Vaccine delivery systems

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ionic polymer surfactants, some MDP analogs and saponins, suggests that acceptable compounds may be developed in the future. Emulsions, principally water in oil emulsions represent an optimal balance of safety, efficacy and cost, especially if more metabolizable oils are employed and less viscous and stable emulsions obtained.

As research progresses providing answers to the many questions that remain concerning the mechanistic aspects of immunostimulation, new and novel approaches to the development of new generations of adjuvants will be within our reach.

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Vaccine delivery systems. J Haensler *(Pasteur Mérieux Sérums et Vaccins, 1541, av Marcel-Mérieux, 69280 Marcy-l'Étoile, France)*

Introduction

Important advances in the development of carrier systems for the delivery of macromolecules have made it possible to apply these systems to vaccine delivery. Many strategies for the ajuvantation of poorly immunogenic purified antigens and for the development of mucosal (especially oral) and single shot vaccines rely on micro-

particulate carriers such as microspheres, liposomes, cochleates and Iscoms. These formulations should lead to improve vaccines, improved vaccination coverage as well as to a reduction in vaccination costs.

Microparticulate carrier systems for antigen delivery

Microparticles

Microparticles are spherical polymeric carriers ranging from 0.1 to 300 µm in size. Microparticles are usually classified into 2 main groups: the reservoir type (microcapsules), with a polymeric membrane surrounding a solution of the active agent, and the monolithic type (microspheres), with the active agent evenly dispersed throughout the polymeric matrix. Microspheres are usually preferred over microcapsules because they allow better control of the release patterns.

The most intensively investigated system for vaccination involves the entrapment of the immunogen into microspheres prepared from the copolymer poly(DL-lactide-co-glycolide) (DL-PLG) (Eldridge *et al,* 1991). DL-PLG is a biocompatible and biodegradable polyester which is utilized in resorbable surgical sutures and controlled-release drug delivery systems.

Liposomes

Liposomes (Gregoriadis, 1990; Alving, 1991) are bilayered membrane vesicles made of water-insoluble amphipathic molecules, usually phospholipids and sterols, surrounding an aqueous solution. Water soluble agents can be entrapped within the inner aqueous space of the vesicle, and alternatively lipid soluble agents can be incorporated into the liposomal membrane. Thus, almost any substance can be entrapped in liposomes.

Although there are many ways of making liposomes, the end product is basically one of 3 types, based on its size and lamellar structure: multilamellar vesicles (MLV), the most frequently used as carriers of vaccines; small unilamellar vesicles (SUV, from 20–60 nm); and large unilamellar vesicles (LUV from 60 nm–10 µm).

Cochleates

Cochleates (Gould-Fogerite and Mannino, 1994) are precipitated sheets of calcium-chelated phospholipid bilayers. They result from the addition of calcium to liposomes made of cholesterol and phosphatidylserine. Since hydrophobic antigenic determinants can be incorporated into the lipid bilayers of cholesterol/phosphatidylserine liposomes to form proteoliposomes, it is possible to prepare cochleates containing such antigenic determinants. Protein cochleates appear as solid cylinders that have no internal aqueous space. Rather, they are composed of a large, continuous, solid lipid bilayer which is rolled up in a spiral. Calcium ions maintain the cochleate in its rolled-up form, interacting with negative charges in opposing bilayers. Cochleates may be stored in calcium-containing buffer or lyophilized to a powder, and reconstituted with liquid just prior to use. When protein cochleates are used as vaccines, it is believed that they slowly unroll *in situ* due to the diffusion of calcium ions in a low calcium environment, and that the resulting proteoliposomes then interact with the cells of the immune system.

Iscoms

Iscoms (Morein, 1988) are negatively charged cage-like pentagonal dodecahedra, 30–40 nm in size. They form spontaneously when cholesterol is mixed with QuilA, a saponin mixture with intrinsic adjuvant properties. Other lipids, such as phos-

phatidylcholine or phosphatidylethanolamine, and amphipatic proteins can be incorporated in the Iscoms during their preparation. Equimolar ratio of Quil A, cholesterol and protein represents an optimal formulation for the Iscom particle. It has been shown that Iscoms retain their integrity and immunogenic properties over a one year storage period.

In vivo *administration and fate of microparticulate carrier systems*

Intramuscular and subcutaneous administration

When given by intramuscular or subcutaneous routes, a small proportion of the particulate carriers (about 20% for standard systems < 1 μm) reach the blood circulation and are rapidly captured by liver, spleen and bone marrow macrophages. The remaining carriers are retained at the site of injection and are attacked by infiltrating macrophages or intercepted by resident macrophages in the lymph nodes during migration through the lymphatics draining the site of injection.

The larger particles (> 15 μm) do not usually redistribute from the site of injection and aborted attemps at phagocytosis by infiltrating macrophages usually leads to the walling off of the particles and to the appearance of fibrosis and granuloma.

Oral administration

The gastrointestinal transit of orally administered particles (Jenkins *et al*, 1994) is fast and the overall absorption of particles from the gastrointestinal tract is low; particles larger than 10 μm are not absorbed from the intestine. Particles < 10 μm are absorbed in Peyer's patches, the mesenteric lymph nodes and the superior mesenteric duct. The larger particles (3–10 μm) are retained for longer periods (up to 35 d) in Peyer's patches and can be used to induce local (mucosal) immune responses. Smaller particles (< 3 μm) are absorbed and retained in Peyer's patches and the mesenteric lymph and subsequently extravasate and disseminate to the mesenteric lymph nodes and to the major organs (*eg*, liver and spleen). Such particles can be used for the induction of both systemic and local responses *via* oral administration.

Aerosol administration to the lungs

The aerosol particles in the form of solids or droplets greater than 10 μm that are inhaled through the nose are trapped entirely by the mucus blanket in the nasal cavity (O'Hagan and Illum, 1990). The smaller the particles, the further back in the nose the deposition will occur. For 5 μm particles, about 50% of the inhaled dose is trapped in the nasal cavity. Particles smaller than 1 μm will not normally settle in the nasal cavity but will be carried further down in the respiratory tract.

When the aerosol particles are inhaled through the mouth, particles larger than 10 μm are deposited by impaction in the upper airways (mouth and pharyngeal surface). Smaller particles are to some extent deposited in the lower airways and partially exhaled, the size between 1 and 2 μm being optimal for deposition in the alveoli.

Devices normally used therapeutically for aerosol delivery typically create polydispersed aerosol particles with a size range of 1–10 μm.

In a context of vaccine delivery, the particles administered into the respiratory tract are both absorbed into the bronchus-associated lymphoid tissue (BALT), which consists of structures similar to the Peyer's patches of the intestine, and phagocytized by alveolar macrophages that can enter the

draining lymphatics and migrate into the bronchial lymph nodes.

The nasal route is useful for formulations that may be sensitive to the conditions found in the gastrointestinal tract (acids, proteases, bile salts). Liposomes are the most exhaustively investigated pulmonary drug carrier to date and have been proven to be safe in humans (Schreier, 1994).

Adjuvant properties of microparticulate antigen delivery systems

To be optimally immunogenic, there should be several copies of the antigen on the microscopic or submicroscopic particle in order to mimic a natural situation. The delivery system technologies available today allow such a multimeric presentation on a particulate carrier. On the other hand, on the basis of information concerning microparticulate fate *in vivo*, it can be assumed that one possible mechanism of immunopotentiation includes a combination of direct delivery of the antigens to antigen-presenting cells (APCs) with a depot effect that allows, upon erosion of the carrier, a continuous exposure to antigen, as occurs in prolonged infection. In addition, some systems have built-in adjuvants (*eg,* QuilA in Iscoms and poly(CTTH-imminocarbonate microspheres [Kohn *et al,* 1986]) or adjuvants that can be coformulated with the antigen in the same carrier (*eg,* monophosphoryl lipid A (MPL) or muramyl dipeptide (MDP) in liposomes [Alving, 1991]). Finally, because some carriers can destablize cellular membranes and have fusogenic properties (*eg,* Iscoms (Morein, 1988], pH-sensitive liposomes [Zhou *et al,* 1992], cationic liposomes [Chen and McCluskey, 1992], they can deliver the antigens directly into the cytosol for MHC class I presentation which leads to the induction of cytotoxic lymphocytes.

Antigen delivery systems with potential for single-step immunization

It is clear that the current methods for achieving long-lasting protective immunity require primary and booster administrations of the immunogen. An advantage of the copolymer DL-PLG microsphere delivery system is the ability to control the time and/or rate at which the incorporated material is released.

In the case of encapsulated vaccines, this allows the scheduling of antigen release in such a way as to mimic conventional primary and booster immunizations. The release of a given antigen from a DL-PLG microsphere is essentially controlled by the size of the microspheres and the ratio of lactide to glycolide in the matrix; the antigen release rate decreases when the ratio of lactide to glycolide in the copolymer increases. There are, therefore, 2 methods for controlling vaccine delivery in order to achieve pulsed release: (i) mixing vaccine microspheres with different sizes; and (ii) mixing vaccine microspheres with different copolymer ratios.

DL-PLG microspheres of less than 10 μm have been used for priming and early boosting (around 2 months) and larger microspheres for late boosting (around 10 months). Similarly, DL-PLG microspheres with lactide to glycolide ratios of 50:50, 85:15 and 100:0 have been combined for priming and early and late boosting (peak responses at 2, 4 and 8 months) (Eldrige *et al,* 1993).

However, since bioerosion of the DL-PLG matrices generates a strongly acidic microenvironment, antigen microencapsulation into DL-PLG particles in general, and in the above described applications in particular, are most compatible with acid-resistant antigens.

Antigen delivery systems with potential for mucosal immunization

Antigens that are inhaled or ingested enter the secondary lymphoid tissues (*eg*, Peyer's patches of the intestine), which are separated from lumenal spaces by a layer of epithelium that is composed of specialized antigen sampling cells, the microfold cells (M cells). These can take up the particulate antigens and transport them by transcytosis to the underlying lymphoid tissues for induction of appropriate mucosal immunity (McGhee and Mestecky, 1990).

A secretory antibody response can be obtained with mucosally administered antigens but it requires antigen doses which are several orders of magnitude higher than those required for systemic immunization. This is the result of 3 major phenomena: (i) limited absorption of the antigen; (ii) enzymatic degradation; (iii) and inactivation by pre-existing antibodies.

Basically, to be a good immunogen, a mucosally applied antigen must survive in the lumenal spaces and be both recognized and taken up by M cells. It has been shown in many models that entrapment of the antigen into particulate carriers (eg, microspheres [Eldridge *et al,* 1989], liposomes [Michalek *et al,* 1992], Iscoms (Mowat and Donachie, 1991] and cochleates [Gould-Fogerite and Mannino, 1994]) can help prevent antigen degradation and enhance antigen delivery to the M cells, leading to the induction of SIgA responses in the mucosal effector sites as well as to serum IgG responses.

Although most of these formulations have pronounced immunogenic effects, the M-cell uptake of orally administered particles remains poor and most of the material is eliminated without uptake (Jenkins *et al,* 1994). In addition, absorption is influenced by other conditions: disease (*eg,* diarrhea); the ingestion of food and drugs; and the acidic and proteolytic properties of digestive secretions.

Some studies on flux vaccines formulated in liposomes or Iscoms have shown that antigen delivery *via* the nasal/pulmonary route can produce local and systemic immunity (Aramaki *et al,* 1994; Jones *et al,* 1988). However, further research is necessary to determine if some or all of the systems that have shown efficacy when given orally could also be administered by aerosol techniques to the lungs.

Conclusion

Among the techniques now available for antigen formulation/encapsulation, those yielding final products that are easy to sterilize by filtration and those avoiding exposure of the antigens to denaturing processes (organic solvents and gamma irradiation) have the most advantages (Esparza and Kissel, 1992).

The compatibility of the antigen with the encapsulation process, the desired conditions for vaccine storage and the administration, efficacy and quality control of the packaged product are factors that will have implications in the choice of a 'carrier formulation' for immunization programs. Even if this requires the study of many parameters and even if any given technology may have to be optimized for each individual antigen/application, it is clear that encapsulation and delivery technologies will play an important role in the design of future vaccines.

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Molecular events in production and transport of secretory IgA: a basis for design of reagents for passive immune protection. JP Kraehenbuhl [1], S Kerneis [1], J Berdoz [1], B Corthésy [2], L Rindisbacher [2], I Corthésy-Theulaz [3], A Phalipon [4], MR Neutra [5], E Pringault [1,6] *([1] Swiss Institute for Experimental Cancer Research and Institute of Biochemistry, University of Lausanne, 1066 Épalinges, Switzerland; [2] Institute of Animal Biology, University of Lausanne, 1015 Lausanne-Dorigny; [3] Division of Gastroenterology, Centre hospitalier universitaire vaudois, 1011 Lausanne, Switzerland; [4] Department of Molecular Pathogenicity, Institut Pasteur, Paris, France; [5] Childrens' Hospital and Harvard Medical School, Boston, MA, USA; [1,6] Institut Pasteur, unité des membranes, Paris, France)*

The major class of antibody produced by mucosal tissues lining the digestive, respiratory and urogenital tracts is secretory IgA (sIgA), made of 2 IgA monomers, one joining (J) chain and 1 or 2 molecules of secretory component (SC). Two cells cooperate to produce sIgA antibodies: the local plasma cells that secrete dimeric IgA and the epithelial cells that express the polymeric immunoglobulin (poly-Ig) receptor mediating transepithelial transport of the dimeric IgA. During transport, the receptor is cleaved and the SC that corresponds to the 5 receptors' Ig-like domains remains covalently bound to the IgA dimer providing resistance to proteolysis. In order to identify the mechanisms whereby sIgA antibodies protect mucosal surfaces, we have developed an *in vitro* system that mimics the *in situ* situa-