

## Recent Advances In Vaccine Adjuvants; For Effective Immunization

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*Infectious diseases are the world's leading cause of death. Immunization with several vaccines is one of the major achievements of modern medicine. As a result of immunization, diseases such as polio and measles have been controlled and small pox has been eradicated. Vaccines have been shown to be one of the most cost effective, safest and powerful tool that results in reduction in the death rate and increased human survival. When traditional vaccines were developed one knew very little about how diseases were caused and how the immunity worked. Most recent advances in molecular biology, immunology and recombinant DNA technology, one can understand how the antigens are effect to the immune system and how that responds immune activity. Although vaccines produced by recombinant DNA technology are effective, safer than traditional vaccines, which are based on attenuated or inactivated bacteria or viruses, they are often poorly immunogenic. Therefore, adjuvants are often required to enhance the immunogenicity of these vaccines. A number of particulate adjuvants such as emulsions, microparticles, iscoms, liposome's, virosomes, niosomes nanoparticles have been shown to be effective in enhancing the immunogenicity of weak antigens in animal models. The current development researches in vaccinology with DNA vaccines, transgenic vaccines, combination vaccines, transdermal vaccines, cancer vaccines, subunit vaccines, promises on exciting area in prevention and control of infections diseases. The review discusses all recent vaccine adjuvants and delivery systems for effective immunization.*

**Keywords:** Vaccination, Immunization, Adjuvants, nanocarriers, delivery systems.

### Introduction

The science of vaccinology and immunology was born two centuries ago when Edward Jenner proved scientifically the prevention of small pox by inoculation of cow pox virus. This weakened form of *small pox* was called a "Vaccine" from *vacca*, the Latin word for "Cow". Subsequently it was Louis Pasteur who suggested that all the "Immunization" is preferred instead of vaccination. Vaccines are biological products which act by reinforcing the immunological defense of the body against foreign agencies. Used for prophylaxis, they impart active immunity and act as antigens which induce production of specific antibody by the recipient himself since they impart active immunity, they are more efficacious and longer lasting<sup>1,2</sup>. Vaccination works by manipulating the body's own defenses, readying them for the rapid elimination of infections agents and / or their toxic products and providing often lifelong protection from disease for the vaccinated individual. More precisely, vaccine select, activate and expand immunological memory B and T cells which are then poised to respond rapidly and specifically to subsequent pathogen exposure. Immunization with several vaccines is one of the major achievements of modern medicine. Vaccines have been

shown to be one of the most cost effective, safest and powerful tools that result in reduction in death rate and increased human survival. A number of particulate adjuvants such as micro emulsions, microparticles, iscomes, liposomes, virosomes, niosomes have been shown to be effective in enhancing the immunogenicity of weak antigens in animal models<sup>3,4</sup>.

The wide spread use of vaccines over the last few decades has resulted in a reduction in the incidence of many infections diseases in developed countries and especially in the prevention of small pox yellow fever. It is now possible to control hepatitis-B virus infection with recombinant hepatitis vaccine. Development of a large number of new vaccines could greatly reduce the estimated 12 million deaths in children caused by infections diseases in each year world wide

### Novel Approaches to The Development of Vaccine Adjuvants And Delivery Systems

The most challenges for modern vaccine design is to develop strategies for efficiently targeting the innate immune

response to mimic more closely a natural infection and achieve potent responses to recombinant antigens. Improved adjuvants and vaccine delivery systems are required to meet this challenge. The field of adjuvant /delivery system design and development has exploded recently due to the new understanding of how and why adjuvants work. The intense interest in improved adjuvants and delivery systems has been driven by an appreciation of the central role of innate immunity in initiating, amplifying and steering antigen-specific responses. Moreover, the recent identification of numerous pathogen associated molecular patterns (PAMPs) and their PRR which rapidly activate innate immune cells, has provided specific targets for adjuvant discovery and development. These breakthroughs in innate immunology and their local application in modern vaccine design.

Immunological adjuvants were originally described as substances used in combination with specific antigen that produced a more robust immune response than the antigen alone. This broad definition encompasses a very wide range of materials including number of particulate delivery systems eg: emulsions, liposomes, iscoms, virus-like particles and microparticles, whose principal mode of action is to deliver antigens

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into the key cells and /or sites which are responsible for the induction of immune responses.

A key issue in adjuvant development is toxicity, since safety concerns have restricted the development of adjuvants, since Alum was first introduced more than 50 years ago. Many experimental adjuvants and delivery systems have advanced to clinical trials and some have demonstrated high potency, but most have proven too toxic for routine clinical use. For prophylactic immunization in healthy individuals, only adjuvants that induce minimal adverse effects, will prove acceptable. In contrast, for adjuvants which are designed to be used in life-threatening situations eg: as a component of cancer vaccines the acceptable level of adverse events would likely be increased. This might also be true for vaccines to be used therapeutically in people already infected with pathogens with life threatening consequences eg. HIV and HCV. Hence an important contribution of particulate delivery systems may limit the toxicity of new generation adjuvants by limiting their distribution in vivo. Additional practical issues which are important for the development of adjuvants and delivery systems include biodegradability, stability, ease of manufacture, cost and applicability to a wide range of vaccines. Ideally, for ease of administration and enhanced patient compliance, the adjuvant should allow the vaccine to be administered by a mucosal route, preferably orally<sup>5,6</sup>.

## The Role Of Delivery Systems In Vaccine Development

Although the precise mechanisms of action of most adjuvants still remains only partially understood, if the geographical concept of immune reactivity is accepted in which antigens that do not reach the local lymph nodes do not induce responses, it becomes easier to propose mechanistic interpretations of the important effects of adjuvants which work, primarily as delivery systems. Delivery systems may function to improve antigen access to lymph nodes in a number of ways; they may increase cellular infiltration into the injection site so that more cells are present to take up antigen, they may directly promote the uptake of antigen into antigen presenting cells (APC) through activating phagocytosis, or they may directly deliver antigen to the lymph node by exiting from the injection site and moving into lymphatics. The most important APC involved in antigen capture are thought to be dendritic cells (DC) which have unique ability to present antigen to native T cells. Immunization with delivery systems including emulsions, microparticles, liposomes and iscoms has been shown to result in

recruitment of significant numbers of APC's into the injection site, which are then able to take up the delivery system, along with associated antigens and adjuvants, prior to trafficking to the local lymph nodes<sup>7</sup>.

## Need For Vaccine Delivery Systems

The many changes needed in vaccinology are new vaccine formulations that afford very rapid, i.e. within days and also long-lasting immunity after one or two doses. The search for novel and improved vaccines has traditionally been centered on the discovery of new and highly immunogenic antigens, while formulation aspects have received increasing attention only a few years ago in vaccinology. It is now fully recognized that, in addition to the optimal antigen, a successful vaccine requires a stable and adjuvanting formulation, a properly tuned delivery kinetics and an optimal delivery route. Therefore formulation and delivery aspects play an increasing role in vaccine development.

A general difficulty of most current vaccines is that primary immunization requires multiple injections; a child needs to be vaccinated upto 18 times to be considered fully protected against major infection childhood diseases. Failure to the complete booster injections in infant vaccination regimens in a vast majority of the population can have pronounced effect disease state<sup>8</sup>.

New vaccines based on recombinant proteins and DNA, are safer than traditional vaccines, but they are less immunogenic. In recent years, microparticles constructed from biodegradable polymers have shown considerable promise as antigen delivery systems, particularly for DNA vaccines. Microparticles also offer unique opportunities for the development of single dose vaccines, due to the controlled release of entrapped antigens. However, progress has been slow in this area, largely due to problems of instability of entrapped antigens and due to inefficiencies of microencapsulation for many antigens. therefore, there is an urgent need for development of potent and safe adjuvants and delivery systems that can be with new generation of vaccines<sup>9</sup>.

## Delivery Systems For Mucosal Immunization

Although most vaccines have traditionally been administered by intramuscular or subcutaneous immunization, mucosal administration of vaccines offer a number of important advantages; including easier administration, reduced adverse effects and the potential for frequent boosting. In addition, local immunization induces mucosal immunity at the sites where many pathogens initially establish infection

of hosts. Oral immunization would be particularly advantageous in isolated communities, where access to health care professionals is difficult.

Moreover, mucosal immunization would avoid the potential problem of infection due to the re-use of needles. Several orally administered vaccines are commercially available, which are based on live attenuated organisms, including polio, *vibrio cholerae* and *salmonella typhi*. In addition, a wide range of approaches are currently being evaluated for mucosal delivery vaccines, including many approaches involving adjuvants and delivery systems<sup>10</sup>.

The most attractive route for mucosal immunization is oral, due to the ease and acceptability of administration through this route. However, due to the presence of low acidity in the stomach, an extensive range of digestive enzymes in the intestine and a protective coating of mucus which limits access to the mucosal epithelium, oral immunization have proven extremely difficult with non-living antigens. However, novel delivery systems and adjuvants may be used to significantly enhance responses following oral immunization<sup>11</sup>.

Mucosal vaccines have currently been investigated using a broad spectrum of nanocarrier systems such as multiple emulsions, liposomes, polymeric nanoparticles, dendrimers ISCOM's etc<sup>12</sup>.

## Liposomal Delivery Systems

Liposomes are organized phospholipids vesicles than 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<sup>12</sup>. 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 reticuloendothelial 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 GI tract, also offers potential for use in mucosal vaccination<sup>13</sup>.

A formulation for oral delivery of vaccines using polymerized liposomes is taught in the patent of Okada et al. 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 I of this patent states that the invented technology teaches "a method of delivering an antigen to the GI tract of an animal, which comprises of orally administering to said animal, polymerized liposomes comprising a phospholipids 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, secretary or cell mediated immune response against the antigen 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 mucosal tissue<sup>14,15</sup>.

Avantis Pasteur<sup>16</sup> is the assignee of a delivery system consisting of nucleic acid encoding at least a portion of the D 15 outer membrane portion 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. 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, suspension, patches, emulsions, implants, and encapsulated particles, among others, and contains various forms of 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.

Purified and isolated nucleic acid molecules are disclosed, which encode a basal body Rod protein of a strain of *Campylobacter*, particularly *Campylobacter jejuni*, a fragment 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<sup>17</sup>.

Several patents have been cited in literature which describe vaccine preparations for the prevention of Chlamydia infections<sup>18,19</sup>. In one embodiment of the main claims, 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 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<sup>20</sup>. In one of the disclosed invention, vaccine compositions are based on traditional bilayer or multilamellar liposomes, and are phospholipids 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<sup>21</sup>. The invention is based on the discovery that nanoparticle vaccines having multivalent surface antigens (presented on the exterior or interior of 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.

## Micro-/Nano-/Multiple – Emulsion Delivery Systems

Emulsions are mainly used as depot agents in the experimental production of polyclonal antibodies. These heterogeneous 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, micro emulsions 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. 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<sup>22</sup>.

In an invention by Aucouturier, et al.<sup>23</sup>, 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) emulsions, or a water-in-oil-in water (W/O/W) multiple emulsion.

The invention of Baker et al.<sup>24</sup>, 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 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.

## 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 and oral delivery of vaccines<sup>25</sup>. 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.<sup>26</sup> They 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 $\mu$  suitable for delivery of an active material has been described by Calvo, et al.<sup>27</sup> 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 $\mu$  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 phospholipids 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.<sup>28</sup> 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.<sup>29</sup> 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 of hydrophobicity. Shefer, et al.<sup>30</sup> 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 nanospheres. Nanospheres comprise a surface active agent, and a bioadhesive material. The invention 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 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, polysaccharide-based spermine, alginate capsules, which are natural polymers and synthetic biocompatible and biodegradable poly(D,L-lactide-co-glycolide) copolymer<sup>31</sup>.

## Micellar Delivery Systems

Micelles have been well investigated as potential antigen carriers and are well reviewed. In an embodiment by Scharrenburg, et al.<sup>32</sup> 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 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-potentiator 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<sup>33</sup>. 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.<sup>34</sup> 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 in which a pH-responsive component and a temperature-responsive component are combined. The 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<sup>35</sup>.

## Dendrimer-based Delivery Systems

Dendrimers are branched, synthetic polymers with layered architectures that show promise in a number of biomedical applications.<sup>36</sup> 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.

A new method of adjuvant delivery using a variety of materials has been developed by Wright. 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.<sup>37</sup>, 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.<sup>38</sup> 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.

## ISCOMS

Immunostimulating complex (ISCOMs) were first described by Morein and co-workers in 1984 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, phospholipids, 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 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. 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. Other ISCOM based vaccines are invented for infections by Moraxella, Helicobacter infections, Campylobacter infections.<sup>39,40</sup>

## Conclusions

In this article, we have discussed 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, and dendrimers. Development of vaccine or antigen engineered nanocarriers are expected to be immunogenically 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 adjuvants 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 can play a major role in treatment of infectious diseases.

## References

1. Arntzen C.J. Mason H.S. Oral Vaccine production in the edible tissues of transgenic plants in New Generation Vaccines, 2nd Edn, Marcel Dekker Inc, New York; 1997; 263-277
2. Moxon E.R. The Scope of Vaccination, Lancet 1990;335; 448-451.
3. Medzhitor. R, Janewary C.A. Jr. An ancient system of host defence, Annu. Rev. Immunol; 1994;10(1); 12-36.

4. David R Friedn, Drug delivery, pharm news, 2002; 9; 373-375.
5. Udaya Bhaskara Rao Recent Advances in Vaccinology, Ind. J. Biotech, 2003; 2; 494-498.
6. Plotkin S.L. plotkin S.A. A short history of vaccination. Vaccine 3rd edn W.B. Saunders Co. 1999; 1-12.
7. Derek T. Hagam O' Nicholas M.V. Recent advances in Vaccine adjuvants and delivery systems, Pharm, News; 2002; 9; 397-405.
8. Prof. Hans. P. Markle, Dr. Bruno Gander, Dr Lorenz Meinel, Novel opportunities of microparticulates for the delivery of therapeutics and vaccines, Pharm. Tech. 2002; 188-192.
9. Hagam. O' D.T., Singh M, Gupta R.K, Poly (lactide-co-glycoside) Microparticles for development of single-dose controlled release vaccine Adv. Drug. Rev. 1998; 32; 225-246.
10. Derek T., Hagam O' Recent advances in Vaccine Adjuvants for systemic and Mucosal administration, J. Pharm. Pharmacol, 1997; 49; 1-10.
11. Leuine M.M. Dougan G. Optimism over Vaccines administer Via Mucosal surfaces, Lancet. 1998; 351; 1375-80.
12. Chikh. G, Schutze - Redelmeier M.P. Liposomal delivery of CTL epitopes to dendritic cells, Biosci, Rep 2002; 22; 339-353.
13. Gregoriads G. The Immunological adjuvant of Vaccine Carrier properties of liposomes. J. Drug. Target 1994; 2; 351-356.
14. Ambrosch. F. et al Immunogenicity and protectivity of new liposomal hepatitis A Vaccine, Vaccine, 1997; 15; 1209-1213.
15. Hongming C, Torchilin V, Langer R, Polymerized liposomes as potential oral Vaccine Carriers; Stability and bioavailability J. Control. Release 1996; 42(3); 263-272.
16. Pasteur L. De l' attenuation du virus du Cholera des poules in new generation Vaccines, 2nd edn, Marcel Dekker Inc, 1995; 141-228.
17. Chan, V.L, Louie H, Monoclonal antibodies, US patent, 20006020125, 2000.
18. Murdin A.D. Oomen R.P., Wang.J, Dunn P, Vaccine preparations for prevention of Chylamydia infections, US patent, 2005202048, 2005.
19. Maisonneuve J.F, Lucien Prieels Vaccine Comprising a CD8+ T cell immunoprotective, US patent 2005208123; 2005.
20. Tiwari S.B., AMiji M.M. A review of nano carrier - based C.Ns delivery systems, Curr. Drug. Deliv. 2006; 3(2); 219-232.
21. Langer, et. al Polymerized liposome nanoparticles as Vaccine delivery; US patent; 6004534; 1999.
22. Freud J. Thomson K.J. Hough H.B, Sommer H.E. Antibody formation and sensitization with the aid of adjuvants, J. Immunol, 1948; 60; 383-398.
23. Aucouturier. J. et. al. Vaccine formulation in a form of water-in-oil (W/o) emulsions. US patent 2002048587; 2002.
24. Baker et al methods and Compositions for the stimulation of immune response using nanoemulsions, US patent 2003194412, 2003.
25. Illum. L, Nanoparticlelate systems for nasal delivery of drugs; A real improvement over simple systems. J. Pharm. Sci. 2006; 1(1); 16-20.
26. Mumper, et. al Nanoparticles as potential oral delivery systems of proteins and vaccines; A mechanistic approach J. Control release 2006; 116(1) 1-27.
27. Calvo, et al US patent 5843509, 1998.
28. Lowell G.H. Vancott T.C., Brix, D.L. US patent 2002155120; 2005.
29. Nagy J.O. et al US patent 2003223938; 2003
30. Shefer. A. Samuel D. US patent 200440062778;2001.
31. Schwendeman S, Cui C, US patent 20016326021;2001.
32. Scharrenburg et al US patent 200446793928;2004.
33. Quay S.C, US patent 20055232897; 2004.
34. Berzofsky et al, US patent 20055232897;2004.
35. Shanker. G.N, Burke R.L. US patent 2006115532;2006.
36. Boas U, Heegaard P.M. Dendrimers in drug research, Chem. Soc Rev; 2004; 33(1) 43-46.
37. Bellegrun A., Tso C.L, US patent 20022058041; 2006.
38. Lowe T. et al. US patent 2005169882; 2005.
39. Moein B. et al Iscom, a navel structure for antigenic presentation of membrane proteins from enveloped virus, Nature, 1984;308;457-460.
40. Claassen. I. Osterhaus A. The Iscom structure as an immune - enhancing moiety; experience with viral systems, Res immunol; 1992;143;531-541.