J. Pharm. Pharmacol. 1997, 49: 1–10
Received September 15, 1997

Recent Advances in Vaccine Adjuvants for Systemic and Mucosal Administration*

DEREK T. O'HAGAN

Chiron Corporation, 4560 Horton Street, Emeryville, CA 947608, USA

Abstract

Although vaccines produced by recombinant DNA technology are 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 adjuvants which are particulates of defined dimensions ($< 5\,\mu m$) have been shown to be effective in enhancing the immunogenicity of weak antigens in animal models. Two novel adjuvants which possess significant potential for the development of new vaccines include an oil-in-water microemulsion (MF59) and polymeric microparticles. MF59 has been shown to be a potent and safe adjuvant in human subjects with several vaccines (for example HSV-2, HIV-1 and influenza virus). An MF59 adjuvanted influenza has been recommended for approval in Italy.

Microparticles prepared from the biodegradable polymers the poly(lactide-co-glycolides) (PLG) are currently undergoing extensive pre-clinical evaluation as vaccine adjuvants. Because of their controlled release characteristics, microparticles also possess considerable potential for the development of single dose vaccines. The development of single dose vaccines would offer significant advantages and would improve vaccination uptake rates in at risk populations, particularly in the developing world. In addition to systemic administration, microparticles have also also been shown to enhance the immunogenicity of vaccines when administered by mucosal routes. Therefore microparticles may allow the development of novel vaccines which can be administered by non-parenteral routes. Mucosal administration of vaccines would significantly improve patient compliance by allowing immunization to be achieved without the use of needles.

An alternative approach to the development of mucosally administered vaccines involves the production of genetically detoxified toxins. Heat labile enterotoxin (LT) from *Escherichia coli* and cholera toxin from *Vibrio cholerae* are two closely related bacterially produced toxins, which are the most potent adjuvants available. However, these molecules are too toxic to be used in the development of human vaccines. Nevertheless, these toxins have been modified by site-directed mutagenesis to produce molecules which are adjuvant active, but non-toxic. The most advanced of these molecules (LTK63), which has a single amino acid substitution in the enzymatically active subunit of LT, is active as an adjuvant, but non-toxic in pre-clinical models. The approach of genetically detoxifying bacterial toxins to produce novel adjuvants offers significant potential for the future development of mucosally administered vaccines.

The widespread use of vaccines, which can be defined as agents that induce protective immunity against a pathogen, has had a significant impact on man's health for more than a hundred years. With the exception of the provision of clean water supplies, vaccines represent the most cost-effective public health intervention strategy and their use results in the prevention of many millions of deaths a year (Plotkin & Mortimer 1988; Levine et al 1997). The most notable vaccine success stories are the global eradication of smallpox in 1977, the planned eradication of poliomyelitis by the year 2000 and the Expanded Programme on Immunization (EPI). In the early 1970s, fewer than 5% of the world's infants were immunized against routine childhood infections, resulting in millions of deaths a year from vaccine-preventable diseases. In 1974, the World Health Organization and other interna-

*Conference Science Medal 1997 lecture presented at the British Pharmaceutical Conference, Scarborough, September 15–18, 1997.

Table 1. The characteristics of an 'ideal' vaccine: the Children's Vaccine Initiative, New York, 1990.

Effective after a single-dose
Can be administered early in life
Administered by a mucosal route, preferably orally
Heat-stable during transport and storage
Affordable throughout the world
Applicable to a wide range of diseases

tional agencies initiated the EPI and by 1990, global infant immunization coverage had risen to 80%. More recently, in 1990, the Children's Vaccine Initiative (CVI) was established in New York. Within the CVI, the characteristics of an 'ideal' vaccine for future development were defined (Table 1). Although the goal of developing such a vaccine is a daunting task, significant steps have already been made towards this objective (Levine et al 1997).

New Strategies in Vaccine Development

Vaccines have traditionally consisted of live attenuated pathogens, whole inactivated organisms or inactivated toxins (Table 2). Despite the successes of traditional approaches to vaccine development, alternative approaches are attractive or necessary for a number of reasons, particularly those related to safety. Some live attenuated vaccines can cause disease in immunosuppressed individuals, or in the general population, at very low incidence, through reversion to a more virulent phenotype. Whole inactivated vaccines (e.g. *Bordetella pertussis* and influenza virus) contain reactogenic components which can cause undesirable side-effects in some individuals. Furthermore, some pathogens (e.g. hepatitis B, hepatitis C, human papillomavirus and *Plasmodium* spp) are difficult or even impossible to grow in culture, restricting the opportunity to develop vaccines by traditional methods.

In the last decade several new approaches to vaccine development have emerged which might solve some of the problems associated with traditional vaccines. The approaches include:

- recombinant sub-unit vaccines based on proteins produced in mammalian cells, yeast, bacteria or baculovirus;
- synthetic peptides representing important epitopes from pathogens;
- conjugate vaccines, based on bacterial polysaccharides conjugated to carrier proteins; and
- DNA vaccines, in which genes encoding antigens from pathogens are administered directly.

These new approaches have already resulted in the development of several new vaccines (Table 3).

Table 2. Examples of the traditional approaches to vaccine development.

	Viral	Bacterial
Live, attenuated pathogens	Measles Vaccinia	Mycobacterium BCG *Salmonella typhi*
Inactivated pathogens	Rabies Hepatitis A	*Bordetella pertussis* *Vibrio cholerae*
Toxoids	Not applicable	Tetanus toxoid Diphtheria toxoid
Combination vaccines	MMR	DPT

MMR measles, mumps and rubella vaccine; DPT, diptheria, tetanus and pertussis (whole cell) vaccine.

The first recombinant sub-unit vaccine was developed after the cloning and expression of hepatitis B surface antigen into yeast cells by two of the founders of Chiron Corporation (Valenzuela et al 1982). More recently, the first rationally designed vaccine was developed at Chiron, using site-directed mutagenesis to render the toxin from *B. pertussis* immunogenic but non-toxic (Pizza et al 1989). Polysaccharide-protein conjugate vaccines were first introduced world-wide in the early 1990s against *Haemophilus influenzae* type b, the principal cause of bacterial meningitis in young children. Bacterial polysaccharides are poorly immunogenic, particularly in young children, because they are not recognized by T helper cells. Therefore, the polysaccharides were chemically cross-linked to a protein carrier molecule (usually diphtheria or tetanus toxoid) which was recognized by T cells. These vaccines have proven to be tremendously successful, eliminating Hib disease in some countries and causing a decline in the disease of greater than 95% in others, depending on the immunization level in the population at risk (Rothbrock et al 1995). This success has motivated the development of conjugate vaccines against meningococcus A and C and against pneumococcus. New approaches to vaccine development, in combination with recent advances in adjuvants and delivery systems, have resulted in an expansion of the potential markets for vaccine products and in the establishment of new market areas (Table 4).

DNA immunization

It was discovered in the early 1990s that direct intramuscular injection of plasmid DNA encoding an antigen resulted in the induction of antibody and cell-mediated immune responses and protective immunity (Donnelly et al 1997). The use of DNA

Table 3. Recent successes in vaccine development.

Hepatitis B vaccine	Recombinant hepatitis B surface antigen expressed in yeast or mammalian cells (Valenzuela et al 1982)
Acellular pertussis vaccine	Including a genetically detoxified pertussis toxin (Pizza et al 1989)
Polysaccharide-conjugate vaccines	Bacterial polysaccharides from *Haemophilus influenzae* type b conjugated to a protein carrier (normally tetanus or diphtheria toxoids). Similar vaccines for Meningococcus A and C and Pneumococcus are in development

for vaccination has the potential to revolutionize vaccine development. Plasmid-based DNA vaccines would be very stable and easy and inexpensive to produce. In addition, they would enable the inclusion of many antigens simultaneously, should work even in the presence of maternal antibodies and should induce potent cytotoxic T-cell responses, because antigens produced within host cells should have access to the major histocompat, cability (MHC) class I antigen presentation pathway. DNA immunization is likely to be the safest approach to the development of vaccines against dangerous pathogens (e.g. Ebola virus), because researchers would not be required to work with the pathogen directly. Because antigens encoded by DNA are expressed by host cells, this approach might be optimally exploited to vaccinate against viruses or intracellular bacteria, particularly if attenuated vaccines are not considered an acceptable approach because of safety concerns (e.g. HIV).

However, DNA vaccination remains largely unproven in large-animal models, including man, and there are significant safety concerns which need to be addressed. The potential for genomic integration of DNA and the possibility of insertional mutagenesis is a concern, as too is the induction of an immune response against DNA and the potential for the subsequent generation of autoimmunity. Moreover, in studies performed so far in small-animal models, DNA vaccines have not out-performed alternative approaches, involving immunization with recombinant proteins or attenuated organisms (Manickan et al 1997). Therefore, it seems prudent in the short-term to focus the use of DNA vaccines on to pathogens for which no vaccines are currently available and for which alternative approaches have not proven successful (e.g. HIV, HSV, herpes complex virus (HCV), malaria and *Chlamydia trachomatis*).

Table 4. Potential market growth areas for vaccine development.

Adolescent vaccines	e.g. HIV, HSV, HPV, aP.
Vaccines for the elderly	e.g. Influenza, VZV.
Travellers vaccines	e.g. ETEC, *V. cholerae, S. typhi*, Shigella.
Combination vaccines	e.g. DTaP/Hib, DTaP/Hib/IPV.
Mucosal delivery systems	e.g. microparticles, LT/CT mutants.

HSV, herpes simplex virus; HPV, human papilloma virus; aP, acellular pertussis, VZV, Varicella zoster virus, ETEC, entertoxigenic *E. coli*, DTap, diptheria, tetanus and acellular pertussis-combined vaccine, IPV, inactivated polio virus, LT/CT, heat labile enterotoxin/cholera toxin.

New Directions in Vaccine Development: Vaccines as Drugs

An area with significant potential for vaccine development involves the use of vaccines as therapeutic agents, or 'drugs'. Rather than preventing disease, therapeutic vaccines (or immunotherapeutics) would be designed to eliminate or ameliorate existing diseases, including chronic infectious diseases. The main disease situations in which therapeutic vaccines might prove useful include:

- chronic infections e.g. those caused by HSV, HIV, HCV, hepatitis B vaccine (HBV), HPV or *Helicobacter pylori*;
- tumour vaccines e.g. melanoma, breast or colon cancer; and
- vaccines for allergy and autoimmunity e.g. multiple sclerosis, Type I diabetes and rheumatoid arthritis.

Preliminary studies have indicated that the potency of novel adjuvants (e.g. MF59 emulsion), might promote the development of therapeutic vaccines against chronic infectious diseases (Traquina et al 1996). Moreover, there has been renewed optimism that advances in our understanding of the complexities of the immune response might lead to the development of more effective immunotherapeutic approaches for the control and elimination of tumours (Williams 1996). In addition, studies in pre-clinical animal models have indicated that oral administration of antigens responsible for autoimmune diseases can result in amelioration of the disease process (Weiner 1997). DNA vaccines might have a significant role to play in the development of therapeutic vaccines. It has been suggested that the greatest potential for DNA vaccines is in the re-adjustment of the immune response when the 'normal'

response is inappropriate or ineffective (Manickan et al 1997).

Although the potential development of therapeutic vaccines offers exciting market opportunities, no therapeutic vaccine has yet proven sufficiently effective in a phase III clinical trial to justify the award of a licence. Future developments in this area of vaccinology are very much dependent on our ability to control and manipulate the immune response to a greater extent than has so far been achieved. However, selective manipulation of the immune response might be achieved through the development of novel adjuvants and delivery systems.

The Role of Adjuvants in Vaccine Development

Although newer approaches to vaccine development, particularly the use of recombinant proteins, offer significant advantages over more traditional approaches, a general problem is that the newer generation vaccines are often poorly immunogenic. This is mainly because these vaccines are more highly purified than traditional vaccines and, therefore, do not contain extraneous bacterial or viral components, which often function as built-in adjuvants. These residual components within vaccines e.g. the many proteins administered in the whole-cell *B. pertussis* vaccines, are often the main cause of reactogenicity.

Adjuvants were originally described by Ramon (1924) as "substances used in combination with a specific antigen that produced more immunity than the antigen alone" and this definition remains valid. Despite considerable research over many years, the only adjuvants currently approved for use with vaccines by the Federal Drug Administration of the USA are aluminium compounds (generically called alum). Alum has an excellent safety record, but comparative studies show that it is a relatively weak adjuvant for antibody induction and a poor adjuvant for the induction of cell-mediated immunity (Gupta et al 1995). Therefore, there is an urgent need for the development of new and improved adjuvants and delivery systems which are potent and safe and can be used with new generation vaccines.

The characteristics of an ideal adjuvant

The successful development of adjuvants requires consideration of a number of issues; the characteristics of an 'ideal' vaccine adjuvant are shown in Table 5. The most important issue is safety, because safety concerns have restricted the widespread use of adjuvants in man since alum was first introduced more than 50 years ago (Gupta et al 1995). Many experimental adjuvants have high potency, but are too toxic for clinical use. Adverse events associated with vaccine adjuvants can be a direct consequence of the inclusion of toxic or non-degradable components, or the inclusion of agents that over-activate the immune or inflammatory systems. For standard prophylactic immunization in healthy people, only those vaccine adjuvants that induce a minimum number of side-effects will be generally acceptable. However, for therapeutic vaccination against chronic viral, bacterial, neoplastic or autoimmune diseases more significant side-effects might be acceptable. Overall, an acceptable balance between vaccine potency and vaccine associated side-effects will need to be established in the clinic for each new vaccine application.

Additional issues important for adjuvant development include stability, ease of manufacture, cost, and applicability to a wide range of vaccines. A new adjuvant should have a shelf-life of at least a year, ideally at room temperature. The materials and processes used for adjuvant production will be subject to standard pharmaceutical constraints and suitably pure components should be available in sufficient quantity. Moreover, an ideal adjuvant for world-wide distribution should not add significant cost to the manufacture of a vaccine and should be capable of being administered with a range of antigens by a variety of different routes, including oral or intranasal.

Recent developments in vaccine adjuvants

The two adjuvants which have generated the most data in animal models are alum and Freund's

Table 5. The characteristics of an 'ideal' vaccine adjuvant.

Biodegradable and biocompatible
Should not be toxic, carcinogenic, teratogenic or abortogenic
Non-antigenic and not immunologically cross-reactive with tissue antigens
Induces a minimum of injection site reactogenicity
Simple well defined chemical structure
Induces a minimum of non-specific effects on the immune system
Acceptable for administration to man
Safe to administer to young and immunocompromised individuals
Effective for peptide, protein, polysaccharides and DNA
Effective after a single-dose
Induces both humoral and cell-mediated immunity
Capable of being administered orally
Induces systemic and mucosal immunity
Promotes antigen uptake by lymphoid tissues
Stable formulation which is inexpensive to manufacture
Can be manufactured reproducibly on a large scale
Good shelf-life, preferably without refrigeration
Easy to mix with antigen or combination of antigens

adjuvants. Freund's adjuvants are water-in-oil emulsions, which are generally more potent than alum. Freund's complete adjuvant contains immunostimulatory compounds from *Mycobacterium tuberculosis*, while the less toxic incomplete Freund's adjuvant contains only mineral oil and the emulsifier Arlacel A. Although incomplete Freund's adjuvant has been widely used in man, it has never been approved for commercial use despite the absence of significant toxicity (Gupta et al 1993). Alum is widely used in vaccines for man, but is a relatively poor adjuvant for most antigens and does not induce cell-mediated immunity (Gupta et al 1995). A simplified representation of the immune response to a vaccine antigen is shown in Figure 1.

The adjuvant activity of the immunostimulatory peptidoglycan from *M. tuberculosis* in Freund's complete adjuvant has been shown to reside in an *N*-acetyl muramyl-L-alanyl-D-isoglutamine (MDP) fraction. However, MDP also has significant toxicity. Nevertheless, a number of derivatives of MDP with reduced toxicity have been described and several have been evaluated in clinical trials (Ott et al 1992). A second class of immunostimulatory compounds which have been evaluated as adjuvants are derived from the lipopolysaccharide of Gram-negative bacteria. The most extensively evaluated member of this family, monophosphoryl lipid A, obtained from *Salmonella minnesota*, has

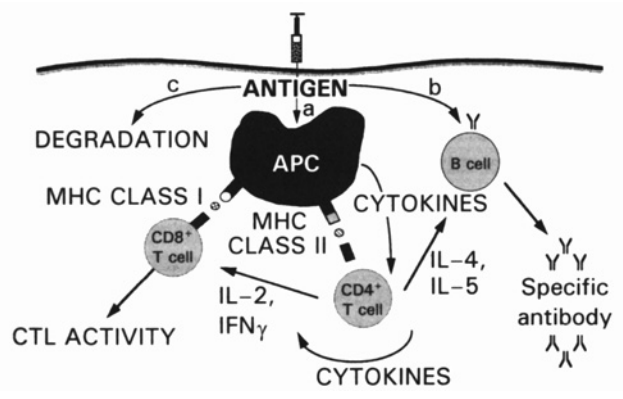

Figure 1. A simplified representation of the immune response to a vaccine antigen. Antigen injected intramuscularly might be: (a) taken up by antigen-presenting cells (APC); (b) bound to surface antibody on B cells; or (c) degraded. In general, adjuvants act to upregulate the immunological activity of any of the cell-types depicted. Antigen-delivery systems modify the distribution of antigen (or adjuvant) through these pathways. Antigen taken up by antigen-presenting cells is processed into peptide epitopes, which use two discreet pathways to MHC molecules (class I and II), which present peptide for interaction with either $CD8^+$ or $CD4^+$ T cells, respectively. The stimulated T cells might generate cytokine signals to upregulate the immune response or act as cytotoxic T lymphocytes. Antibodies are produced by B cells with help provided by the cytokines (interleukins (IL) and interferons (IFN) produced by the $CD4^+$ T cells.

been defined as penta- and hexaacyl derivatives of diglucosamine monophosphate (Ulrich & Ulrich 1995). Monophosphoryl lipid A has been evaluated in the clinic with a range of antigens and shown to have adjuvant activity, with tolerable side-effects (Ulrich & Ulrich 1995). A third group of immunostimulatory compounds which have been widely evaluated are the triterpenoid glycosides derived from *Quillaja saponaria*. The fraction QS21 was isolated by Kensil, who defined the structural groups responsible for adjuvant activity (Soltysik et al 1995). Clinical testing has shown some adjuvant activity for QS21, but also 'flu-like' side-effects (Livingston et al 1994).

The agents which the immune system has evolved to combat (viruses, bacteria and parasites) are particulate in nature and are normally efficiently phagocytosed by macrophages and other antigen-presenting cells. Therefore, it seemed rational to develop particulate antigen delivery systems (e.g. microparticles, iscoms, liposomes and emulsions) which can be manufactured in a size-range comparable with that of natural pathogens (20 nm to 2 μm).

The development of MF59, a submicron emulsion adjuvant

Despite the potency of Freund's adjuvants, unacceptable toxicity prompted researchers to pursue alternative approaches to the development of adjuvants. Liposomes have been evaluated extensively as vehicles for both antigens and adjuvants (Gregoriadis 1990; Alving 1992), but no liposomal vaccines have yet been commercially developed. Immunostimulatory fractions from *Quillaja saponaria* have been incorporated into particulate structures which contain antigen (iscoms) or are co-administered with free antigen (iscomatrix) (Bengtsson & Sjölander 1996). A successful vaccine against equine influenza has been developed using the iscoms approach and a vaccine for man is currently under clinical evaluation. The submicron squalene-water emulsion (MF59) was initially designed as a delivery vehicle for a synthetic MDP derivative, sodium *N*-acetyl-muramyl-L-alanyl-D-isoglutaminyl-L-alanyl-2-(1′, 2′-dipalmitayl-sn-glycero-3′phospho) ethylamide (MTP) (Ott et al 1995). The chemical components of MF59, the oil squalene (a terpenoid cholesterol precursor) and the surfactants sorbitan trioleate (span 85) and polyoxyethylene sorbitan mono-oleate (Tween 80) have all been used in medicinal products for man. In a number of animal models MF59/MTP was a significantly more potent adjuvant than alum and in some studies approached the potency of Freund's. However, no significant toxicity was observed with

MF59/MTP (Ott et al 1995). Rather surprisingly, further studies showed that the MTP was not necessary for the induction of potent antibody responses and that the emulsion alone was a potent adjuvant. Moreover, reduction of the size of the emulsion droplets from 1–2 μm to 200–300 nm resulted in a significant increase in adjuvant activity. Preclinical evaluation of MF59 resulted in the induction of antibody titres in a number of animal models, including primates, which were 5–50-fold higher than those achieved with alum (Ott et al 1995). The occurrence of unacceptable side-effects after administration of MTP to individuals previously exposed to HSV or influenza (Couch 1993) and the observation of the adjuvant effect of the emulsion alone, resulted in clinical evaluation of MF59 as a vaccine adjuvant.

Experience in the clinic (>8000 subjects immunized), with vaccines including HIV, HSV, CMV, HBV and influenza, has shown that MF59 is safe and well tolerated in both seropositive and seronegative individuals (Kahn et al 1994; Langenberg et al 1995). Local effects were limited to transient erythema at the injection site and systemic effects were limited to flu-like symptoms, such as headache and fever, which tended to resolve within two days (Langenberg et al 1995). These effects were judged to be sufficiently minor to allow the vaccination of newborn infants with MF59 in a HIV vaccine trial. The potency of MF59 for HSV and HIV vaccines in phase I/II clinical trials has been shown to be comparable with preclinical models (Kahn et al 1994; Langenberg et al 1995). In the HSV clinical trial, the antibody titres induced by recombinant glycoproteins were significantly higher than those induced by the same antigens in combination with alum. Moreover, the neutralizing titres were similar to those induced after natural infection with the virus. In both the HIV and the HSV trials, strong helper T-cell responses were observed in seronegative individuals. The overall conclusion from all clinical trials with MF59 is that the adjuvant is safe and effective in man in combination with a variety of antigens. In a recent preclinical study in primates the range of antigens for which MF59 is effective was extended to include polysaccharide-protein conjugate vaccines (Granoff et al 1997).

Biodegradable microparticles as vaccine adjuvants

Over the last twenty years, the adjuvant effect achieved through the association of antigens with polymeric microparticles has been repeatedly demonstrated (O'Hagan 1994, 1997). Encapsulation of antigens into microparticles, including submicron particles, promotes their entry into lymph nodes and provides a high local concentration of antigen over an extended time-period. Microparticles also promote the interaction of encapsulated antigens with antigen-presenting cells e.g. macrophages.

The poly(lactide-co-glycolides), biodegradable and biocompatible polyesters, are primary candidates for the development of microparticles as vaccines, because they have been used in man for many years as suture material and as controlled-release delivery systems for peptide drugs (Wise et al 1979). However, the adjuvant effect achieved by the encapsulation of antigens into poly(lactide-co-glycolide) microparticles has been demonstrated only relatively recently (O'Hagan et al 1991a, b; Eldridge et al 1991). Particle-size was shown to be an important factor affecting immunogenicity, because smaller microparticles (<10 μm) were significantly more immunogenic than larger particles (>10 μm) (Eldridge et al 1991; O'Hagan et al 1993). The adjuvant effect of microparticles can also be enhanced by co-administration with additional adjuvants (O'Hagan et al 1991b). Recent studies have shown that microparticles also exert an adjuvant effect for cell-mediated immunity, including the induction of cytotoxic T-cell responses after both systemic and mucosal administration (Maloy et al 1994; Moore et al 1995). The induction of cytotoxic T-cell responses are important for the eradication of virally infected cells and for immune responses against alternative intracellular pathogens.

In the long-term one of the most attractive features of microparticles for vaccine development is their use to control the rate of release of entrapped antigens (O'Hagan 1997). Ultimately, this might enable the development of single-dose vaccines, through the preparation of microparticles which release entrapped antigens at the times when booster doses of vaccines would normally be administered. The development of a single-dose vaccine would represent a significant step towards the development of an ideal vaccine (Table 1) and would result in improved vaccine compliance, particularly in the developing world. In a recent study with rats a single immunization with tetanus toxoid entrapped in controlled-release microparticles induced immunity comparable with that after three doses of tetanus toxoid adsorbed on alum (Singh et al 1997). In addition, a single-dose of microparticles with an entrapped peptide (O'Hagan et al 1995) or protein (Cleland et al 1994) from HIV-1 induced neutralizing antibodies for at least one year. Nevertheless, further research is needed to promote the stability of antigens during microencapsulation and after in-vivo adminis-

Table 6. The advantages of poly(lactide-co-glycolide) microparticles for vaccine development.

Safety: biodegradable and biocompatible polymers
Acceptable for administration to man
Controlled-release might enable the development of single-dose vaccines
Adjuvants might be entrapped in the microparticles
Many antigens can be entrapped simultaneously in the micro particles
Microparticles might be administered by mucosal routes, including oral delivery
Antigens are protected from degradation in the intestine
Antigens are targeted to lymphoid tissue
Microparticles induce serum and secretory antibodies
Microparticles induce cell-mediated immunity
Freeze-dried formulations, with enhanced stability for entrapped antigens
Large scale manufacture of microparticles has already been achieved

tration. The potential advantages of microparticles for vaccine development are shown in Table 6.

Future developments in vaccine adjuvants: mucosal delivery

Mucosal administration of vaccines is an attractive approach which offers several significant advantages over the traditional approach to vaccine delivery, intramuscular injection. The advantages of mucosal delivery include easier administration, reduced side-effects and the potential for frequent boosting without the need for trained personnel. Moreover, mucosal delivery of vaccines is the only effective mean of inducing immune responses in the mucosal secretions of the body. This is important, because the majority of pathogens initially infect hosts through the mucosal tissues of the gut or the respiratory or genital tracts. In addition, because the protective barrier of the skin is not breached during mucosal administration, the potential for the introduction of infection through the use of 'dirty' needles is eliminated.

In mice, oral immunization with poly(lactide-co-glycolide) microparticles induced potent serum IgG, secretory IgA and systemic cytotoxic T-cell responses (Eldridge et al 1990; Challacombe et al 1992; Maloy et al 1994). Although relatively large doses of antigens were used in these studies (at least 100 μg), a single oral dose of 10 μg fimbriae from *B. pertussis* in microparticles protected mice from intranasal challenge (Jones et al 1996). In addition, intranasal immunization with 1–10 μg *B. pertussis* antigens in microparticles also induced protective immunity in mice against aerosol challenge (Cahill et al 1995; Shahin et al 1995). In primates, intra-tracheal or oral delivery of micro-encapsulated inactivated SIV in parenterally primed animals induced protective immunity against intra-vaginal challenge with the virus; systemic immunization alone did not protect (Marx et al 1993). Also in a primate study, intra-tracheal immunization induced protection against aerosol challenge with staphylococcal enterotoxin B (Tseng et al 1995). Recently, microparticles have also been shown to be effective for the oral delivery of plasmid DNA in mice (Matiowitz et al 1997).

Initial observations in small-animal models have indicated that rectal immunization might also be exploited using particulate antigen delivery systems (Zhou et al 1995). Delivery via the rectal route targets antigens to the abundant lymphoid tissues present in the local mucosal epithelium and avoids exposure to the enzymes and low pH of the upper gastrointestinal tract. Nevertheless, there are considerable problems associated with rectal delivery, including lack of cultural acceptability in some areas. In addition, the dosage form containing the vaccine might be expelled from the rectum before it has time to be effective and this might be difficult to control, particularly with young children.

Several different approaches to the mucosal delivery of vaccines have recently been evaluated, particularly for the oral route. These include live genetically attenuated bacterial vectors, including Salmonella and Lactococci spp, and live viral vectors, e.g. adeno, polio and vaccinia. In addition, non-living delivery systems have also been evaluated for the mucosal delivery of vaccines, including the toxins of *Vibrio cholerae* and *Escherichia coli*, lectins, liposomes and iscoms. These alternative approaches to mucosal vaccine delivery have recently been reviewed in multi-authored books (O'Hagan 1994; Kiyono et al 1996). A novel approach to the development of mucosal adjuvants is represented by the genetic manipulation of bacterial toxins, e.g. those of *V. cholerae* and *E. coli*, to render them non-toxic but still adjuvant-active (Douce et al 1995, 1997). The genetic manipulation of bacterial toxins was pioneered by Rappuoli's group (Pizza et al 1989), and was first applied to *B. pertussis* toxin for the development of a rationally designed, genetically engineered vaccine (Rappuoli 1997). Subsequently, the same approach has been applied to *V. cholerae* and *E. coli* toxins. A single amino acid substitution in the enzymatically active A sub-unit of *E. coli* toxin enabled the development of a completely non-toxic molecule (LTK63), which retained adjuvant activity when administered by several mucosal routes (Di Tommaso et al 1996). Native *V. cholerae* and *E. coli* toxins had previously been shown to be potent adjuvants after oral and intranasal administration to man, but they are too toxic to be used in vaccines. The modification of these

potent toxins to reduce or eliminate toxicity might enable their use as adjuvants for a wide range of vaccines, to be administered by several mucosal routes.

The prospects for the development of novel adjuvants

The successful use of MF59 in the clinic and the absence of significant adverse effects suggests that several MF59-adjuvanted vaccines are likely to be developed in the near future. Indeed, the first product incorporating MF59 as an adjuvant, an influenza vaccine, was introduced on to the market in Italy in 1997. It is confidently expected that additional vaccine products incorporating MF59 as an adjuvant will be introduced in the coming years.

Although microparticles offer considerable promise for the development of new and improved vaccines, much work still needs to be done. The most important issue in relation to the potential development of single-dose vaccines is the stability of antigens in microparticles. Recent work has demonstrated that human growth hormone is stable in-vivo for at least one month after administration in microparticles (Johnson et al 1996). Nevertheless, additional work is needed to obtain a better understanding of the mechanisms governing protein stability in microparticles and the steps which can be taken to overcome specific problems with antigens.

A possible limitation of the use of microparticles as oral vaccines is their apparently low efficiency of uptake across the intestinal epithelium (O'Hagan 1996). Hence, it might prove attractive to target microparticles to the cells which are responsible for their uptake, the M cells of the Peyer's patches (Kato & Owen 1994). Traditionally, the oral route has proven difficult to exploit for vaccines, because of enzymatic degradation, dilution effects in the intestine, the low pH, and the poor absorption of proteins and peptides. Nevertheless, the oral route remains the most attractive approach and research will continue in this area. If oral delivery of microparticles proves too difficult to exploit clinically, intranasal delivery of microparticles might be an attractive option. The nasal cavity is readily accessible for administration and presents fewer problems than the gut. Moreover, many important pathogens initially infect the host through the nasal cavity. It seems likely that genetically detoxified toxins, e.g. those of *V. cholerae* and *E. coli*, will play an important role in the future development of intranasal vaccines. However, these molecules are likely to require formulation into specialized delivery systems to optimize their efficacy in the nasal cavity of man. Developments in oral delivery systems to protect mutants of the toxins of *V. cholerae* and *E. coli* against low pH and enzymatic degradation might also enable the development of orally administered vaccines.

It seems likely that future success in the mucosal delivery of vaccines will be driven by partnerships between those generating novel biological approaches and those able to deliver these molecules optimally to the relevant anatomical sites. Hence, it should be clear that pharmaceutical scientists have a valuable role to play in the future development of optimum delivery systems for both systemic and mucosal vaccines.

Conclusions

The only adjuvants currently approved for administration to man are aluminium compounds, which are weak adjuvants for antibody induction and are ineffective for the induction of cell-mediated immunity. In contrast, MF59 has been shown to be a potent and effective adjuvant in more than 8000 subjects in clinical trials, without inducing significant adverse effects. Hence, MF59 shows considerable promise for approval as a new vaccine adjuvant for a number of vaccines for man. In addition, microparticles represent an alternative approach to vaccine delivery which might have some advantages over MF59. Microparticles might prove particularly advantageous for mucosal delivery of vaccines or for the induction of cell-mediated immunity. In addition, microparticles might enable the development of single-dose vaccines through the use of controlled-release technology. Genetically detoxified bacterial toxins (e.g. LTK63) also show considerable promise for the future development of mucosally administered vaccines. However, the efficacy of microparticles and mutant toxins has yet to be demonstrated in man.

Through the advent of genomics and rapid screening techniques, the discovery of new antigens from pathogens has become quicker and more routine than ever before. Consequently, perhaps the greatest current challenge for vaccine development lies in the delivery of antigens for the induction of the optimum immune responses. This might be achieved through the use of novel adjuvants and delivery systems and might involve the selective induction of a specific antibody isotype or a T-cell subset, or it might involve the induction of mucosal immunity. Pharmaceutical scientists have much to contribute to the formulation of delivery systems for mucosal administration and to the selective targeting of antigens and adjuvants to specific antigen-presenting cells after systemic administration.

Acknowledgements

I would like to acknowledge the significant contributions made by Rino Rappuoli, Gary Van Nest, Gary Ott and Dan Granoff to the ideas contained in this review. I would also like to acknowledge the significant contributions made by Ph.D. students, research fellows, colleagues and collaborators to our joint publications.

References

Alving, C. R. (1992) Immunologic aspects of liposomes. Presentation and processing of liposomal protein and phospholipid antigens. Biochim. Biophys. Acta 1113: 307–322

Bengtsson, K. L., Sjölander, A. (1996) Adjuvant activity of iscoms: effect of ratio and co-incorporation of antigen and adjuvant. Vaccine 14: 753–760

Cahill, E. S., O'Hagan, D. T., Ilum, L., Barnard, A., Mills, K. H. G., Redhead, K. (1995) Immune responses and protection against *Bordetella pertussis* infection after intranasal immunization of mice with filamentous haemagglutinin. Vaccine 13: 455–462

Challacombe, S. J., Rahman, D., Jeffery, H., Davis, S. S., O'Hagan, D. T. (1992) Enhanced secretory IgA and systemic IgG after oral immunization with biodegradable microparticles. Immunology 76: 164–168

Cleland, J. L., Powell, M. F., Lim, A., Barron, L., Berman, P. W., Eastman, D. J., Nunberg, J. H., Wrin, T., Vennari, J. C. (1994) Development of a single-shot sub-unit vaccine for HIV-1. AIDS Res. Hum. Retro. 10: S21–S26

Couch, R. B. (1993) Advances in influenza virus vaccine research. Ann. NY Acad. Sci. 685: 803–812

Di Tommaso, A., Saleti, G., Pizza, M., Rappuoli, R., Dougan, G., Abrignani, S., Douce, G., De Magistris, T. (1996) Induction of antigen-specific antibodies in vaginal secretions by using a nontoxic mutant of heal labile enterotoxin as a mucosal adjuvant. Infect. Immun. 64: 974–979

Donnelly, J. J., Ulmer, J. B., Liu, M. A. (1997) DNA vaccines. Life Sci. 60: 163–172

Douce, G., Turcotte, C., Cropley, I., Roberts, M., Pizza, M., Domenghini, M., Rappuoli, R., Dougan, G. (1995) Mutants of *Escherichia coli* heat-labile enterotoxin lacking ADP-ribosyl transferase activity act as non-toxic mucosal adjuvants. Proc. Natl Acad. Sci. USA 92: 1644–1648

Douce, G., Fontana, M., Pizza, M., Rappuoli, R., Dougan, G. (1997) Intranasal immunogenicity and adjuvanticity of site-directed mutant derivatives of Cholera toxin. Infect. Immun. 65: 2821–2828

Eldridge, J. H., Hammond, C. J., Meulbroek, J. A., Staas, J. K., Gilley, R. M., Tice, T. R. (1990) Controlled vaccine release in the gut associated lymphoid tissues. I. Orally administered biodegradable microspheres target the Peyer's patches. J. Contr. Rel. 11: 205–214

Eldridge, J. H., Staas, J. K., Meulbroek, J. A., Tice, T. R., Gilley, R. M. (1991) Biodegradable and biocompatible poly (DL-lactide-co-glycolide) microspheres as an adjuvant for staphylococcal enterotoxin B toxoid which enhances the level of toxin-neutralizing antibodies. Infect. Immun. 59: 2978–2986

Granoff, D. M., McHugh, Y. E., Raff, H. V., Mokatrin, A. S., Van Nest, G. (1997) MF59 adjuvant enhances antibody responses of infant baboons immunized with haemophilus influenzae Type b and *Neisseria meningitidis* Group C oligosaccharide-CRM197 conjugate vaccine. Infect. Immun. 65: 1710–1715

Gregoriadis, G. (1990) Immunological adjuvants: a role for liposomes. Immunol. Today 11: 89–97

Gupta, R. K., Relyveld, E. H., Lindblad, E. B., Bizzini, B., Ben-Efraim, S., Gupta, C. K. (1993) Adjuvants – a balance between toxicity and adjuvanticity. Vaccine 10: 96–97

Gupta, R. K., Rost, B. E., Relyveld, E., Siber, G. R. (1995) Adjuvant properties of aluminium and calcium compounds. In: Powell, M. F., Newman, M. J. (eds) Vaccine Design, The Sub-unit and Adjuvant Approach. Plenum Press, New York, pp 229–248

Johnson, O. L., Cleland, J. L., Lee, H. J., Charnis, M., Duenas, E., Jaworowicz, W., Shepard, D., Shahzamani, A., Jones, A. J. S., Putney, S. D. (1996) A month-long effect from a single injection of microencapsulated human growth hormone. Nature Med. 2: 795–799

Jones, D. H., McBride, B. W., Thornton, C., O'Hagan, D. T., Robinson, A., Farrar, G. H. (1996) Oral immunization with microencapsulated pertussis fimbriae induces protective immunity in mice. Infect. Immun. 64: 489–494

Kahn, J. O., Sinangil, F., Baenziger, J., Murcar, N., Wynne, D., Coleman, R. L. (1994) Clinical and immunologic responses to human immunodeficiency virus (HIV) Type I SF2 gp120 sub-unit vaccine combined with MF59 adjuvant with or without muramyl tripeptide dipalmitoyl phosphatidylethanolamine in non HIV-infected human volunteers. J. Infect. Dis. 170: 1288–1291

Kato, T., Owen, R. L. (1994) Structure and function of intestinal mucosal epithelium. In: Ogra, P. L., Lamm, M. E., McGhee, J. R., Mestecky, J., Strober, W., Bienenstock, J. (eds) Handbook of Mucosal Immunology. Academic Press, San Diego, pp 11–26

Kiyono, H., Ogra, P. L., McGhee, J. R. (1996) Mucosal Vaccines. Academic Press, San Diego

Langenberg, A. G. M., Burke, R. L., Adair, S. F., Sekulovich, R., Tigges, M., Dekker, C. (1995) A recombinant glycoprotein vaccine for herpes simplex type 2: safety and immunogenicity. Ann. Intern. Med. 122: 889–898

Levine, M. M., Woodrow, G. C., Kaper, J. B., Cobon, G. S. (1997) New Generation Vaccines. 2nd edn, Marcel Dekker, New York

Livingston, P. O., Adluri, S., Helling, F., Yao, T. J., Kensil, C. R., Newman, M. J., Marciani, D. (1994) Phase I trial of immunological adjuvant QS-21 with a GM2 ganglioside-KLH conjugate vaccine in patients with malignant melanoma. Vaccine 12: 1275–1280

Maloy, K. J., Donachie, A. M., O'Hagan, D. T., Mowat, A. M. (1994) Induction of mucosal and systemic immune responses by immunization with ovalbumin entrapped in poly(lactide-co-glycolide) microparticles. Immunology 81: 661–667

Manickan, E., Karem, K. L., Rouse, B. T. (1997) DNA vaccines – A modern gimmick or a boon to vaccinology. Crit. Rev. Immunol. 17: 139–154

Marx, P. A., Compans, R. W., Gettie, A., Staas, J. K., Gilley, R. M., Mulligan, M. J., Yamschchikov, G. V., Chen, D., Eldridge, J. H. (1993) Protection against vaginal SIV transmission with microencapsulated vaccine. Science 260: 1323–1327

Matiowitz, E., Jacob, J. S., Jong, Y. S., Carino, G. P., Chickering, D. E., Chaturvedi, P., Santos, C. A., Vijayaraghavan, K., Montgomery, S., Bassett, M., Morrell, C. (1997) Biologically erodible microspheres as potential oral drug delivery systems. Nature 386: 410–414

Moore, A., McGuirk, P., Adamd, S., Jones, W. C., McGee, J. P., O'Hagan, D. T., Mills, K. H. G. (1995) Immunization with a soluble recombinant HIV protein entrapped in biodegradable microparticles induces HIV-specific $CD8^+$ cyto-

toxic T lymphocytes and $CD4^+$ Th1 cells. Vaccine 13: 1741–1749

O'Hagan, D. T. (1994) Microparticles as oral vaccines. In: O'Hagan, D. T. (ed.) Novel Delivery Systems for Oral Vaccines. CRC Press, Boca Raton, pp 175–205

O'Hagan, D. T. (1996) The intestinal uptake of particles and the implications for drug and antigen delivery. J. Anat. 189: 477–482

O'Hagan, D. T. (1997) Prospects for the development of new and improved vaccines through the use of microencapsulation technology. In: Levine, M. M., Woodrow, G. C., Kaper, J. B., Cobon, G. S. (eds) New Generation Vaccines. 2nd edn, Marcel Dekker, New York, pp 215–228

O'Hagan, D. T., Rahman, D., McGee, J. P., Jeffery, H., Davies, M. C., Williams, P., Davis, S. S., Challacombe, S. J. (1991a) Biodegradable microparticles as controlled-release antigen delivery systems. Immunology 73: 239–242

O'Hagan, D. T., Jeffery, H., Roberts, M. J. J., McGee, J. P., Davis, S. S. (1991b) Controlled-release microparticles for vaccine development. Vaccine 9: 768–771

O'Hagan, D. T., Jeffery, H., Davis, S. S. (1993) Long-term antibody responses in mice following subcutaneous immunization with ovalbumin entrapped in biodegradable microparticles. Vaccine 11: 965–969

O'Hagan, D. T., McGee, J. P., Boyle, R., Gumaer, D., Li, J., Potts, B., Wang, C. Y., Koff, W. C. (1995) The preparation, characterization and pre-clinical evaluation of an orally administered HIV-1 vaccine, consisting of a branched synthetic peptide immunogen entrapped in controlled-release microparticles. J. Contr. Rel. 36: 75–84

Ott, G., Van Nest, G., Burke, R. L. (1992) The use of muramyl peptides as vaccine adjuvants. In: Koff, W., Six, H. R. (eds) Vaccine Research and Development. Marcel Dekker, pp 89–114

Ott, G., Barchfeld, G., Chernoff, D., Radhakrishnan, R., van Hoogevest, P., Van Nest, G. (1995) MF59: design and evaluation of a safe and potent adjuvant for human use. In: Powell, M., Newman, M. (eds) Vaccine Design: The Sub-unit and Adjuvant Approach. Plenum Press, New York, pp 277–294

Pizza, M., Covacci, A., Bartoloni, A., Perugini, M., Nencioni, L., De Magistris, T., Villa, L., Nucci, D., Manetti, R., Bugnoli, M., Giovannoni, F., Olivieri, R., Barbieri, J., Sato, H., Rappuoli, R. (1989) Mutants of pertussis toxin suitable for vaccine development. Science 246: 497–500

Pizza, M., Fontana, M. R., Giuliani, M. M., Domenighini, M., Magagnoli, C., Giannelli, V., Nucci, D., Hol, W., Manetti, R., Rappuoli, R. (1994) A genetically detoxified derivative of heat-labile *E. coli* enterotoxin induces neutralizing antibodies against the A sub-unit. J. Exp. Med. 180: 2147–2153

Plotkin, S., Mortimer, E. A. (1988) Vaccines. W. B. Saunders and Company, New York

Ramon, G. (1924) Sur la toxine et sur l'anatoxine diphtheriques. Ann. Inst. Pasteur 38: 1–10

Rappuoli, R. (1997) Rational design of vaccines. Nat. Med. 4: 374–376

Rothbrock, G., Smithee, L., Rados, M., Baughman, W. (1995) Progress toward the elimination of *Haemophilus influenzae* type b disease among infants and children – United States, 1993–1994. J. Am. Med. Assoc. 274: 1334–1338

Shahin, R., Leef, M., Eldridge, J. H., Hudson, M., Gilley, R. (1995) Adjuvanticity and protective immunity elicited by *Bordetella pertussis* antigens encapsulated in poly(DL-lactide-co-glycolide) microspheres. Infect. Immun. 63: 1195–1200

Singh, M., Li, X.-M., Wang, H., McGee, J. P., Zamb, T., Koff, W., Wang, C.-Y., O'Hagan, D. T. (1997) Immunogenicity and protection in small-animal models with controlled-release tetanus toxoid microparticles as a single-dose vaccine. Infect. Immun. 65: 1716–1721

Soltysik, S., Wu, J.-Y., Recchia, J., Wheeler, D. A., Newman, M. J., Coughlin, R. T., Kensil, C. R. (1995) Structure/function studies of QS21 adjuvant: assessment of triterpene aldehyde and glucuronic acid roles in adjuvant function. Vaccine 13: 1403–1410

Traquina, P., Morandi, M., Contorni, M., Van Nest, G. (1996) MF59 adjuvant enhances the antibody response to recombinant hepatitis B surface antigen vaccine in primates. J. Infect. Dis. 174: 1168–1175

Tseng, J., Komisar, J. L., Trout, R. N., Hunt, R. E., Chen, J., Johnson, A. J., Pitt, J., Ruble, D. L. (1995) Humoral immunity to aerosolized staphylococcal enterotoxin B (SEB), a superantigen, in monkeys vaccinated with SEB toxoid-containing microspheres. Infect. Immun. 63: 2880–2885

Ulrich, J. T., Ulrich, K. R. (1995) Monophosphoryl lipid A as an adjuvant: past experiences and new directions. In: Powell, M., Newman, M. (eds), Vaccine Design: The Subunit and Adjuvant Approach. Plenum Press, New York, pp 495–524

Valenzuela, P., Medina, A., Rutter, W. J., Ammerer, G., Hall, B. D. (1982) Synthesis and assembly of hepatitis B virus surface antigen particles in yeast. Nature 298: 347–350

Weiner, H. L. (1997) Oral tolerance: immune mechanisms and treatment of autoimmune diseases. Immunol. Today 19: 335–343

Williams, N. (1996) An immune boost to the war on cancer. Science 272: 28–30

Wise, D. L., Fellman, T. D., Sanderson, J. E., Wentworth, R. L. (1979) Lactide/glycolide polymers used as surgical suture material, raw material for osteosynthesis and in sustained release of drugs. In: Gregoriadis, G. (ed.), Drug Carriers in Medicine. Academic Press, New York, pp 237–270

Zhou, F., Kraehenbuhl, J.-P., Neutra, M. R. (1995) Mucosal IgA response to rectally administered antigen formulated in IgA-coated liposomes. Vaccine 13: 637–644