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REVIEW ARTICLE

Vaccine adjuvants – Current status and prospects on controlled release adjuvancity

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Abstract The strategy of World Health Organization is to develop efficient and inexpensive vaccine against various infectious diseases amongst children's population. Vaccination is considered as the most cost effective health intervention known to public. Since 90 years various substances have been added in vaccine formulation but still alum is considered as the safest adjuvant for human use licensed by United States Food and Drug Administration. MF 59 and ASO4 are the adjuvants were developed recently and approved for human use. Due to poor adjuvancity, conventional vaccines require multiple recall injection at approximately time intervals to attain optimal immune response. For past approximately two decades the vaccine research has been focused towards the alternation of alum type of adjuvant in order to increase the immunogenicity. The development of new vaccines, is more efficacious or easier to deliver, or both have become an area of research that can certainly benefit from controlled release technology. Especially, the conversion of multiple administration vaccine into single administration vaccine may represent an improved advancement towards the betterment of human health care and welfare. Biodegradable polymer microparticles have been evaluated for delivering antigens in native form, sustained release keeping in mind the safety aspects. In this article we review the overall concept of adjuvants in vaccine technology with special focus towards the prospects of controlled release antigens.

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Contents

1. Vaccines and immunization

Vaccines are considered as one of the most successful medical inventions against various infectious diseases (Hilleman, 2000). In 1974, the World Health Organization (WHO) officially launched a global immunization programme called Expanded Programme on Immunization (EPI). The ultimate aim of EPI was to protect the child population across the globe against vaccine preventable diseases in general and particularly diphtheria, whooping cough, tetanus, polio, tuberculosis and measles by the year 2000. Particular in the year 1984, World Health Organization established the uniform vaccine schedule for diphtheria, whooping cough, tetanus, polio, tuberculosis and measles. The high proportion of chronic infection that is acquired during childhood can be prevented by routine infant immunization programme.

The Expanded Programme on Immunization is now renamed as Universal Immunization Programme (UIP). The EPI was launched in 1978 in India after successful global eradication of small pox in 1975 through effective vaccination programmes and strengthened surveillance. The WHO global advisory group of Expanded Programme on Immunization in the year 1991 recommended that by the year 1997, accordingly, hepatitis B vaccine should be introduced into national immunization programme across countries around the globe because of high rate of incidence of chronic infectious diseases that could affect during childhood by hepatitis B virus. Accordingly, this vaccine (hepatitis B) has been included in the national immunization programme in more than 130 countries (Kane, 1998). Later, yellow fever and Haemophilus influenzae meningitis (Hib) vaccines have been added in countries with a high burden of disease (Fiore et al., 2009).

Vaccine development and immunization constitute critical component of public health policy in any nation. Accordingly, the Global Programme for Vaccines and Immunization (GPV) was established in 1994 and this programme thus had the terms of orientation that span from vaccine research through vaccine production and quality control to help policy matter of health plan and provide their services to control vaccine preventable diseases. The Children's Vaccine Initiative (CVI) is a companion organization to the Global Programme for Vaccines and Immunization and has its own strategic plan. The purpose of CVI is to develop new technologies to progress and develop a single shot efficient vaccine. It should be given as a single dose (preferably by oral). It is effective when administered near birth, heat stable, contains multiple antigens and highly effective against wide variety of diseases. With a particular focus on the world BPL (below poverty line) children, GPV aims at strengthening routine immunization services, increasing wide coverage and introducing novel generic vaccines. Global Alliance for Vaccines and Immunization (GAVI), an advisory group comprising National Governments and International agencies such as WHO, World Bank, UNICEF and industries was launched on January, 2000. GAVI's mission statement is "To save children's lives and protect people's health through wide spread use of vaccines with a particular emphasis on developing countries". As an alliance of major leaders in International health and development, GAVI has great potential in decision making among policy makers and donors on the value of vaccination for reducing poverty and infant mortality in the developing countries.

The decision to introduce a vaccine into EPI is greatly influenced by a number of factors such as bio-burden, epidemiological aspects with special reference to transmission, vaccine factors that include safety, efficacy and availability, feasibility of introduction, financial implications and projected or expected benefits in terms of morbidity, mortality and cost effectiveness. Thus a vaccine that is ideal for introduction in to EPI would be one that is highly efficacious, economical, safe and protects against disease. Therefore, in designing effective vaccines certain key elements are essential such as:

1. An antigen that can stimulate good immune response.
2. Presentation of antigens in order to augment the immune response.
3. Presentation of antigens in native form.

Stimulation of innate immune system is an important role in the evolution of adaptive immune response (Hobe et al., 2004). Therefore, inclusion of immune potentiators (adjuvants), which triggers a robust and long lasting immune response, is of primary importance.

2. What are adjuvants and their status?

The ultimate principle of vaccination is to generate a strong immune response against infectious diseases. Adjuvant is a term coined from the Latin word adjuvare, which means to help or to augment. A vaccine adjuvant is a component that can improve the effectiveness of vaccines by inducing robust immune responses (Vogel, 1998). Therefore, traditionally, adjuvants have been used for vaccine formulations by the industries in order to augment the immune response. The concept of adjuvants was first focused by Ramon (1925, 1926) emerged from observations that an abscess at the inoculation site produced higher specific antibody titers. Generally, adjuvants can be used for various purposes (Aguilar and Rodriguez, 2007):

1. To augment the immune response of any antigens by delivering in native form.
2. To reduce the multiple immunization protocol for protective immunity. In particular to develop single step vaccination coverage that can reduce the vaccination costs.
3. To enhance the immune response of immune compromised adults and weakened immune system of children, to elicit cytotoxic T lymphocytes response and generate local immune response (Achal et al., 2005).

During the last 90 years many adjuvants have been developed among them aluminum and calcium salts are licensed for human use (Achal et al., 2005). But recently MF 59 and ASO4 have been approved for human use (Tagliabue and Rappuoli, 2008) being considered as the adjuvants of the new era of vaccinology. Because of higher toxicity no other adjuvants have been licensed for human vaccination programme. Since the safety of adjuvant is the prime importance for routine vaccination, Edelman (1980) listed various criteria such as general toxicity, hypersensitivity reactions, carcinogenicity, teratogenicity, etc. Therefore, the ideal adjuvant would be non-toxic, biodegradable, cheap, non-immunogenic by itself and must not have any interaction with the antigen.

2.1. Types of adjuvants

There are several types of adjuvants with differing modes of action. These include mineral salts, oil emulsions, immune stimulating complexes (ISCOM), bacterial derivatives, carbohydrate adjuvants, liposomes, cytokines, virus like particles and polymeric microparticle adjuvants.

2.1.1. Mineral salts

Mineral salts such as alum and calcium phosphate have been used as adjuvant in vaccine formulations. Glenney et al. (1926) have demonstrated the addition of potassium alum to diphtheria toxin resulted in a precipitate. The precipitated diphtheria toxin when injected into guinea pigs resulted in a higher number of antibody production when compared to normal non-precipitated diphtheria toxoid. Relyveld et al. (1985) and Relyveld (1986) demonstrated calcium phosphate as vaccine adjuvant. Alum is chemically potassium aluminum sulfate was initially used for purifying the protein antigen such as tetanus toxoid and diphtheria toxoid by precipitating them. However, aluminum compounds used as vaccine adjuvants are aluminum phosphate or aluminum hydroxide. In practice the aluminum phosphate and aluminum hydroxide imperfectly referred as alum but they have different physical characteristics and differ in their adjuvant property. Among this, aluminum hydroxide showed higher adsorption property and found to be more potent than aluminum phosphate. Aluminum salts are effective forming a short term depot (Table 1) at the site of injection, slowly releasing antigen to the body's immune response system (Gupta et al., 1993, 1995). Since last 90 years, various substances have been tried and used as vaccine adjuvant, most of these substances were never accepted for human vaccines due to their high level of toxicity and still the alum salts remain the safest adjuvant approved by United States Food Drug Administration (US FDA) for human vaccine products (Gupta et al., 1995). But the use of aluminum adjuvant in manufacturing vaccines is a difficult task because the adsorption of antigen on aluminum type adjuvant is based on physico-chemical characteristics of antigen. Although, aluminum salts remain the only adjuvant approved for human use it has some limitation such as lacking in inducing cyto toxic T-lymphocyte (CTL) responses especially to protect from viral infection (Doherty et al., 2006). Besides, there are well documented problems of aluminum adjuvant that induce inflammation and stimulate local production of erythema, granuloma, subcutaneous nodules and contact hypersensitivity (Men et al., 1995; Baylor et al., 2002) in addition to that alum cannot be frozen or lyophilized. Walls (1977) and Nagel et al. (1977) reported that aluminum hydroxide has attraction towards eosinophil that leads IgE mediated allergic reaction at the site of injection. On the other hand, Gupta et al. (1995) stated that aluminum adjuvants have been used for many years for hyposensitization of allergic patients without adverse effects. Clements and Griffth (2002) reported that the alum has been established as a safety adjuvant for vaccine delivery since last 90 years. Furthermore, aluminum consumption from vaccines is far less than that received from the diet or medications such as antacids (Baylor et al., 2002). Nonetheless, in recent years, the adjuvants received much attention because of the development of protein subunits made by rDNA technology (Hanes et al., 1997). The sub unit vaccines are weakly immunogenic when compared to whole-cell vaccines and therefore, need suitable adjuvants for delivering the antigens. Even though alum has good properties it is not much suitable for small proteins because the alum adsorbed vaccines elicit a short term immune response requiring many boosters for attaining minimum optimal threshold immune response. Consequently, it is necessary to find a new adjuvant that can replace the alum type adjuvant.

2.1.2. Oil emulsions

Le Moignic and Piony (1916) demonstrated that the suspension of killed *Salmonella typhimurium* with mineral oil emulsion elicited increased immune response. Later Freunds et al. (1937) prepared a w/o type of emulsion adjuvant using paraffin oil mixed with killed Mycobacteria called Freund's complete adjuvant (FCA) and without mycobacteria referred as Freund's incomplete Adjuvant (FIA). The FIA forms depot at the site of injection (Table 2) and slow release of antigen with the stimulation of antibody producing cells led to poor immunomodulatory effect (Freund, 1956). Earlier, FIA was used in human vaccine formulations such as influenza and killed poliomyelitis vaccines (Davenport, 1968; Salk and Salk, 1977). However, FIA is not currently used due to poor immunogenic effect, induces local irritation, may cause granuloma and cyst

Table 1 Mineral adjuvants and their properties.

Adjuvant type	Representing examples	General description	Mechanism of action	Advantages	Disadvantages
Mineral salts	Aluminum salts – aluminum hydroxide or aluminum phosphate	1. Licensed and approved by US FDA for human use 2. Misreferred as alum 3. It is widely used as human and veterinary vaccines 4. Aluminum hydroxide is more potent than aluminum phosphate due to their adsorption property 5. Considered as the safest adjuvant	1. Sort term depot effect 2. Induction of cytokine network 3. Complement activation 4. Delivery of antigens to different APC 5. Strong Th_2 response	1. In expensive 2. Safe 3. Efficient uptake of alum adsorbed antigens by antigen presenting cells due to particulate nature with optimum size 4. Long lasting immune response when compared to soluble antigens	1. Not suitable for recombinant proteins and sub unit vaccines 2. Adverse effects have been reported such as inflammation and stimulate local production of erythema, granuloma, subcutaneous nodules, contact hypersensitivity and IgE mediated hypersensitivity. Alum cannot be frozen or lypholized 3. Unable to induce cytotoxic T cell response
	Calcium salts	1. Calcium salts in the form of Calcium phosphate have been used as human vaccine adjuvant especially DTP, polio, yellow fever and BCG vaccines 2. Approved for human use in European countries	1. Short term depot effect 2. Adsorbs soluble antigens and presents them in a particulate form to the immune system	It is a normal constituent of the body and therefore well tolerated, elicit high IgG type antibodies	1. It lacks immunomodulatory response 2. Neurological reactions have been observed 3. Local irritation and active inflammatory reaction at the site of injection

Table 2 Properties of oil emulsion and microbial derivatives as vaccine adjuvant.

Adjuvant type	Representing examples	General description	Mechanism of action	Advantages	Disadvantages
Oil emulsion	Freund's complete adjuvant (FCA)	w/o type of emulsion adjuvant using paraffin oil mixed with killed Mycobacteria	1. Short term depot effect 2. Strong Th_1 and Th_2 response by producing	Strong immune response	Highly toxic
	Freund's incomplete adjuvant (FIA)	w/o type of emulsion adjuvant using paraffin oil mixed without killed Mycobacteria	1. Short term depot 2. It induces weak Th_1 and Th_2 response	Lesser side effects than FCA	1. Poor immunomodulatory effect 2. Local irritant effect may induce granuloma and cyst formation
	MF 59	1. o/w type emulsion contains 4.3% of squalene oil, tween 80 and span 85 2. Licensed for human use in European countries	Inducing local immune stimulatory effect at the site of injection, regulates cytokines, chemokines, recruitment of $CD11b^+$, MHC II^+ cells and enhance antigen uptake by dendritic cells	It is a superior than alum adjuvant for influenza vaccine	1. Pain at injection site 2. Reactogenicity 3. Induces inflammatory arthritis
Microbial derivatives	ASO4	1. It is 3-*O*-desacyl-4′-monophosphoryl lipid A obtained from the cell wall LPS of Gram-negative *Salmonella minnesota* R595 2. Licensed for human use in European countries	Local activation of NF-kB activity, cytokine production, optimal activation of APC and generation of Th_1 response	It is superior than alum adjuvant for cancer, HBV, malaria and HPV	1. Reactogenicity 2. Issues on consistency of preparation of vaccines from bacteria 3. Cost effective

formation at the site of injection and reported carcinogenic in mice (Murray et al., 1972; Potter and Boyce, 1962; Gupta et al., 1993). The FCA can induce strong Th_1 and Th_2 immune response because of the combination of Mycobacterium tuberculosis with w/o type of emulsion (Freund et al., 1948). However, inducing toxic effect is the major disadvantage of this adjuvant. There are some other oil emulsions which have also been demonstrated adjuvant properties include Montanide (Aucouturier et al., 2002), Adjuvant 65 (Hilleman et al., 1972; Weibel et al., 1973) and Lipovant (Byars and Allison, 1990). But these adjuvants are also not much useful for routine human prophylactic vaccine because of their toxic properties (Aguilar and Rodriguez, 2007). MF 59 is an adjuvant developed by the ex-Chiron now Novartis Vaccines (O'Hagan et al., 2007) consists of oil-in-water emulsion (Table 2), comprising 4.3% of squalene oil as dispersed phase, which is stabilized by two non-ionic surfactants (Tween 80 and Span 85), and a low ionic strength citrate buffers as continuous phase. MF 59 is originally developed as delivery agent that contains muramyl tripeptide phosphatidylethanolamine (MTP-PE), which activates non-TLR sensing receptors known as NOD LRRs (Akira et al., 2006) that leads toxicity. But the MF 59 without MTP-PE proved as a potent adjuvant for influenza vaccine (Fluad) tested in animal model and later human trials have been shown successful results. Thus it becomes first new adjuvant for human use after alum (Tagliabue and Rappuoli, 2008).

2.1.3. *Immune stimulating complexes (ISCOM)*

The immune stimulating complexes (ISCOMs) were first described by Morein et al. (1984). ISCOM and ISCOMs (Table 3) are trade marks of ISOTEC AB, Uppsala, Sweden that composed of saponin, cholesterol, phospholipid and immunogen (Sjolander et al., 1998). Saponin such as Quil A has also been used as a component of immune stimulating complexes (Kensil, 1996). Quil A is a triterpenoid saponins obtained from *Quillaia saponins* is less toxic than crude saponins (Dalsgaard, 1978; Dalsgaard et al., 1990). Quil A was not satisfactory for human use because it is a heterogeneous mixture of many closely related saponins that vary in their chemical and biological activities lead to unpredictable effects (Sjolander et al., 1998). QS-21 is a purified form of crude extracts of Quil A have the ability to induce strong cellular immune response against various pathogen derived antigens and HIV-1 (Allison and Byars, 1991; Takahashi et al., 1990). QS-21 is less toxic when compared to Quil A but it has same toxic properties of Quil A that leads to unsuitable for human uses other than cancer vaccines where higher toxicity may be accepted (Kensil et al., 1995).

2.1.4. *Bacterial derivatives*

Bacterial toxins and toxoids from *Escherichia coli* and *Vibrio cholera* have been reported as mucosal adjuvants (Walker, 1994; McGhee et al., 1992). Bacterial cell wall components like peptidoglycan or lipopolysaccahrides (LPS) enhance the immune response by mediating through activation of Toll-like receptors (TLR) that activates the host immune system (Audibert and Lise, 1993). LPS containing the active lipid A moiety can act as potent mucosal adjuvants but it is too toxic for human vaccines. GlaxoSmithKline developed an adjuvant ASO4 contains 3-*O*-desacyl-4′-monophosphoryl lipid A (MPL) that obtained from the cell wall LPS of Gram-negative

Table 3 Particulate delivery systems as vaccine adjuvant.

Adjuvant type	Representing examples	General description	Mechanism of action	Advantages	Disadvantages
Particulate delivery vehicle	ISCOM	It contains a triterpenoid saponins obtained from *Quillaia saponins*, a sterol and optionally a phospholipid. The saponins are Quil A or QS-21	1. Generate CTL response, induce cytokines 2. Directly phagocytosed by macrophages	Induces strong immune response	1. Severe pain at the site of injection 2. Severe toxicity includes severe haemolysis, granulomas
	Liposomes	Contains synthetic phospholipids. Liposomes based hepatitis A vaccine approved in Europe	Fuse with cell membrane of macrophages, enable antigen in to the cyto plasma, enter MHC class I path way and activate CD8 CTL response	Induces strong immune response	1. Manufacturing difficulties due to stability 2. High cost 3. Severe pain at the site of injections
	Polymeric microparticle	1. It is made by biodegradable polymers 2. Antigens encapsulated inside the microparticles 3. It is considered as next generation of adjuvants 4. Potential for single shot vaccines	1. Long term depot effect from weeks to months 2. Pulsatile release of antigens 3. Target to antigen presenting cells	1. It can mimic the priming and boosting effect of conventional vaccine 2. Potential for single shot vaccines and can reduces the cost of vaccination	1. Issues on stability of antigens during micro encapsulation and storage 2. Issues on dose optimization

Salmonella minnesota R595 and is detoxified by mild hydrolytic treatment and purification (Table 2). ASO4 is absorbed on either aluminum hydroxide or aluminum phosphate and it became second new adjuvant for human use after MF 59. Recently, a recombinant hepatitis B vaccine (FEN-Drix) has been formulated with ASO4 and compared their efficacy with Energix-B in healthy young adults (Levie et al., 2002) of European region. Cerevarix is a vaccine against human papilloma virus, a causative agent of cervical cancer is formulated with ASO4 to prove high and sustained level of antibodies aimed to provide long term protection. ASO3 is also an adjuvant developed by GlaxoSmithKline contains squalene. It is used as an adjuvant for swine flu vaccine being used in Canada and Europe.

2.1.5. Carbohydrate adjuvants

Several complex carbohydrates of natural origin such as gamma inulin derived from plant root of the composite family stimulate both humoral and cellular immunity. It activates complement pathway and increases the production of activated C3 and thereby activating macrophages (Cooper, 1995). Gamma inulin can be combined with variety of other adjuvants to get a better adjuvant, for example algammulin. Algammulin is a combination of Gamma inulin with aluminum hydroxide a potent inducer of Th_1 and Th_2 activity when compared to gamma inulin alone (Cooper et al., 1991; Cooper and Steele, 1991). Inulin can be easily metabolized in to simple sugars fructose and glucose and does not suffer from the adverse effects that are associated with alum based adjuvants (Petrovsky and Aguilar, 2004). Other polysaccharides such as glucans, dextrans, glucomannons, galactomannans, levans and xylans (Tizard et al., 1989; Sheets et al., 1991) also enhance the immune response.

2.1.6. Liposomes

Liposomes are synthetic spheres containing lipid layers that can encapsulate antigens that are desired and act as adjuvants (Table 3). Liposomes can induce both humoral and cellular immunity to protein and polysaccharide antigens (Allison and Gregoriadis, 1974; Rooijen and Nieuwmegen, 1983). The potency of liposomes varies with the number of lipid layers (Shek et al., 1983), electric charge (Allison and Gregoriadis, 1974), composition and method of preparation (Heath et al., 1976; Tyrrel et al., 1976). Liposome based hepatitis A vaccine (Epaxal) was developed by Swiss Serum and Vaccine Institute (Ambrosch et al., 1997) demonstrated that two doses of the liposomal hepatitis A vaccine administered at months 0 and 12 early protection within 14 days and long lasting immunity can be attained. The liposomal based vaccines fuse with cell membranes of macrophages, enabling the delivery of proteins into the cytoplasm and enter in to MHC class I pathway and activate CD8 cells (Owis and Gupta, 2000; Zheng et al., 1999). The major drawbacks of liposomes are manufacturing difficulties such as stability, high cost and may produce pain at the site of injection.

2.1.7. Cytokines

Cytokines are low molecular weight soluble proteins that are formed in response to antigens and regulate the innate and adaptive immune response. Cytokines are grouped in the modern classification of adjuvants for example Granulocyte–macrophage colony stimulating factor (GM-CSF) enhances the primary immune response by stimulating and activating the antigen presenting the cells (Heufler et al., 1988). Cytokines are much useful only in cancer vaccines but its application is limited due to toxicity.

2.1.8. Virus like particles (VLP)

Viruses like particles are inert, empty capsids of viruses that lack nuclear material but retain the structure of a virus. By means of genetic engineering technology the desired antigens can be attached to VLP. The VLP presents the antigens are capably engaged by dendritic cells that induce strong immune response (Antonis et al., 2006; Young et al., 2006; Dell et al., 2006). Recombinant hepatitis B surface antigen (HBsAg) has been produced as VLPs in *Saccharomyces cerevisiae*. Recently human papilloma virus vaccine has been produced by this VLP technology and approved by US Food and Drug Administration for clinical use (Aguilar and Rodriguez, 2007).

2.1.9. Polymeric microparticle adjuvants

Biodegradable polymers are being used as sutures and drug carriers, because of the biocompatible, nontoxic nature and their biodegradable properties. The polymers which are chosen as excipients (adjuvants) for parenterally administered vaccines should meet some of the requirements, including being biodegradable, safe (tissue compatible, no secondary reaction), antigen compatible and permeable, stable *in vitro* easy to process, alone responsible formulations and ideally inexpensive. The biodegradable synthetic and natural polymers have been investigated for the control release of macromolecular drugs and proteins. These polymers in the form of microspheres/microparticles seem to be preferred for better controlled release of antigen (Table 3). Novel vaccines based on recombinant proteins and DNA, are safer than traditional vaccines, but they are unfortunately less immunogenic. Therefore, there is an urgent need for the development of potent, safe adjuvants as delivery systems that can be used to boost up the immune response of vaccines. In recent years a great effort has been made to improve the efficacy of vaccine by using novel adjuvant or antigen carrier as delivery systems.

Polymer microspheres are solid, spherical or approximately spherical particles ranging in size from 1 to 1000 μm. Microsphere-based antigen deliver systems can now be made to deliver subunit protein and peptide antigens in their native form in a continuous or pulsatile fashion for periods ranging from weeks to months. Polymer microspheres have shown great potential as a next generation adjuvant to replace or complement existing aluminum salts for vaccine potentiation. The use of polymers to control the release of an antigen to stimulate the immune response was first reported by Peris and Langer (1979). Controlled release of antigens from polymer microparticles has been of great interest to the development of vaccine adjuvant which could be effective in a single dose (single-step immunization). Controlled vaccine delivery system significantly enhances immunity without invoking a vigorous inflammatory response. But instead it follows a phenomenon known as depot therapy. In fact, controlled release systems can provide a release of antigens for weeks to months, a time far exceeding the depot effect of aluminum salts or w/o emulsions such as Freund's adjuvants. In addition, the prolonged and pulsatile release of vaccine from microparticles may mimic the priming and boosting effect of conventional vaccines. The antigen released from microparticles could regulate the desired immunological effect. However, the release rate is greatly influenced by

loading capacity, cross-linking, molecular weight, particle size and the porosity of microparticles (adjuvants).

Poly(D,L-lactide-co-glycolic acid) is a polymer ester of the two hydroxyl acids, viz., lactic and glycolic acids. PLGA degrades through bulk erosion by hydrolysis of its ester linkages in the presence of water, which gives lactic acid and glycolic acid. These two monomers under normal physiological conditions are byproducts of various metabolic pathways in the body. Therefore, the human body effectively deals with these monomers, which can be metabolized via the TCA cycle. It is approved by food and drug administration, United States for therapeutic devices for many years as resorbable sutures and bone plates (Austin et al., 1995; Pihlajamaki et al., 1992; Winde et al., 1993).

Maria Alonso et al. (1999) developed a single dose tetanus vaccine based on poly(lactide-co-glycolic acid) microspheres, which became complicated because of instability of tetanus toxoid. They attempted to redesign PLGA microspheres by co-encapsulating together with tetanus toxoid in dry solid state together with potential stabilizers such as the haloes, bovine serum albumin, alginate, heparin, dextran or poloxamer 188 by employing an appropriate technique. The PLGA microspheres were able to release *in vitro* antigenically active tetanus toxoid for at least 5 weeks. The efficacy of the strategy was demonstrated by high, long lasting, titers of neutralizing antibodies achieved after *in vivo* administration of dextran containing microspheres with a fraction of alum adsorbed tetanus toxoid as compared to the commercial absorbable tetanus toxoid vaccine. These findings suggest that future developments in the area of vaccinology depend on the ability to combine a detailed knowledge of the microencapsulation technology with rational choice of stabilizing excipient or combination of excipients.

Feng et al. (2006) investigated that the feasibility of a single-dose hepatitis B vaccine based on three kinds of PLGA microspheres. PLGA microspheres loaded with recombinant hepatitis B surface antigen (HBsAg) were formulated using a double emulsion microencapsulation technique. The pharmaceutical characteristics of size, surface morphology, loading efficiency, antigen integrity and release from PLGA microspheres and their *in vitro* degradation were evaluated. The degradation of the polymer corresponded with the composition of the polymer (lactide/glycolide ratio), molecular weight of the polymer (viscosity) and morphology of the microspheres, were able to continuously release antigen under conditions that mimic the environment *in vivo*. The single subcutaneous injection of HBsAg-loaded PLGA 50/50 microspheres, PLGA 75/25 microspheres and a mixture of PLGA 50/50, PLGA 75/25, and PLGA 50/50-COOH microspheres in mice resulted in comparable serum antibody titers to those of three injections of the conventional aluminum adjuvant formulated HBsAg vaccine. Based on these findings *in vitro* and *in vivo*, it was concluded that HBsAg was successfully loaded into the PLGA microspheres, which can auto-boost an immune response, and the HBsAg-loaded PLGA microsphere is a promising candidate for the controlled delivery of a vaccine. Poly(lactic acid) (PLA) is biodegradable, biocompatible, thermoplastic, aliphatic polyester derived from lactic acid. It is frequently used in bone repair applications. The polymer chains are cleaved by hydrolysis to form monomeric acids and are eliminated from the body through Krebs's cycle, as carbon dioxide through respiration and water in urine. The rate of hydrolysis of the polymer chain is dependent on significant temperature, pH or presence of catalyst. The utilization of PLA as sustained release vaccine carrier system was demonstrated by some researchers (Sivakumar et al., 2010).

Chitosan is a natural polymer has great potential for pharmaceutical applications due to its biocompatibility, biodegradability, high charge density and non-toxicity (Sinha et al., 2004). It also has antacid and anti-ulcer activities (Ito et al., 2000), wound healing properties (Tachihara et al., 1997); it is also used in artificial kidney membranes because of their suitable permeability and high tensile strength (Amiji, 1995). Chitosan has been used as a vehicle for directly compressed tablets (Kristmundsdoittr et al., 1995). Chitosan microspheres are widely studied drug delivery system for the controlled release of drugs such as antibiotics, anti-cancer agents, proteins and vaccines. Vaccine research is often focused to find out novel antigens, their delivery methods, including variation of adjuvants used, the dose and number of injections (Lofthouse, 2002). Many classical adjuvants such as bacterial cell wall extracts are having adjuvant properties and can stimulate nonspecific inflammatory response (Hanes et al., 1997). The ability of chitosan to enhance both the systemic and local immune responses against diphtheria toxoid after oral and nasal administration was demonstrated by Vander Lubben et al. (2003). DT associated to chitosan microparticles resulted in systemic humoral and local immune responses against DT oral vaccination and insignificant enhancement of IgG production after nasal administration. Hence these experiments demonstrated that chitosan microparticles were very promising mucosal delivery system. Tiyaboonchai and Nanteetip (2007) fabricated a new nanoparticulate delivery system for amphotericin B. In their work, two opposite charged polymers were used to form nanoparticles through electrostatic interaction, chitosan a positively charged particle and dextran sulfate a negatively charged polymer linked together and hardened by zinc sulfate.

The importance of developing new vaccine systems with proper attention to developing controlled delivery system was demonstrated by Stanley Davis (2006). They stated polymer microspheres and lamellar particle based on the biodegradable materials PLA and PLGA could be employed for the improved parenteral and mucosal administration of antigens. Like wise soluble biopolymers such as chitosan could be used for the nasal delivery of various antigens as well as DNA. The process of optimization is one of the most important biological barriers to controlled drug delivery (Donald Owens and Nicholas Peppas, 2006). Sangmook and Jae Wook (2005) investigated the thermal, rheological, morphological and mechanical properties of a binary blend of poly(lactic acid) and poly(butylenes succinate adipate). Yong-Hong et al. (2005) investigated the physical stability of spray dried proteins with surfactant free hydrofluroalkane pressurised metered dose inhalers during prolonged storage. The results indicated that the presence of PVA in the spray dried stabilized protein particles could enhance the physical stability of microparticles. Recently chitosan microspheres have been demonstrated as better adjuvant for HBsAg when compared to PLGA and PLA (Sivakumar et al., 2010).

3. Vaccine encapsulated microspheres and immune response

Potent adjuvants can improve the efficacy of vaccines by inducing robust immune responses for longer duration.

Microspheres are capable of providing enhanced antigen processing through their ability to target phagocytosis by antigen presenting cells (APCs). The encapsulation of antigen in a biodegradable polymer particulate system or on their surface adsorption can lead to antigen presentation by MHC class I pathway (Men et al., 1995; Moore et al., 1995; Nixon et al., 1996; Bandowiski et al., 1993; Falo et al., 1995; Rock and Clark, 1996). The important function of MHC class I molecule is bind to the processed endogenous antigen and present it to the immunocompetent cells that lead to cytotoxic T lymphocyte (CTL)-mediated immune response. Presentation of antigen to the immunocompetent cells by MHC class II molecule generally leads to enhanced antibody production, i.e., induction of humoral immune response.

The biodegradable polymer microspheres form depot that retains antigens for an extended period and exposing towards the immune system for longer duration. Early reports showed that the interaction between antigen and the cells of the draining lymph node is one of the basic parameters that determine the outcome of any immune response (Zinkernagel et al., 1997; Zinkernagel, 2000). On the other hand, the duration of antigenic stimulus has been demonstrated by various scientists and suggested that the continuous release of antigen is a major factor for determining the fate of both naïve and effector T cells. Naïve T cells require prolonged signaling for at least 2 h (Iezzi et al., 1998) and up to 2–3 days (Zinkernagel, 2000) to become committed to proliferation, while effector T cells require only 1 h of antigen stimulation (Iezzi et al., 1998) but prolonged stimulation may induce death of effector cells (Cleland, 1999). It is noted that low dose of i.v. injections has been shown to induce immunity where as high dose in i.v. results immunotolerance (Zinkernagel et al., 1997). Earlier reports showed that a mature immune response can be induced by continuous antigen delivery and lead to extended effector responses but the activation of immune memory requires booster stimulation with antigen (Lofthouse, 2002). However, pulsatile delivery of antigen has been stimulated through the use of microencapsulated vaccines in biodegradable polymers that vary in density or size (Cleland, 1999; Sanchez et al., 1996). Although there is a number of a biological and synthetic polymer available for use in microencapsulation, a vaccine formulation intended for human requires the use of biodegradable polymers and their safety is an important parameter. Microspheres are capable of protecting the vaccine and presenting in its native conformation to the various cells of the immune system. The need of improved method of delivering antigens has spurred research aimed at the development of future generation adjuvants. The significance of stable antigen delivery from microspheres has been highlighted by an enormous number of investigators. Therefore, the development of newer vaccine delivery system, both efficacious and easier to deliver has an area of research that can certainly benefit from controlled release technology.

4. Conclusion

Prevention of diseases through immunization programme is the most cost effective health arbitration. Alum, MF 59 and ASO4 are the adjuvants used in vaccine formulation for human usage but requires multiple dose regimens. The development of single contact vaccines mainly administered soon after birth to combat various infections will be an important advancement towards the betterment of human health care. Though many reports proved that microspheres based vaccines provide a long term antigen release by forming a depot at the site of injection, and target the antigen presenting cells leading to the enhancement of humoral or cell mediated immunity. Unfortunately it is not applicable in practice till date and it is under study level only. Let us hope for the best in future to get a better adjuvant for vaccine delivery to develop single contact vaccines.

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