NANOPARTICLE VACCINES

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FIELD OF THE INVENTION

The present invention relates to nanoparticle vaccines, comprised of polymerized liposomes, that carry multiple copies of an antigen or combinations of different antigens or carry antigens inside of targeted liposomes and are capable of producing a protective immune response.

BACKGROUND OF THE INVENTION

Infectious diseases have plagued human populations throughout history and still cause the death of millions each year. Both human and other vertebrate organisms become infected with a broad array of microbial pathogens including bacteria, viruses, fungi, and protozoa. Products, which we have developed to protect against infectious diseases, consist primarily of antibiotics and vaccines. However, conventional antibiotics continue to become less effective due to the increased resistance of infectious organisms.

The prevention of clinical symptoms and pathogenic processes via the use of vaccines is considered one of the most effective and desired procedures to combat illness. In this art, antigens or immunogens are introduced in a manner that stimulates an immune response in the host organism prior to infection in order to protect against the infectious disease. However, for many infectious diseases, including malaria, tuberculosis, anthrax, tularemia, brucellosis, Hepatitis C infections, histoplasmosis, coccidioidomycosis, viral hemorrhagic fevers, bubonic plaque, viral encephalitis, Yellow Fever, and viral and bacterial gastroenteritis, there remains no available or effective vaccine.

Multivalent Carriers and Liposome Nanoparticles

In any composition suitable for use as a vaccine, it is essential that the conformational integrity and immunogenic epitopes and antigenic sites be preserved intact. Changes in the structural configuration, chemical charge, or spatial orientation of these molecules and compounds may result in partial or total loss of antigenic activity and utility. The ability of an associated carrier particle to have minimal undesirable reactions in the vaccine and yet facilitate interaction of the antigenic compound with the immune system are primary concerns. All of these factors must be taken into account when preparing a composition as a conjugate that is to be used as a vaccine or as biomaterial for recognition of specific receptors.

It is also well known that many biological systems interact through multiple simultaneous molecular contacts. See, e.g., a comprehensive review by Mammen, et al., "Polyvalent Interactions in Biological Systems: Implications for Design and Use of Multivalent Ligands and Inhibitors," *Angew. Chem. Int. Ed.* 37:2754-2794 (1998). These authors describe a wide variety of polyvalent reagents and the binding interactions between such reagents and various targets, but not in the context of vaccines.

Numerous multivalent constructs have been described in the literature. Brennan, et al., "Cowpea Mosaic Virus as a Vaccine Carrier of Heterologous Antigens," *Mol. Biotechnol.* 17(1):15-26 (2001), discusses chimeric virus particles as carriers of heterologous antigens. In particular, the viral capsid shell was used as a presentation system for antigenic epitopes derived from a number of vaccine targets and inmunizations and resulted in humoral and cellular immune responses against the antigens. U.S. Pat. No. 6,060,064 to Adams, et al., also describes use of a protein carrier used to display immunogenic amino acid sequences for use as a vaccine. Although protein carriers can be effective, it is widely known that it is difficult to produce protein carriers using synthetic chemical methods, resulting in their use being time-consuming and expensive. Additionally, the coupling of an antigen to a protein carrier can alter the immunogenic determinants of the antigen. In many cases a robust immune response can be generated toward the protein carrier and a very minimal response to the hapten.

Other carrier types that have been used as multivalent vaccine constructs include metallic oxide particles (U.S. Pat. No. 6,086,881 to Frey, et al.); polysaccharide-based spermine, alginate capsules, which are non-synthetic (U.S. Pat. No. 5,686,113 to Speaker, et al.); and synthetic biocompatible base polymer of poly lactide-co-glycolide (U.S. Pat. No. 6,326,021 to Schwendeman, et al.). Each of these materials relies on a method of derivatizing a pre-formed particle and the loading of antigen is difficult to control.

Nanoparticle carriers for use as vaccine have also been made from lipids or other fatty acids (U.S. Pat. No. 5,709,879 to Barchfeld, et al.; U.S. Pat. No. 6,342,226 to Betbeder, et al.; U.S. Pat. No. 6,090,406 to Popescu, et al.; Lian, et al., Trends and Developments in Liposome Drug Delivery Systems, *J. of Pharma. Sci.* 90(6):667-680 (2001), and van Slooten, et al., Liposomes Containing Interferon-gamma as Adjuvant in Tumor Cell Vaccines, *Pharm Res.* 17(1):42-48 (2000)), as well as non-lipid compositions (Kreuter, "Nanoparticles and Microparticles for Drug and Vaccine Delivery," *J. Anat.* 189:503-505 (1996)). These described compositions are traditional bilayer or multilamellar liposomes, and are phospholipid based. Such liposomes are physically and chemically unstable, and rapidly leak encapsulated material and degrade the vesicle structure. Without stabilization of the liposome structure, they are not good candidates for oral drug or antigen delivery.

Phospholipids make up the bulk of cell membranes in the body. Phospholipid liposome based carriers have several disadvantages. Being natural-occurring substances, utilized in the membranes of a wide range of pathogenic organisms, the body has devised sensitive ways for differentiating between self and non-self membranes. Part of the protection of "self" comes from the decorations (such as carbohydrates) found on the extracellular side of the phospholipid membranes. Things entering with altered or different "decorations" are recognized as foreign and targeted for opsinization (clearance). Naked (undecorated) phospholipid membranes such as phosphotidylcholine (PC) liposome are rapidly cleared from circulation. This is accomplished by recognition by the RES cells and enzymatic degradation by the body's phospholipases. These enzymes rapidly metabolize phospholipid materials (Waite, *The Phospholipases* Plenum Press, NY (1987)). To retard this process, decoration of the PC membrane with "stealthing" agents, such as polyethylene glycol polymers has been applied. These large polymers shield the phospholipid surface from being "seen" by the immune system. If one uses a phospholipid based carrier, one must employ the cumbersome technique of