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New Delivery Systems for Vaccines

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Immunization against infectious diseases has saved innumerable lives and contributed to today's increased life expectancy. In spite of these impressive results, there is still considerable potential for improved vaccines. New strategies to achieve safe and effective immunization are under investigation. Many vaccination guidelines call for multiple dosing schedules. Reduction of injection frequency, by having controlled release, peroral or nasal vaccine delivery systems could lead to better immunological protection of the population and might facilitate in eradication of some infectious pathogens. Some aspects of safety are closely related to the route of administration, such as granuloma formation or allergic reaction at the injection site and storage conditions. Serious failures of smallpox and measles immunizations have resulted from inadequate refrigeration. New vaccine delivery systems have shown to possess greater effectiveness, improved adjuvanticity, *in vitro* and *in vivo* stabilization of antigens, safety from risk of infections and side effects. Furthermore, results obtained by delivering the new subunit vaccines against diseases such as Hepatitis B, HIV, malaria by novel delivery systems have shown encouraging results. Biodegradable microphereres made up of PLGA, liposomes, nanoparticles, immunostimulating complexes and nonionic surfactant vesicles have been reviewed here and these have found to be promising modes of vaccine delivery.

When Edward Jenner began injecting an extract of cowpox lesions into patients to prevent small pox infection in the eighteenth century, little could he have known how his crude inoculation would revolutionize the science of disease prevention and control. Since those humble beginnings, the science of vaccination has both spurred and adapted biotechnological advances to produce vaccines, which are efficacious and safe. Novel drug delivery systems form a part of these advances.

An ideal vaccine is characterized by the following features[1]; 1) single dose administration, 2) life long immunity, 3) safety from risk of infections and side effects, 4) greater stability, 5) ease of administration (more oral vaccines), 6) simple and cost effective technology for mass production and 7) multipotent hybrid vaccines or formulations such as DPT, so that a single preparation can offer protection for a range of diseases.

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Since the discovery of the "adjuvant effect", i.e. potentiation of the immune response to antigen by addition of certain adjuvants by Le Moignac and Pinay (1916), a host of substances and formulations have been studied for their adjuvanticity[2,3]. However the only licensed adjuvants for vaccines, for human use as on today are, aluminium salts commonly referred to as "alum". Though they are safe, they have a number of disadvantages which include, production of abscesses or nodules at the injection site, instability, failure to work with certain antigens and limited immunostimulatory properties especially with respect to cell mediated immunity. Vehicles such as Freund's adjuvant though have elicited high levels of antibodies and cell mediated immunity, have not been approved for human use because of severe local adverse reactions at the injection site.

In order to challenge the pitfalls of the classical vaccine delivery, the following newer vaccine delivery

systems have been introduced recently :

1) Biodegradable micropheres
2) Liposomes
3) Nanoparticles
4) Immunostimulatory complex (ISCOMS)
5) Nonionic surfactant vesicles

1) BIODEGRADABLE MICROSPHERES

Biodegradable microspheres have met with increasing interest because of their characteristics like protection of sensitive proteins from degradation, prolonged or modified release of the antigen, pulsatile release patterns and intrinsic adjuvant effects of carrier system itself. Choice of an appropriate polymer plays an important role in the design of an injectable microsphere vaccine delivery system. Biodegradable polymers are preferred because surgical removal of the spent device is unnecessary. Biodegradation kinetics of the polymer, mode and rate of antigen presentation, its toxicity and tissue compatibility as well as antigen stability under *in vitro* and *in vivo* conditions would influence the performance of the vaccine delivery system.

A variety of synthetic and naturally occurring polymers have been intensively studied over the past thirty years, but polyesters of poly(lactic acid) (PLA), poly (glycolic acid) (PGA), and their copolymer, poly(lactic-co-glycolic acid) (PLGA) have found widespread use due to their lack of toxicity and their ability to prepare micropheres under mild conditions to protect encapsulated antigen from any harm and they are approved by regulatory authorities as polymeric excipients for microparticles. Copolymers of PLA and PGA are very versatile polymers and by controlling the copolymer composition, monomer stereochemistry, polymer molecular weight and fabrication conditions, biodegradation and antigen release of micropheres can be manipulated to release antigen continuously or in discrete pulses, over one week to one year, thus making these systems promising candidates for use as efficacious vaccines. These systems could also be used as excellent tools for studying the effects of antigen release patterns and vaccine formulation parameters on immune response.

PLGA micropheres have shown to potentiate the immune response, though, the mechanism has not been clearly elucidated. Eldridge *et al.*[4,5,6] determined that subcutaneous enterotoxin B (SEB) encapsulated in DL-PLGA microspheres stimulated immunoglobulin G (Ig G) antitoxin response in mice 500-fold greater than the response induced by an optimal dose of nonencapsulated SEB toxoid. Eldridge also determined that it was necessary for the antigen to be contained within the microspheres to potentiate antibody response. Double walled microparticles[7] for Hepatitis B with inner coat of hydroxy propyl cellulose and outer coat of PLGA elicited higher antibody titre in guinea pigs than two shots of alum formulation. Offit *et al.*[8] have also demonstrated enhancement of rotavirus immunogenicity after microencapsulation.

Controlled delivery of diphtheria toxoid using biodegradable PLGA microspheres have been demonstrated by Singh *et al*[9,10]. It is possible to achieve a pulsatile release of antigens by administering combinations of different size of antigen loaded PLGA. SEB toxoid[4], when administered as a mixture of small (1-10 μm) and larger (20-50 μm) particles together, was able to elicit a strong secondary immune response surpassing those resulting from administration of either size alone or of antigen and alum. These results support the rationale of using smaller particles which are taken up by macrophages to generate primary antibody response and primed memory B-cells. The larger microsphere which cannot be phagocytised release their antigen more slowly and provide the long term persistent levels of antigen that stimulate a strong sustained secondary antibody response.

A pulsatile release pattern which mimics antigen levels obtained with multiple injections was attainable with a single injection in a study with tetanus toxoid[11,12]. After one injection of a mixture of PLGA micropheres of different sizes, with different monomer ratios and antigen loading, antigen levels could be achieved similar to those achieved clinically with injecting one strong initial antigen dose followed by two subsequent booster doses at 1 and 3 months. Cleland *et al.*[13] microencapsulated HIV-subunit antigen MN rgp 120 with DL-PLG in formulation designed to yield an *in vivo* auto boost at 1,2,3, and 4 or 6 months. Pulsed release for ricin toxoid[14] and Staphylococcal enterotoxin[15,16] have also been reported.

A birth control vaccine inducing antibody against pregnancy hormone, Human Chorionic Gonadotropin

(HCG) has reached advanced stage of development;its safety, reversibility and efficacy in preventing pregnancy in women has been demonstrated. Singh and Ganga[16] demonstrated the immunogenic potency of the HCG vaccine delivered through biodegradable polymer DL-PLG. The mean response was compared to that obtained with three injection schedule of alum adsorbed vaccine. The preliminary result showed that a single shot of microsphere encapsulated vaccine produces anti-HCG response comparable to those generated by conventional three injection schedule, indicating the feasibility of delivering the HCG vaccine by biodegradable microspheres.

Biodegradable micropheres for mucosal immunisation:

A majority of infectious disease agents are first encountered through the body's mucosal surfaces including HIV, hence the induction of mucosal immunity has become a central theme of vaccine development today. Eldridge *et al.*[4,18] demonstrated an effective delivery induction of both systemic and mucosal antibody response and immunopotentiating action after oral administration of Staphylococcal enterotoxin B antigen in PLGA micropheres.

A number of investigations have been carried out to show the efficacy of biodegradable microspheres for mucosal immunization. McQueen *et al.*[19] have demonstrated the efficacy of oral immunization with microencapsulated pili of *E. coli* RDEC-1 after intraduodenal administration. O'Hagan *et al.*[20,21] orally immunized mice with cholera toxin B (CTB) entrapped in microspheres. Free CTB does not induce any immunity. A synthetic branched peptide containing the main neutralizing domain of the V3 loop of HIV-1[22] has been incorporated into microparticles prepared from two different PLGA copolymers and poly (DL-lactide) resulting in microspheres similar in size~1μm. Each of these microsphere preparation was administered to a single baboon as a series of one systemic and three doses over four days. Two other baboons were subcutaneously immunized twice with the peptide absorbed into alum, four weeks apart; V3 peptide specific IgG antibody titers and HIV neutralization were superior in sera obtained from baboons immunized with microencapsulated antigen.

Struresson *et al.*[23] incorporated L-α phosphatidyl choline in PLG microspheres making the microsphere more hydrophilic and more efficient in targeting the Rotavirus antigen to the Peyer's patch. Enteric coated microspheres of *Mycoplasma hyopneumoniae*[24] antigen showed effective protection under simulated gastric juices and intestinal conditions. The antigen was enteric coated using cellulose acetate phthalate. Apart from PLGA other materials have also been used to prepare microsphere for oral immunisation. Santiago *et al.*[25] prepared microspheres using derivatised amino acids and demonstrated oral immunisation against ovalbumin antigen. Pang *et al.*[26] showed stimulation of systemic immune response after oral administration of tetanus toxoid in oil based carriers. Alginate-based microspheres[27] has shown enhanced virus-specific humoral immune response following intranasal immunisation in mice. Polyacylamide and poly (butyl-2-cyanoacrylate) microparticles containing OVA have been used by O'Hagan et al.[28] to boost rats orally following an intraperitoneal prime. Degradable starch microparticles containing a glycoprotein fragment from influenza virus have been used to intravaginally immunize sheep. Agarose beads have been used to immunize rats intrabronchially against *Bordetella pertussis*[29].

2) LIPOSOMES :

Liposomes, which represent a new type of fat based encapsulation technology developed for parenteral drug delivery have also been used successfully as carriers for vaccines and adjuvants[30,31]. Liposome is a structure consisting of one or more concentric spheres of lipid bilayers separated by water or aqueous buffer compartments. The most common liposome vaccine composition is dipalmitoyl phosphatidylcholine, cholesterol, diacetylphosphate and antigen. Manufacture of liposome-vaccine formulations is accomplished by gently mixing neutral fat in the presence of phospholipid and dispersing the mixture with aqueous solution containing the antigen by vigorous shaking resulting in spontaneous formation of phospholipid stabilized liposome containing antigen in the core.

Recent experimental evidence suggests that liposomes may indeed play a double role as safe carriers and potent adjuvants for vaccines against viral, bacterial and parasitic infections and various forms of cancer. Liposomes have a number of potential advantages to be used as carriers for vaccine delivery[32].

1) They have tremendous flexibility for incorporating hydrophilic as well as hydrophobic materials under mild conditions with minimized risk of vaccine denaturalization during the encapsulation procedure.

2) An important feature of liposomes as adjuvants is their ability to induce cell mediated immune response, they are naturally taken by macrophages.

3) Typical of the multifarious nature of liposomes is their ability to accommodate a variety of approaches leading to the amplification of immunoadjuvant action. Thus immunity to antigen can be drastically improved in some cases selectively through the administration of liposome together with other adjuvants for example IL-2, muramyl dipeptide Lipid soluble adjuvants such as Lipid A obtained from gram negative bacterial lipopoly-saccharide.

4) It has been found to induce mucosal immunity by stimulating IgA secretion specifically in the oral mucosa. Michelek and coworkers[33] have designed liposomes carrying soluble antigens of *S. mutans* for oral delivery and have demonstrated a significant increase in salivary IgA levels and reduction in *S. mutans* colonization.

Liposomes can serve as a vehicle that allow expression of the adjuvant activity of Lipid A and can simultaneously reduce certain unwanted side effects of Lipid A[34]. Incorporation of Lipid A into liposomes greatly reduces many of the toxic effects normally associated with endotoxins. A successful human trial of alum-adsorbed liposomes containing monophosphoryl lipid A demonstrated that a formulation consisting of a combination of oil in water and adsorbent adjuvants can have considerable safety and efficacy and maybe useful in the development of a potential vaccine against the human malarial parasite *Plasmodium falciparam*[35].

Immunoadjuvant action of liposomes have been studied with other antigens such as *Neisseria gonorrhoeae*[36], Tetanus toxoid[37], Influenza virus[38], *Mycobacterium leprae*[39], Cholera[40], *S. Pneumoniae*,[41] Hepatitis B[42] and many more. It was observed that liposomes in addition to promoting immunity to antigens injected through a variety of parenteral routes also increase IgA immunity to antigens given orally. Liposomes have also been used for the controlled delivery of cytokines which can prove effective in cancer vaccination, the area of vaccination that has not received much attention until recently. Interleukin 2 was among the first cytokines to be encapsulated in liposomes and used as an adjuvant to boost the antitumour immunity[43,44]. Cytokine containing liposomes have also been used as adjuvant for HIV subunit vaccines[45,46].

The susceptibility of conventional liposomes to bile salt dissolution and enzymatic degradation in the gastrointestinal tract may lead to exposure of encapsulated vaccine, resulting in the loss of their protective functions. Hence, polymerised liposomes have been suggested where the liposomes were polymerized with 1,2-di(2,4 octadecadienoyl) sn-glycerol 3-phosphorylcholine. Stability was found to be more than the unpolymerised liposomes. It was observed that the antibody levels induced by Hepatitis B antigen in microencapsulated liposomes were sixty times higher than those induced by Hepatitis B antigen in liposomes[47]. The prolonged production of high levels of antigen specific antibodies when administered in microencapsulated liposomes suggest that they can be used as a long lasting step in immunization strategy.

3) NANOPARTICLES

These are solid colloidal particles ranging in size from 10-100 nm. It has been shown by Kreuter *et al.*[48] that the particle size of the adjuvant can significantly influence the immune response. The most promising nanoparticulate polymer used is poly (methyl methacrylate) (PMMA). The observed slow degradation rate seems to be very promising for vaccines.

The adjuvant effect of whole virus and of subunit influenza vaccines[49] were tested in mice and guinea pigs. Both nanoparticle preparation incorporated as well as adsorbed products were significantly superior to aluminium hydroxide and to fluid vaccines[50]. Nanoparticles have also been found to be promising candidates for HIV. Enormously higher adjuvant effects in comparison to the results obtained with influenza were observed with HIV-I and especially with HIV-2. The antibody response with HIV-2 was 10-200 fold higher than that obtained with aluminium hydroxide[51].

4) IMMUNOSTIMULATING COMPLEXES (ISCOMS):

ISCOMS are stable molecular structures with a mean

diameter of 35 nm in which peptide antigens are incorporated into a matrix of glycoside Quil A, cholesterol and phospholpids[52]. Advantages of ISCOMS as vaccine carriers include no granuloma at the injection site, very low amounts of antigens are immunogenic in ISCOMS with as little as one μg protein being required for optimal responses after parenteral immunization[53]. A unique property of ISCOMS is its capacity to present antigens via both the major histocompatibility complex (MHC) class I and class II pathways. By the intranasal route also, the influenza virus ISCOMS induced cytotoxic T cell responses[54].

One of the most striking properties of ISCOMS is the ability to induce HIV-I dependent cytotoxic T cell response. Very antibody titers as well as strong cell mediated immune response including MHC class I restricted cytotoxic T cell response was induced by ISCOMS containing gp 160 of HIV-I[55]. Combined parenteral and oral immunization with ISCOMS is extremely effective at inducing immunity in the gut and elsewhere offering the potential for combining vaccination routes.

5) NONIONIC SURFACTANT VESICLES (NIOSOMES):

The nonionic block copolymers examined for oral vaccine research have typically consisted of polymers of polyoxypropylene which is hydrophobic and polyoxyethylene which is hydrophilic. Nonionic copolymers of various lengths have been constructed and evaluated as adjuvants in oil-in-water emulsions containing malaria peptides. Features which make non ionic block copolymer attractive as vaccine vehicles are: 1) they are readily produced using standard laboratory procedures, 2) stringent preparation conditions such as organic solvents, shear force and other denaturing procedures are not required, and 3) they are stable. Tomasi *et al.*[56] demonstrated high titer, long lasting mucosal and systemic immunity in mice was induced using multiple emulsion containing antigen and nonionic block polymer PL005 cholera toxin. Rentel *et al.*[57] investigated the potential of new vaccine delivery system based on niosomes. The model protein ovalbumin was encapsulated. Group of female BALB/c mice were fed intragastrically with ovalbumin loaded niosomes, empty niosomes and ovalbumin. Only encapsulation of ovalbumin in niosomes resulted in significant increase in specific antibodies. Thus, the development of peroral vaccine delivery systems based on niosomes seem to be possible.

In conclusion, the delivery of adjuvant active microparticle vaccine delivery system seeks to combine the versatility in release profiles obtained with microparticulate release systems with additional adjuvant effect. The next generation of vaccine vehicles may combine an optimized depot effect with adjuvant active system to improve the immune response. The end result will be a new generation vaccine systems that may not only improve vaccination with existing antigen but may very well make the difference between success and failure with poorly immunogenic subunit antigens.

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