarriers, liposomes, micro- and nanoemulsions, polymeric nanoparticles, micelles, dendrimers.

1. INTRODUCTION

Vaccination against debilitating infectious diseases has proven remarkable in prevention of these diseases and has contributed significantly to an increase in life expectancy, especially in children, in many parts of the world [1, 2]. Despite these impressive results and remarkable accomplishments, there is still a need to further improve on vaccine research and development to combat deadly and emerging infectious diseases, such as AIDS, tuberculosis, and malaria, and provide complete protection, especially in developing nations [3, 4]. Majority of pathogens invade into the body via one or more of the mucosal routes. Oral, nasal, pulmonary, and urino-genital routes are the most common pathways for entry of infectious pathogens into the human host. Therefore, the importance of generating a "first-line of defense" at the site of entry has been well recognized. In order to have adequate mucosal protection, there are several factors that can influence the effectiveness of vaccines. The most critical factor in mucosal vaccine effectiveness is the route of administration and potential for the antigen to be processed by the antigen-presenting immune cells, such as macrophages and dendritic cells. Presently, most vaccines are administered via the parental route or via other invasive routes. Invasive mode of vaccine administration can trigger the systemic immune response, but may not essentially provide adequate mucosal immune protection. On the other hand, effective mucosal vaccines will not only elicit superior local immune protection, but has been shown to trigger systemic response analogous as that of parenterally-delivered vaccine. As such, it is critically important to examine the

development of mucosal vaccination strategies that can effectively trigger systemic as well as mucosal immunity.

This review summarizes the inventions disclosed in patent literature related to mucosal delivery of vaccines or antigens using nano-sized delivery systems. In reviewing the patent literature, we have used the Claims/U.S. Patents Abstracts database. This review is intended to inform readers about the developments in nanocarrier-based formulations in the field of mucosal delivery of vaccines. The article contains only brief summaries of the inventions. In order to access the full-text and claim language of the respective patents, the readers should do an online search using the patent numbers provided in the reference list.

The article is divided into specific nanocarrier vaccine delivery systems, which include liposomes, micro-, nano-, and multiple-emulsions, polymeric nanoparticles, micelles, dendrimers and immunostimulatory complexes (ISCOMs). Fig. 1 shows the schematic of the different delivery systems that will be discussed. It is expected that the readers will be familiar with the fundamentals of the various nanotechnologies described here. For those needing additional information, several excellent references are available that discuss the properties of the nanocarrier systems [5-7].

1.1. The Mucosal Route of Administration

The mucosal route covers aero-digestive and urino-genital tracts as well as the eye conjunctiva and the inner ear and the ducts of all endocrine glands endowed with powerful mechanical and chemical cleansing mechanisms that degrade and repel most foreign matter. It is comprised of anatomical defined lymphoid microcompartments, such as the Peyer patches, the mesenteric lymph nodes, the appendix and solitary follicles in the intestine, and the tonsils and adenoids at the entrance of the aero-digestive tract, which serve as the principal mucosal inductive sites where immune responses

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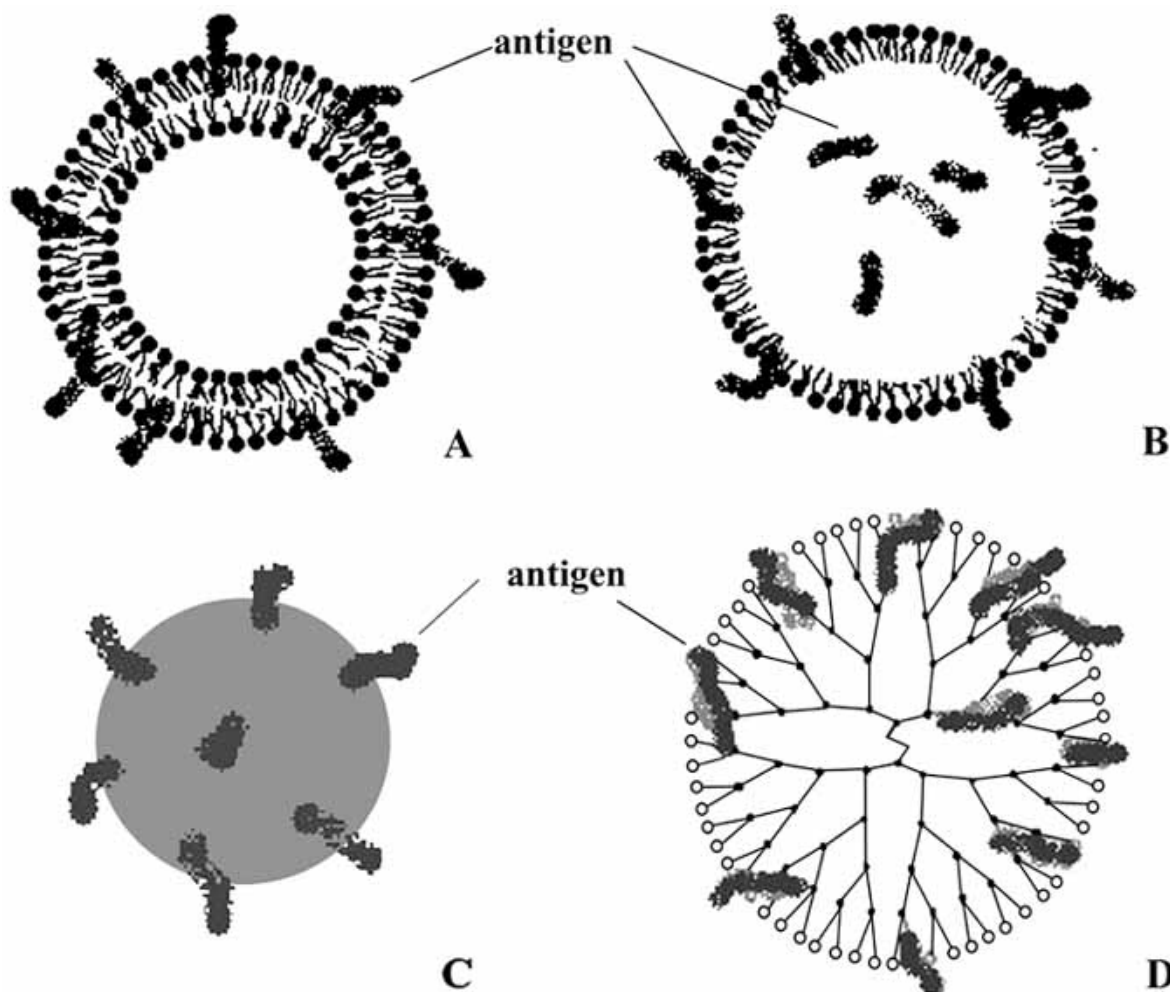


Fig. (1). Schematic illustration of various nanocarrier delivery systems for mucosal vaccines. (A) liposome, (B) micelle/emulsion, (C) polymeric nanoparticles, and (D) dendrimer.

are initiated. In a healthy human adult, mucosal immune system contributes almost 80% of all immunocytes. The mucosa-associated lymphoid tissues represent a highly compartmentalized immunological system. The primary reason for using a mucosal route of vaccination is that most infections affect or start from mucosal surfaces, and that in these infections, topical application of a vaccine is often required to induce a protective immune response at the site of pathogen entry.

There is an enormous challenge for development of vaccines targeted to induce immunity that can either prevent the infectious agent from attaching and colonizing at the mucosal epithelium (non-invasive bacteria), or from penetrating and replicating in the mucosa (viruses and invasive bacteria), and/or that can block microbial toxins from binding to an affecting epithelial and other target cells.

1.2. Need for Vaccine Delivery System

New vaccines based on recombinant proteins and DNA, are safer than traditional vaccines, but they are less immunogenic. Therefore, there is an urgent need for the development of potent and safe adjuvants and delivery systems that can be used with new generation of vaccines.

There are two classes of adjuvants: vaccine delivery systems (e.g., emulsions, microparticles, immune-stimulating complexes ISCOMs, liposomes) and immunostimulatory adjuvants (e.g., lipopolysaccharide, monophosphoryl lipid A, CpG DNA, or muramylpeptides). As particulate adjuvants, nanocarriers having comparable dimensions to the pathogens, are efficiently taken up by phagocytes and deliver associated antigen into the antigen presenting cells. Immunostimulatory adjuvants can also be included in nanocarriers to enhance the immune response or to generate specific type of immune response (e.g., Th1 or Th2). In addition, formulating potent immunostimulatory adjuvants into nanocarriers may limit adverse events, through restricting the distribution of the adjuvant.

Mucosal vaccines have currently been investigated using a broad spectrum of nanocarrier systems such as multiple emulsions, liposomes, polymeric nanoparticles, dendrimers, ISCOMs etc. Some examples of literature-cited nanocarrier-based vaccines are presented in Table 1. Griffiths, *et al.*, [8] have developed ricin toxoid vaccine and upon delivery through pulmonary route, they observed that liposome-encapsulated ricin vaccine delivered via pulmonary route was more effective in triggering mucosal and systemic

Table 1. Selective Examples of Vaccines Formulated in Nanocarrier Systems

Nanocarrier Formulation	Antigen(s)	Route of Delivery	Outcome(s)	Reference
Liposomes	Ricin toxoid vaccine	Intratracheal instillation	Higher titers and better protection against ricin toxoid	[8]
Liposomes	Tetanus toxoid	Intranasal administration	Intranasal administration was found more effective for inducing mucosal immunity	[9]
Liposomes	Various antigen carriers	Oral drug delivery	Liposomal preparation may be promising for M cell interaction	[10]
Liposomes	Ferritin antigen	Rectal	Effective mucosal immunization following rectal administration	[11]
Liposomes	Ganglioside GM1	Oral	Vaccine was found promising to induce IgA antibody response	[12]
Water-in-oil-in-water emulsion	HIV-1 envelope protein	Subcutaneous	Higher antibody titers whereas low mucosal immunization	[13]
Multiple emulsion	Cholera toxin	Intranasal	Higher titers both qualitatively and quantitatively in mucosal membranes and systemic circulation	[14]
Polymeric nanoparticles	<i>Salmonella enterica</i> serovar <i>Abortusovis</i>	Subcutaneous	Formulation provided protection in single shot and polymeric nanoparticles may be better alternative	[15]
Alginate coated chitosan nanoparticles	Ovalbumin	Oral delivery to Peyer's patches	Better uptake of nanoparticles which provides higher degree of protection	[16]
Nanoparticulate vesicular formulation	Bovine Serum Albumin	Oral delivery of liposomes and niosomes	High sIgA levels was noticed with carrier-adjuvant system	[17]
Micro- and nano-particles	<i>Toxoplasma gondii</i> tachyzoites	Intranasal	Increased levels and higher mucosal and systemic immunity	[18]
Cationic nanoparticles	Plasmid DNA	Intranasal	25-30 fold higher beta-galactosidase response	[19]
Dendrimers	Cytotoxic T-lymphocytes	Oral	can elicit systemic and mucosal immunoglobulin response	[20]

immunity. Tafaghodi, *et al.*, [9] have successfully developed liposomal intranasal vaccine for tetanus toxoid. Following nasal administration in rabbits, they found that the nanocarrier encapsulated formulation was more effective in inducing the mucosal immune responses. Interestingly, in one of the investigations carried out by Zho, *et al.*, [10] on liposomal encapsulated vaccine formulation for site specific delivery to lymphatic system, they found that the vaccine had adequate interaction with M cells and triggered site-specific lymphatic immune response. In another investigation using gangliosides GMI encapsulated liposomes, an impressive efficacy in inducing mucosal IgA response was demonstrated upon oral administration [12]. In addition to liposomes, polymeric nanoparticles are also actively being investigated for vaccine delivery. For instance, Estevan, *et al.*, [15] have encapsulated extracts of *Salmonella enterica* serovar *Abortusovis* in polymeric nanoparticles. Upon subcutaneous injection in Balb/c mice, the nanoparticles conferred a significant protection and the antibody titer levels were similar to those induced by the attenuated commercial vaccine Rv6. In another study, ovalbumin was encapsulated in alginate-coated chitosan nanoparticles and delivered via the oral route to Peyer's

patches [16]. Jain, *et al.*, [17] observed high sIgA concentrations and better immune response with nanoparticulate vesicular formulation upon oral administration. In a study carried out using micro- and nano-particles encapsulated with *Toxoplasma gondii* tachyzoites, remarkable improvements in both systemic and mucosal immunity were observed [18].

2. LIPOSOMAL DELIVERY SYSTEMS

Liposomes are organized phospholipid vesicles that have been used to encapsulate protein and DNA-based vaccines. Considerable evidence suggests that liposomes, or suspensions of lipids and/or phospholipids, can exert immunomodulatory effects when introduced into the body as a vaccine adjuvant [21-25]. The mechanism by which liposomes elicit their adjuvant effect is not well understood, but passive targeting by virtue of their particulate nature and tendency to interact with macrophages of the reticulo-endothelial system is likely to be an important factor, particularly for non-targeted conventional liposomes. Depending on their lipid composition, liposomes also may interact with macrophages and dendritic cells via cell surface

lipid receptors, such as CD1a, after complement activation. The development of polymerized liposomes, which show enhanced stability in the gastrointestinal tract, also offers potential for use in mucosal vaccination [26].

A formulation for oral delivery of vaccines using polymerized liposomes is taught in the patent of Okada, *et al.* [27]. In this invention, the methods of preparing polymerized liposomes and incorporation of biologically-active substances within the polymerized liposomes as well as methods of oral administration of the formulations to patients are disclosed. Claim 1 of this patent states that the invented technology teaches “a method of delivering an antigen to the gastrointestinal tract of an animal, which comprises of orally administering to said animal polymerized liposomes comprising a phospholipid bilayer having covalently bonded phospholipids therein, an aqueous core and an antigen encapsulated in said polymerized liposome administered in an amount effective to elicit a humoral, secretory or cell mediated immune response against the antigen is disclosed”. Candidate vaccines for encapsulation are selected from a group consisting of viruses, proteins, glycoproteins, nucleic acids, carbohydrates, and lipids. Polymerized liposomes are further modified with a targeting molecule selected from the group consisting of antibodies, antibody fragments, antigens and molecules capable of binding to specific cell surface receptors found in the mucosal tissue.

Aventis Pasteur [28] is the assignee of a delivery system consisting of nucleic acid encoding at least a portion of the D15 outer membrane protein of hemophilus for purposes of diagnosis and medical treatment of hemophilus infection. They further describe immunogenic composition formulated as a microparticle preparation, a capsule preparation, or a liposome preparation. Another invention relates to a unit dosage form of the composition having in a sterile container, an agent with cytokine activity, including natural, recombinant and mutated cytokines, fragments, analogs, and derivatives of the cytokines, and mixtures thereof [29]. The composition is also provided as a kit with single or multiple unit dosages of the various ingredients, instructions, and device(s) for its administration, such as needles and syringes, inhalators, and other identical delivery devices. The composition may be provided in various forms, including topical and systemic dosage forms, such as powders, creams, ointments, sprays, solutions, suppositories, powders, suspensions, patches, emulsions, implants, and encapsulated particles, among others, and contains various forms of the cytokines, useful for prevention and treatment of malignancies, as well as, mild and severe infections afflicting individuals that include viral, fungal, parasitic, and bacterial infections.

Lyfjathroun [30] has been granted a formulation patent for the topical administration of antigens and/or vaccines to mammals via mucosal membranes in liposome or emulsion form. This formulation enhances the immunological response in a mammal following mucosal administration, including nasal, oral, rectal or vaginal application.

Purified and isolated nucleic acid molecules are disclosed [31], which encode a basal body rod protein of a strain of *Campylobacter*, particularly *Campylobacter jejuni*, a frag-

ment or an analog of the basal body rod protein. Peptides corresponding to portions of the basal body rod protein or analogs thereof are useful immunogenic compositions against disease caused by *Campylobacter*, in the diagnosis of infection by *Campylobacter*, and as tools for the generation of immunological reagents. Monoclonal antibodies or antisera raised against these peptides, produced in accordance with aspects of the present invention, are useful for the diagnosis of infection by *Campylobacter*, specific detection of *Campylobacter* in *in-vitro* and *in-vivo* assays, and for use in passive immunization for prevention and treatment of diseases caused by *Campylobacter*. A method is disclosed for producing a vaccine that comprises administering the immunogenic composition formulated in a vaccine for *in-vivo* administration to protect against diseases caused by bacterial pathogens by producing basal body rod proteins to a test host to determine an amount and a frequency of administration of the active component to confer protection against disease caused by a bacterial pathogen that produces the basal body rod protein or produces a protein capable of inducing antibodies in the host specifically reactive with the basal body rod protein. The invention also discloses a composition of at least one adjuvant selected from the group consisting of aluminum phosphate, aluminum hydroxide, QS21, Quil A, calcium phosphate, calcium hydroxide, zinc hydroxide, a glycolipid analog, an octadecyl ester of an amino acid, a muramyl dipeptide and a lipoprotein. The immunogenic composition of the invention is formulated in the form of microparticle, capsule, immuno-stimulating complex (ISCOM), or liposomal preparation.

Several patents have been cited in the literature [32-34], which describe vaccine preparations for the prevention of *Chlamydia* infections. In one embodiment of the main claims [35], there is a statement relating to the composition having liposomes and, associated with the liposomes, nucleic acid operatively encoding an antigenic protein and an assistor protein, wherein the assistor protein shares at least one epitope with the antigenic protein. The composition is for use as a vaccine and provides improved immune response compared to non-vesicular compositions, or mixtures of liposomes some of which are associated with nucleic acid and some of which are associated with assistor protein. A vaccine comprising a CD8+ T cell immunoprotective and/or antibody immunoprotective amount of virus has been described [36], wherein said virus is associated with a nanotube or a liposome.

Nanoparticle carriers for use as vaccine have also been made from lipids or other fatty acids [37-39]. In one of the disclosed invention, vaccine compositions are based on traditional bilayer or multilamellar liposomes, and are phospholipid in nature. Such liposomes are physically and chemically unstable and rapidly allow for leakage of the encapsulated material and degrade the vesicle structure. Without stabilization of the liposome structure, they are not good candidates for oral drug or antigen delivery. Polymerization of lipid-based nanoparticles creates a stable structure that does not readily fuse with other polymerized liposome nanoparticles or cell membranes, and therefore, these nanoparticle vaccine carriers can maintain their small and uniform size even upon oral administration. Polymerized

liposome nanoparticles as vaccine delivery system have been described by Langer, *et al.* [40]. The invention [41] is based on the discovery that nanoparticle vaccines having multivalent surface antigens (presented on the exterior or interior or the particle) or encapsulated antigens elicit significantly increased immune responses. Additionally, simultaneous display of more than one targeting molecule(s) on the polymerized liposome nanoparticle surface for purposes of directing the vaccine to a specific *in-vivo* location, increases the efficiency and effectiveness of the desired immune response.

3. MICRO-/NANO-/MULTIPLE-EMULSION DELIVERY SYSTEMS

Emulsions are mainly used as depot agents in the experimental production of polyclonal antibodies. These heterogenous liquid systems may be water-in-oil emulsions, oil-in-water emulsions, or more complex systems such as water-in-oil-in-water multiple emulsions, microemulsions or nanoemulsions. A variety of oils and emulsifying agents have been used in forming and stabilizing emulsions. Paraffin oil, a crude mineral oil, and the emulsifying agent mannide monooleate (Arlacel A[®]) were used in the original formulation of Freund's vaccine adjuvant [42]. Vaccines prepared by mixing, in equal parts, a Freund's adjuvant and an aqueous antigenic medium are still used as reference standards for laboratory experiments.

In an invention by Aucouturier, *et al.* [43], a vaccine intended for prevention or for treatment of an infectious disease has been described, in particular, an infectious disease engendered by a virus or a micro-organism. The vaccine formulation is encapsulated in a form of water-in-oil (W/O) emulsion, or a water-in-oil-in water (W/O/W) multiple emulsion.

The invention of Baker *et al.*, [44] provides methods and compositions for the stimulation of immune responses using nanoemulsions. Specifically, this invention provides methods and compositions for the use of nanoemulsion as mucosal adjuvants to induce immunity against environmental pathogens, including bioterror pathogens. The invention provides nanoemulsion comprising a nanoemulsion and an inactivated pathogen or protein derived from the pathogen. The vaccine delivery system mainly consist an emulsion and an immunogen, said emulsion comprising an aqueous phase, an oil phase, and a solvent.

4. POLYMERIC NANOPARTICLE DELIVERY SYSTEMS

Polymeric nanoparticles because of their size are preferentially taken up by the mucosa associated lymphoid tissue. They are extensively reviewed for nasal [45] and oral [46] delivery of vaccines. A vaccine delivery system comprising adjuvant selected from the group consisting of cholera toxin, lipid A, and monophosphoryl lipid A and a plurality of nanoparticles comprising immunogenic antigen or nucleic acid encoding an immunogenic antigen. The adjuvant is administered within 24 hours of administering the nanoparticles has been described by Mumper, *et al.* [47]. O'Mahony, *et al.* [48] have described purified synthetic polypeptide ligands for targeting pharmaceutical agents and carriers comprising such agents to intestinal epithelial tissue,

especially Peyer's patch and/or M-Cell tissue. This ligand is non-covalently or covalently bound to a carrier entity comprising a pharmaceutical agent. The carrier entity is selected from the group consisting of a nanoparticle, a microparticle, a liposome, a bacterium, a phage and a virus. A process for the preparation of a colloidal system having a size less than 1 μm suitable for delivery of an active material has been described by Calvo, *et al.* [49], said system comprising a coated member selected from the group consisting of nanodroplet, nanocapsule and nanoparticle, wherein one of said solutions contains said active material and wherein said amino-polysaccharide is between 0.05 and 0.5% by weight and the said phospholipid is between 0.2 and 1% by weight. A colloidal system having a size less than 1 μm comprising a coated member selected from the group consisting of nanodroplet, nanocapsule and nanoparticle comprising a hydrophobic polymer or oil and having a surface coating which is the ionic reaction product of a negatively charged phospholipid and a cationic amino-polysaccharide selected from the group consisting of chitin and chitosan.

A vaccine composition capable of eliciting neutralizing antibodies has been invented by Lowell, *et al.* [50], which has a composition of: (a) an antigen comprising a protein or peptide having (i) an endogenous hydrophobic sequence of between about 3 and about 50 non-polar or uncharged amino acids; (ii) added to the protein or peptide, an exogenous hydrophobic material comprising a sequence of between about 3 and about 50 non-polar or uncharged amino acids or a C₈-C₁₈ fatty acyl group; or (iii) both (i) and (ii), (b) complexed with said antigen, a composition comprising proteosomes, bioadhesive nanoemulsions, or both, wherein said complexed or coupled protein or peptide maintains a native structure of antigenic epitopes such that, upon administration to said subject, the antigen induces neutralizing antibodies in one or more of vaginal secretions, intestinal secretions, lung secretions and feces, capable of neutralizing said pathogenic organism. In another embodiment, Nagy, *et al.* [51] have described nanoparticle formulation comprising a carrier (e.g., polymerized diacetylene); a first ligand displayed on said carrier; and a second ligand, that is different than the first ligand, displayed on said carrier; wherein said first ligand and said second ligand form a polyvalent binding unit that is effective to produce a specific interaction between the nanoparticle and one or more receptors on a target under physiologically relevant shear conditions; and wherein said second ligand interacts specifically with said one or more receptors based on its charge or hydrophobicity. Shefer, *et al.* [52] have invented a controlled release system comprising matrix compositions which control the lag time and release rate of the composition, as well as pharmaceutical and other active ingredients included in the composition, through surface dissolution and/or bulk erosion of the system. The controlled release system can be used to target and control the release of active ingredients onto certain regions of the gastrointestinal tract including the stomach and the small intestine. The matrix compositions of the present invention can be comprised of the following components: a wax material, a fat material, a water sensitive material, and a surface active material. Active agent is encapsulated in a

multicomponent carrier comprising solid hydrophobic nanospheres encapsulated in moisture sensitive or pH sensitive polymeric microspheres. Active ingredients are encapsulated in said hydrophobic nano-spheres. Nanospheres comprise a surface active agent, and a bioadhesive material. The invention [53] is relative to novel means of systemic or mucosal vaccinal therapy against some cancers, viral infections and allergy which are provided by the invention under the form of a family of composite superimmunogenic compounds for bifunctional vaccinal use able to induce an immune response raised towards two distinct targets, respectively, the causal pathogenic antigenic structure, on the one hand, and locally produced factors responsible for a subsequent immunotoxic or neoangiogenic stroma disorder, on the other hand. A use according to any of claims 1 to 8, characterized in that the polypeptide (a) and the polypeptide (b) are both immobilized on one single nanoparticle, or embedded within one single microparticle or within one single nanoparticle.

An effective prophylactic mucosal gene expression vaccine (GXV) described in [54], made up of a cocktail of at least 4 different plasmid DNAs encoding corresponding RSV antigens, coacervated with chitosan to formulate nanospheres. In a murine model of RSV infection, intranasal administration with GXV results in significant induction of RSV-specific antibodies, nasal IgA antibodies, cytotoxic T lymphocytes, and IFN-gamma production in the lung and splenocytes. A single dose of GXV induces a drastic reduction of viral titers. A gene expression vaccine for conferring protection in a host against disease caused by respiratory syncytial virus (RSV) comprising: a plasmid DNA cocktail comprising a combination of at least two RSV antigens selected from the group consisting of F, G, M, M2, SH, NS1, NS2, N, and P; wherein said plasmid DNA cocktail is coacervated with the chitosan to form nanospheres.

Other nanocarrier types that have been used as multivalent vaccine constructs include metallic oxide particles [55], polysaccharide-based spermine, alginate capsules, which are natural polymers [56], and synthetic biocompatible and biodegradable poly(D,L-lactide-co-glycolide) copolymer [57].

5. MICELLAR DELIVERY SYSTEMS

Micelles have been well investigated as potential antigen carriers and are well reviewed [58]. In an embodiment by Scharrenburg, *et al.* [59] composition and formulation of vaccine containing at least one particulate immunogen and an adjuvanting amount of B subunits of heat-labile enterotoxin characteristic of *E. coli*, wherein said at least one immunogen is not covalently coupled to said B subunits, and wherein said at least one immunogen is in the form of aggregates, clusters, micelles, virosomes, or rosettes.

The invention by Moyer [60] teaches methods and systems for generating a safe and effective oral smallpox vaccine for humans using a genetically defective strain of vaccinia virus to confer immunity following oral delivery of the vaccine. This invention is one that expands on current use of vaccinia virus propagation developed for gene therapy applications, and pharmaceuticals and nutraceuticals packaging and formulation technologies. The vaccine

invention can be delivered as a live virus with the ability to express viral proteins but unable to achieve complete, lytic virus replication, or it may be derived from such a virus, contain additional immunogens, or be delivered as viral antigens. Furthermore, the invention establishes innovative methods for formulation and packaging and for preclinical testing of the vaccine invention for safety, efficacy and potency with the use of human intestinal and other test cells and diagnostic test systems and kits. Under the claimed methods, micelles, micro-starch particles, omega-3 fatty acids, and other nanoparticles and immuno-potentiators are methods of preparing the vaccine for use. Methods and systems for generating a safe and effective oral smallpox vaccine for humans using a genetically defective strain of vaccinia virus to confer immunity following oral delivery has been described by Quay [61]. Biologically active agent and permeabilizing peptide are administered in combination with one or more mucosal delivery-enhancing agents such as mixed micelle, liposome, or carrier are one of the examples. The formulation of said biologically active agent with said mucosal delivery-enhancing agents provides for increased bioavailability of the biologically active agent delivered to a mucosal surface of a mammalian subject.

A method for inducing a protective mucosal cytotoxic T-lymphocyte (CTL) response in a mammalian subject has been invented by Berzofsky *et al.* [62] comprising contacting a mucosal tissue of the subject with a composition comprising a purified soluble antigen (cytokine) and an adjuvant cholera toxin (CT), mutant cholera toxin (MCT), or mutant-*E. coli* heat labile enterotoxin (MLT). The absorption-promoting agent is selected from a surfactant, mixed micelle, enamines, nitric oxide donor, sodium salicylate, glycerol ester of acetoacetic acid, cyclodextrin or beta-cyclodextrin derivative, or medium-chain fatty acid.

Formulations and methods for transmucosal delivery of a beneficial agent are described [63] in which a pH-responsive component and a temperature-responsive component are combined. The temperature-responsive component is a component that, in aqueous solutions, is capable of undergoing a temperature-dependent sol to gel phase transition. The formulations may be characterized as having bioadhesive properties, and are suitable for delivery of a variety of beneficial agents. A pharmaceutical formulation for transmucosal delivery of a beneficial agent comprising: (1) a pH-responsive compound; (2) a temperature-responsive compound that in an aqueous medium is capable of undergoing a temperature-dependent sol to gel phase transition; (3) a base; (4) an effective amount of a beneficial agent; and (5) water. The temperature-responsive compound is an alkylene oxide copolymer capable of forming micelles in aqueous solution.

6. DENDRIMER-BASED DELIVERY SYSTEMS

Dendrimers are branched, synthetic polymers with layered architectures that show promise in a number of biomedical applications [64]. Advances in the understanding of the role of molecular weight and architecture on the *in vivo* behavior of dendrimers, together with recent progress in the design of biodegradable chemistries, has enabled the application of these branched polymers as scaffolds for presenting vaccine antigens [64].

A new method of adjuvant delivery using a variety of materials has been developed by Wright [65]. The present invention features a vaccine having a starburst dendrimer as an adjuvant. The preferred vaccine is for influenza and contains an effective amount of a composition in form of an influenza antigen and a starburst dendrimer in a physiologically compatible carrier. The use of the starburst dendrimer makes it possible to adjuvant Influenza without producing a toxic complex since even a small amount of the dendrimer acts as an effective adjuvant. The use of a dendrimer as an adjuvant makes it possible to use an amount of influenza antigen which is substantially reduced from the amount necessary to yield a compatible antigenic response if the antigen is given without the dendrimer. Mid-Generation dendrimers are preferred and yield high antibody titer levels with reduced antigen dosage.

A novel approach to the treatment of renal cell carcinomas using a chimeric molecule comprising a granulocyte macrophage colony stimulating factor (GM-CSF) attached to a G250 kidney cancer specific antigen has been described by Beldegrun *et al.*, [66], which provides a highly effective "vaccine" that raises an immune response directed against renal cell cancers.

The method of transfecting cell is by use of an agent that transfects a cell, said agent selected from the group consisting of a viral vector, a lipid, a liposome, a dendrimer, and a cationic lipid. Advantages of using multifunctional polymers having a smart segment and a biodegradable segment are disclosed Lowe *et al.*, [67]. Advantageously, the biodegradable segment includes a hydrophilic segment and a hydrophobic segment. Embodiments include combining the multifunctional polymeric material with a biologically active substance in an aqueous loading environment and administering the composition as a drug delivery vehicle to a human subject. The polymeric materials of the present invention can have a variety of structures, such as a hydrogel structure, a dendritic structure and other structures including micro- and nano-particulates. A dendritic structure has been disclosed comprising a poly(*N*-isopropylacrylamide) segment or derivative thereof, a poly(L-lysine) segment or derivative thereof, and a poly(lactic acid) segment or derivatives thereof.

7. ISCOMS

Immunostimulating complex (ISCOMs) were first described by Morein and co-workers in 1984 [68] to form a vaccine delivery system that combined certain aspects of virus particles such as their size and orientation of surface proteins, with the powerful immunostimulatory activity of saponins. ISCOMs are open cage-like complexes typically with a diameter of 30-80 nm made up of saponin, cholesterol, phospholipid, and immunogen, usually protein. Unlike other vaccine adjuvants, ISCOMs have shown to promote a broad immune response by simultaneously promoting high levels of antibody and strong T cell responses, including enhanced cytokine secretion and activation of cytotoxic T lymphocyte responses in a variety of experimental animal models [69,70], and have now progressed to phase I and II human trials.

Brunham and Murdin have a number of patents that teaches on two-step immunization procedure against *chlamydia* infection by initial administration of *chlamydia* protein followed by administration of a *chlamydia* protein in ISCOMs [71-74]. Immunogenic compositions have utility as *chlamydial* vaccines and in diagnostic applications, comprising an outer membrane antigen extract of a strain of *Chlamydia* and ISCOM has also been described [75]. Other ISCOM based vaccines are invented for infections by *Moraxella* [76-80], *Helicobacter* infections [81], *Campylobacter* infections [82].

8. CURRENT AND FUTURE DEVELOPMENTS

Liposomal vaccines based on viral membrane proteins (viroosomes) have been approved as products in Europe for hepatitis A and influenza. Squalene O/W emulsion containing influenza vaccines was approved in Italy in 1997 and in several additional countries in 2000. ISCOM-based veterinary vaccine against equine influenza is commercially available. Developments in DNA vaccines, novel proteins and peptides based antigens developed by recombinant technology should open a new frontier for nanocarrier-based vaccine delivery. The fact that nanocarriers can be easily modified for active targeting, i.e., tissue specific delivery to local lymph nodes, cell specific targeting to antigen presenting cells, or targeting to subcellular compartments like nucleus for DNA vaccines. Additional emphasis need to be placed on development of efficient target specific nanocarriers that can preferentially interact with antigen presenting cells upon mucosal administration. In addition, development of novel materials used for nanocarrier design could be synthesized to include potent adjuvant effects. In this case, parallel synthesis methods recently introduced in development of biodegradable polymers should be very useful. Nanocarriers can also be directed for specific type of immune response either Th1 or Th2 depending on the pathogen of interest.

The discovery of more potent and safer adjuvants may allow for development of better prophylactic and therapeutic vaccines against chronic infectious (e.g., Herpes simplex virus, human immunodeficiency virus, hepatitis C virus, hepatitis B virus, human papilloma virus *Plasmodium*, or *Helicobacter pylori*) and non-infectious diseases such as multiple sclerosis, insulin-dependent diabetes, rheumatoid arthritis, allergy and cancer (e.g., melanoma, breast, or colon cancer).

9. CONCLUSIONS

In this article, we have discussed patent literature on various nanocarrier technologies that are used for broad spectrum vaccine or antigen formulation using various nanosized delivery systems like liposomes, micro- and nanoemulsions, polymeric nanoparticles, micelles, and dendrimers. Development of vaccine or antigen engineered nanocarriers are expected to be immunologically more effective over conventional dosage forms since, they can be fabricated to specifically target and be retained at the desired site of action. More importantly, mucosal delivery of nanocarrier antigens and vaccines can trigger immunization at different mucosal barriers which is body's imperative first line defense in addition to systemic immune response. From

the future perspective, development of vaccines using combined strategic approach like nanocarriers delivered by mucosal route of delivery can play a major role in the treatment of infectious diseases.

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