Bulletin of Pharmaceutical Research 2011;1(3):49-62
An Official Publication of Association of Pharmacy Professionals
ISSN: 2249-6041 (Print); ISSN: 2249-9245 (Online)

REVIEW ARTICLE

FORMULATION AND DEVELOPMENT OF VACCINES AND THEIR SELECTION FOR NEXT GENERATION

Amish A. Dangi*, Navin R. Sheth, Hima H. Sodha, Purvi C. Joshi, Divyesh S. Bhalodiya, Ankita C. Panchal and Pooja R. Ramanuj

Department of Pharmaceutical Sciences, Saurashtra University, Rajkot-360 005, Gujarat, India

**E-mail*: amishdangi@gmail.com
Tel.: +91-9428236404

Received: November 15, 2011 / Revised: December 30, 2011 / Accepted: December 31, 2011

The goal of advancement in vaccine formulation is to generate a strong immune response to the administered antigens. To achieve this objective with vaccines based on insufficiently immunogenic antigens, adjuvant and other formulation materials are alternatives. Vaccines contain various types of additives, excipients, antigen and adjuvants which in combination provide maximum protection against various types of infectious diseases. Vaccine contains various types of lives or killed viruses, inactivated bacterial toxin and polysaccharides. Selection of excipients and adjuvants is a serious task having an implication towards safety, stability and storage of vaccine. Preservatives are used in vaccines to prevent microbial growth. Stabilizers are required in vaccine formulation to keep the vaccine homogenous and stop the components separating. Surfactants or emulsifiers are very important to alter the surface tension of a liquid. Animal products are commonly used in formulation of vaccines and are necessary for growing the vaccine pathogens. Moreover, new vaccine modalities such as DNA vaccines and multiple vaccines are currently being explored for future scope. Novel delivery technologies will be essential component for next generation vaccines.

Key words: Adjuvant, Stabilizer, Animal product, DNA vaccine, Multiple vaccine.

INTRODUCTION

While the development and widespread use of effective vaccines has an extraordinary impact on global health, there remain many infectious and other diseases for which vaccines are not available. Increasing understanding of the immune system and the nature of particular immune responses that are associated with protection from infection or disease are being put to use by vaccine developers who now produce increasingly sophisticated vaccine candidates for complex diseases (Lang and Wood, 1999). Many of the newer vaccine candidates are based on protective antigens which are inherently less immunogenic than the whole cell inactivated or live attenuated vaccines or multicomponent conjugate vaccines that were developed in the past (Luke and Subbarao, 2006). Therefore, adjuvant has become an increasingly important ingredient in novel vaccines being developed today (Podda and Del Giudice, 2003). Vaccines available in the market contain various types of additives (Brewer, 2006), antigens and adjuvants (Garcon *et al* 2007) which in combination provide maximum protection against various types of infectious disease (Lindblad, 2004). This vaccine contains various types of live or killed viruses, inactivated bacterial toxin and polysaccharides (Guy, 2007). This diverse nature of antigens requires various types of excipients to stabilize them (Pashine *et al* 2005). Selection of various types of excipients is a serious task having huge implication towards their safety, stability and storage (Treanor *et al* 2006). Like any other pharmaceutical exicipients intended for human use, the excipients used in vaccines must comply with some rigorous standard of quality, purity, availability and compatibility (Burdin *et al* 2004). Pharmaceutical excipients further

evaluated to meet higher purity and safety standard because these are injected into human body, because most commonly used vaccines are administered parenterally. Excipients must comply with strict guideline set by the U.S Food and Drug Administration (FDA) for any vaccine formulation development. The upcoming section will contain all detail about each component with a vaccines formulation and describe its use, source and limitation.

Vaccine additives

Vaccines may contain the following (if required): 1) Adjuvant: Immune enhancer 2) Excipients: Usually inert substances other than the active ingredient included in the manufacturing process of vaccine.

Adjuvants:

Adjuvant is a term derived from the latin word *adjuvare*, which means to aid or to help and it was first coined by Ramon in 1926, who observed that horses developed abscesses at the site of an injection of diphtheria toxoid produced higher antitoxin titers than animals without abscesses (Vogel and Hem, 2004).

In 1926, Glenny demonstrated the adjuvant activity of aluminum compounds utilizing an alum-precipitated diphtheria toxoid (Bernstein *et al* 2007). In the mid-1930s, Freund developed a powerful immunologic adjuvant composed of a water-in-mineral oil emulsion containing killed mycobacteria, known as Freund's complete adjuvant (FCA).

Adjuvant has traditionally been defined as agents added to vaccine formulations that enhance the immunogenicity of antigens *in vivo*. A proposed update of this definition divides adjuvant into two classes: delivery systems and immunopotentiators, based on their dominant mechanism of action (**Table 1**). Adjuvants may exert their activities by their impact on the presentation of the antigen to the immune system (*e.g.* adsorbents, particles and emulsions), the antigen/adjuvant uptake (*e.g.* emulsions), the distribution (targeting to specific cells), the immune potentiation/modulation (*e.g.* microbial, synthetic and endogenous adjuvants) or protection of the antigen from degradation and elimination.

Table 1. Examples of mechanistic classes of adjuvants

Antigen delivery systems	Immunopotentiators
Insoluble aluminum compounds	MPL and synthetic derivates
Calcium phosphate	MDP and derivatives
Liposomes	Oligonucleotides (CpG etc.)
Virosomes™	Double-stranded RNA (dsRNA)
ISCOMS®	Alternative pathogen-associated molecular patterns (*E. coli* heat labile enterotoxin (LT); flagellin)
Microparticles (*e.g.* PLG)	Saponins (Quils, QS-21)
Emulsions (*e.g.* MF59, Montanides)	Small-molecule immune potentiators (SMIPs) (*e.g.* resiquimod [R848])
Virus-like particles and viral vectors	Cytokines and chemokines

Role of adjuvant in vaccine development

1) To increase the total antibody titer or functional titers
2) To decrease the dose of antigen needed
3) To decrease the total number of doses of vaccine necessary for complete immunization
4) To overcome competition in combination vaccines
5) To enhance immune responses in the young or older populations
6) To increase the speed and duration of the vaccine specific protective response
7) To induce potent cell mediated immunity
8) To induce mucosal immunity
9) To induce broader immune response

Aluminum salts:

Aluminium is the 8th most abundant element on earth and the most common metallic element. It is found in the blood of all animals, including humans, and we are constantly exposed to it. Aluminium salts have been used as adjuvants for over 70 years. Most commonly of these are aluminium hydroxide, aluminium phosphate and potassium aluminium sulphate (alum). Aluminium adjuvant work by inducing a range of inflammatory factors to the local injection site and also appear to help retain the antigen at the injection site long enough for an immune

response to be generated (Glenny *et al* 1926). Additionally, the use of aluminium adjuvants in vaccines generally means that less antigen is required.

Some studies have found aluminium containing vaccines to be associated with local reactions and, less often, with the development of subcutaneous nodules at the injection site. This is particularly, so if the injection is given too superficially. Other studies have reported fewer reactions with aluminium containing vaccines than those without, and in some cases, fewer vaccine doses are needed. An individual's exposure to aluminium from vaccines is far less than that received from a normal diet. The average daily intake is 10-15 mg. The Hepatitis B vaccine has 0.235 mg of aluminium; average water has about 0.2 mg of aluminium per liter. Aluminium in vaccines is absorbed into the blood and excreted in urine via the kidneys.

A recent review of all the available studies of aluminium containing diphtheria, tetanus and pertusis vaccines (either alone or in combination) found that there was no evidence that aluminium salts in vaccines cause any serious or long-term adverse events (Petrovsky and Aguilar, 2004). Most of our current inactivated and subunit vaccines contain the aluminium salts and they have an impressive safety record (Janeway and Medzhitov, 2002).

MF59:

Another new adjutants used in a flu vaccines in Europe is MF59. This adjutants emulsion is safe and non toxic for use in humans and has been tested in several million subjects. The vaccine formulation contains MF59 (FLUAD)™ which is a licensed product in Europe and has been shown to be safe and well tolerated in patients over the last seven years. MF59 is oil in water emulsion (Stephenson *et al* 2005). It is made with squalene, hydrocarbon oil, which is common in foods as well as being produced in the body as precursor to cholesterol and steroid hormones (Nicholson *et al* 2001). MF59 significantly enhances immune response to a variety of antigens (Atmar *et al* 2006). It is used in some influenza vaccines (Podda and Del Giudice, 2003), pandemic vaccines and it has been trialed in newer vaccines (**Table 2**).

Emulsions and lipids:

Liposomes are phospholipids vesicles that have been evaluated both as adjuvant and as vehicles for antigens. A liposomal hepatitis A (Hep A) vaccine (virosomes) has been extensively evaluated in the clinic and is currently licensed for hepatitis A vaccine. Alternative adjuvants that have been used in a few products include L-tyrosine (allergy vaccines) and MPL (cancer treatment). The various adjuvants used in vaccines formulation are mentioned in **Table 2**.

Table 2. List of types of adjuvant and their examples

Sr. No.	Type of adjuvant	Examples
1.	Gel-type	Aluminium hydroxide/phosphate, Calcium phosphate
2.	Microbial	Bacterial exotoxins, Bacterial DNA
3.	Particulate	Polymer microspheres, liposome, ISCOMS
4.	Oil emulsion and surfactant based adjuvants	Freunds incomplete adjuvant, Microfluidised emulsions (*i.e.* MF59), Saponins
5.	Synthetic	Synthetic polynucleotide
6.	Cytokines	Interleukin 2, 12 Granulocyte-macrophage-colony-stimulating-factor, Interferon gamma
7.	Genetic	Cytokine genes or genes encoding co-stimulatory molecules derives as plasmid DNA

Immunostimulatory adjuvant:

Immunostimulatory adjuvant exerts their effects predominantly at the cytokine level or through the activation of co-stimulatory signals. The type of response required for optimal protection depends on the pathogen (Mbawuike *et al* 2007). One class of immunostimulatory adjuvant is derived from the lipid polysaccharide of gram negative bacteria. The most extensively evaluated member of this family monophosphoryl lipid A (MPL) is obtained from *Salmonella minnesota*. MPL has been shown to include the synthesis and release of cytokines which promote the generation of specific immune responses (Neuzil *et al* 2006). It has recently been established that bacterial DNA but not vertebrate DNA has direct immuno-stimulatory effect on leukocytes. Various

immunostimulatory adjuvant used in human vaccine are tabulated in **Table 3**.

Table 3. Vaccine adjuvant that have been evaluated in humans

Adjuvant/ formulations	Benefits	Comments	Safety/Immunogenicity
Mineral salts Aluminum salts (hydroxide, phosphate) [Alum]	The adjuvant in >80% of vaccines licensed for human use Induction of strong antibody responses, independent of TLR signaling Directly activate DCs to secrete IL-1β and IL-18	Alum has been used for years in vaccines for billions of people of all ages Poor CD8 T-cell induction	May cause mild local reactions at the site of injections, occasional granulomas
Calcium phosphate	Has been used as an adjuvant in vaccine formulations against diphtheria, tetanus, pertusis and poliomyelitis More efficient than aluminum hydroxide when tested as a booster with DT as an antigen Has also been used for absorption of extracts for hyposensitization of allergic patients	Potential alternative to aluminum salts	Calcium phosphate adjuvant contains no components that are not natural constituents of the body, and vaccines containing it are well tolerated
Emulsions MF59 (Microfluidized detergent stabilized squalene oil-in-water emulsion)	Increased flu vaccine immunogenicity in young adults and in elderly as evaluated with HAI titers; broadens response against heterovariant strains Improved immunogenicity over Alum when tested with HBV vaccine, HSV, HIV1 gp120, and CMV gB IM injection in combination with a variety of subunit antigens results in elevated antibody response, increased T-cell proliferation and induction of cytotoxic lymphocytes	Induces chemokines to increase recruitment of immune cells, enhances ag uptake by monocytes and differentiation to DCs MF59 is a component of Fluad®, a licensed subunit influenza vaccine in Europe with >30 million doses distributed Combination of MF59 with MTP-PE enhanced systemic reactogenicity, without improving immunogenicity in Ph1 flu vaccine study	Mild local reactions Minor reactogenicity upon intramuscular injections of humans in combination with various antigens
Incomplete Freund's adjuvant (IFA, stabilized water/Drakeol oil)	Increased anti-p24 titers and DTH responses when used with gp120-depleted inactivated HIV1		May cause granulomas and abscesses at the site of injection

	In seropositive subjects, increased lympho-proliferation and chemo-kine production following p24 stimulation		
Montanide ISA-51 (stabilized water-in-oil emulsion) and ISA-720 (stabilized water/squalene)	Induction of anti-Tat antibodies in 100% of vaccines Strong T cell lympho-proliferative response DTH and lymphoproli-ferative response to Tat was observed in 50% of vaccines	ISA-51 has been used in vaccines for >1000 people and has slow release properties like IFA ISA-720 has been used in vaccines for approximately 1,000 people, >250 in malaria including some children	ISA-720 t-Minor local effects such as local tenderness, swelling and discomfort
Microbial (natural and synthetic) derivatives Monophosphoryl lipid A (MPL)	When combined with polysaccharide-conjugate vaccine, enhanced Th1 responses to the carrier protein, but no impact on antibody responses in humans, despite superior antibody responses observed in animals. Reduced toxicity *vs* LPS	Detoxified derivative of LPS from *Salmonella Minnesota* TLR-4 agonist Component of a melanoma vaccine approved in Canada MPL has been used in vaccines for >20,000 people.	Possible effects on autoimmune and neuro-inflammatory disorders being evaluated. Strong activator of pro-inflammatory cytokines
Detox (MPL + CWS)	Induction of cellular and humoral responses against melanoma associated antigens Increase in survival in patients with metastatic melanoma	Detox has been approved for use in Canada as a component of melacine - a melanoma vaccine Has been used in vaccines for >5,000 people	
Modified LT, CT (Genetically modified bacterial toxins [heat-labile enterotoxin, cholera toxin] to provide non-toxic adjuvant effect)	Enhancement of seric and mucosal IgA production; LT activates Langerhans' cells, causing migration from skin to draining LNs Ongoing evaluation of CT and LT as adjuvant in patch-based transcutaneous immunization, LT induces more balanced Th1/Th2 response than CT	Potential for oral and intranasal adjuvant use LT has been used in human clinical trials, with modest adjuvant effect by oral route and promising results as an antigen in a traveller's vaccine given as a patch	Prototypic mucosal adjuvant, efficient in numerous animal models, but toxic in humans A flu vaccine formulation with LT was withdrawn in Switzerland because of potential safety issues (Bell's palsy) Local rashes with patch

AS02 (Oil-in-water emulsion + MPL + QS-21)	With a candidate malaria vaccine, induced high anti-plasmodium CSP lympho-proliferative and antibody responses but no induction of CD8 T cells, leading to short-lived protection (<6 months) against challenge	AS02 (SBAS2) has been used in vaccine candidates in >1,000 people Used with malaria, TB, HBV, HIV and MAGE-A3 antigens	Significant local (swelling and pain) and systemic reactogeni-city in malaria CSP and MSP-1 antigen trials, but not with RTS, S antigen in Ph1 Local and systemic reactogeni-city more common in children than adults
AS01 (Liposomes + MPL + QS21)	Designed to improve CD8 T-cell responses Data with malaria antigens indicate higher antibody and T-cell response with AS01 than with AS02	AS01 favors Th-1 responses; AS02 elicits more balanced Th-1/Th-2 responses Used with malaria and TB antigens	Limited human safety data
Immuno-adjuvants Cytokines: (IL-2, IL-12, GM-CSF, Flt3)	Enhancement of antibody responses with GM-CSF.	Administration of Flt3 with HBV antigen resulted in the accumulation of immature DCs in peripheral blood, without enhancement of antibody response	Utilization of cytokines as immunoadjuvants in cancer patients as recombinant proteins, with limitations including short biological half-life and some severe toxicity
Accessory molecules (B7.1)	The accessory molecule B7.1 provides costimula-tory signals to T lympho-cytes, has been included in association with the CEA antigen within the canary-pox vector ALVAC, so potentially enhancing cellular responses.		
PLA (polylactic acid) PLG (poly[lactide-co-glycolide]) microparticles	PLGA particles were shown to elicit Th1 (presentation of CTL epitopes) and Th2 responses in mice.	Microparticles function mainly as delivery system Clade B Gag DNA/PLG and Env DNA/PLG Micro-particles vaccine is in an ongoing Ph1 trial in HIV-negative adults Ongoing trial with the tetanus toxoid Difficult to prepare GMP-grade PLGA particles	

Excipients:

Preservatives

For our purposes, preservatives may be defined as compounds that kill or prevent the growth of microorganisms, particularly bacteria and fungi. They are used in vaccines to prevent microbial growth in the event that the vaccine is accidentally contaminated, as might occur with repeated puncture of multi-dose vials. In some cases, preservatives are added during manufacture to prevent microbial growth; with changes in manufacturing technology, however, the need to add preservatives during the manufacturing process has decreased markedly. Moreover, this is an agent added to increase the shelf life of a vaccine. The United States Code of Federal Regulations (CFR) requires, in general, the addition of a preservative to multi-dose vials of vaccines; indeed, worldwide, preservatives are routinely added to multi-dose vials of

vaccine (www.fda.gov). Preservatives cannot completely eliminate the risk of contamination of vaccines. The literature contains several reports of bacterial contamination of vaccines despite the presence of a preservative, emphasizing the need for meticulous attention to technique in withdrawing vaccines from multi-dose vials.

2-Phenoxyethanol:
The most commonly used preservative in vaccines is phenoxyethanol is used to preserve some vaccines. It is also commonly used in cosmetics, baby care products, eye and ear drops. It is used in aromatherapy products to protect against contamination. Phenoxyethanol is absorbed through skin and excreted by being exhaled as well as being metabolized (broken down) and excreted via the urine and faeces. There is little toxicity in humans and some irritation with very high doses in animals. 2-PE contains phenol, which has the ability to inhibit phagocyte activity, meaning it is toxic to all cells. The phenol in 2-PE is capable of disabling the immune system's primary response mechanism. It can also cause systemic poisoning, headache, shock, weakness, convulsions, kidney damage, cardiac failure, kidney failure, or death. 2-PE also contains ethylene oxide, which is an irritant causing dermatitis, burns, blisters, and eczema.

Phenol:
Phenol is a highly poisonous, caustic substance derived from coal tar and used in the production of disinfectants, dyes, pharmaceuticals, plastics, germicides, and preservatives. Exposure may result in systemic poisoning, weakness, sweating, headache, shock, excitement, kidney damage, convulsions, cardiac or kidney failure, and death. Repeated exposure may also cause vomiting and mental disturbances. Phenol is considered to be corrosive to the skin and it is known to be a protoplasmic poison *i.e.* toxic to all cells. Tests indicate that phenol actually inhibits phagocytic activity. The use of phenol in vaccine preparations introduces a poisonous and caustic substance into the body. Since it inhibits phagocytic activity, it actually serves to debilitate, rather than stimulate, the immune response. Phagocytes serve as the body's first line of defense against antigenic activity: they engulf and digest antigens and they cause other elements of the immune system to become activated. Since vaccines are meant to stimulate an immune response, the use of phagocyte-inhibiting phenols contradicts the basic rationale for using vaccines. Furthermore, harm cannot possibly be avoided when it is understood that at the same time that pathogens are being introduced into the body, phenols are acting to inhibit an appropriate immune response. An aromatic alcohol used infrequently as a preservative in vaccines.

Thiomersal:
Thimersal is a mercury containing preservative used in some vaccines and other products since the 1930s. No harmful effects have been reported from thimersal at doses used in vaccines, except for minor reactions like redness and swelling at the injection site. However, in July 1999, the Public Health Service (PHS) agencies, the American Academy of Pediatrics (AAP), and vaccine manufacturers agreed that thiomersal should be reduced or eliminated in vaccines as a precautionary measure. Today, with the exception of some influenza (flu) vaccines, none of the vaccines used in the U.S. to protect preschool children against 12 infectious diseases contain thimersal as a preservative (Frech *et al* 2005).It is no longer present in the vaccines on the New Zealand childhood schedule. There is no evidence that thiomersal causes serious adverse events. Thimersal in concentrations of 0.001% (1 part in 100,000) to 0.01% (1 part in 10,000) has been shown to be effective in clearing a broad spectrum of pathogens. A vaccine containing 0.01% thimersal as a preservative contains 50 micrograms of thiomersal per 0.5 ml dose or approximately 25 micrograms of mercury per 0.5 ml dose.

Stabilizers
Certain additives, salts and bulking agents may be added primarily to improve vaccine stability upon storage. Stabilizers inhibit chemical reactions and prevent components separating or sticking to the vial during transport and storage. Examples of stabilizers include sugars such as lactose and sucrose, amino acids such as glycine and monosodium glutamate (salts of amino acids), albumin which is a protein derived from human or bovine (cow) serum albumin (both proteins). Gelatin, which is partially hydrolyzed collagen, usually of bovine (cow) or porcine (pig) origin, is added to some vaccines as a stabilizer. These excipients such as mannitol, glycine and trehalose have a direct impact on the polypeptide or conjugate and are investigated

for this purpose. Stabilizers are required to keep the vaccine homogenous and stop the components separation. *e.g.* Sodium chloride: salt; Sodium borate: commonly used in mouthwashes, antiseptics and as an astringent; Boron: an essential trace element; Lactose: milk sugar; Magnesium chloride: common dietary supplement; Gelatin: stabilizer used in desserts as a setting agent; Sorbitol: a plant extract commonly used as a bulk sweetener; Medium 199: serum free growth media.

Buffers

These control pH within a specific vaccine formula, but downstream effects on body fluids and tissues after injection have not been thoroughly researched. Local (micro domain) concentrations of Ca^{2+}, Na^{+}, and K^{+} ions can be altered once the buffers are no longer confined to the vaccine. Citric acid is excitotoxic and may harbor mercury. Sodium citrate is a strong blood anticoagulant that converts Ca^{2+} into calcium citrate, a process that increases absorption of aluminum and lead contributes to excitotoxicity. It is a byproduct of citric acid manufacturing so may also harbor mercury depending on its source. Sodium bicarbonate (aka baking soda) is a salt consisting of the Na^{+} ion and the bicarbonate HCO^{3-} anion. At a cellular level this pair is critical in regulating pH, especially in the brain and central nervous system where sodium-bicarbonate (NBC) transport has been shown to govern the function of neuron and glial cells, the production of myelin, and protection against excitotoxicity. People with immature or damaged nervous systems (infants, small children, and people suffering toxic insult) are most likely at risk from the effects of free $NaHCO_3$ received by injection. Sodium borate (aka borax) is neurotoxic and not meant for internal use. Infants are particularly susceptible especially with repeated exposure. Symptoms can be immediate but generally appear hours or days later. Symptoms include nausea, vomiting, diarrhea, flushed skin, changes in respiration/temperature/pulse, hyperactivity, CNS depression, mental confusion, lethargy, seizures, shock, metabolic acidosis, vascular collapse, and death. At the cellular level, it interferes with DNA, RNA and enzymes, and causes cell death. Phosphate buffers contain the element phosphorus. Phosphorus is an important component of all body tissues, plays a role in bone development, supports calcium metabolism, is a buffer for acid-base equilibrium, and is an element in various enzyme systems. Buffers containing phosphorus have the ability to affect various metabolic processes and can trigger or intensify excitotoxicity. There could be some precipitation of calcium phosphate in soft tissue (calcification). Overall these phosphate buffer agents appear less problematic than other ingredients, but they can still add stress to the system when administered. This example shows how exposure from the levels in a vaccine is of similar magnitude to what has shown harm in infants. Effects may be subtle and unobserved; however the timing and location of exposure can have an effect on other events. The elderly, young children and patients with poor kidney function are at increased risk. Buffers serve to resist changes in pH, adjust tonicity and maintain osmolarity. The most commonly used buffer is sodium chloride (table salt).

Diluents

Diluent is a liquid used to dilute a vaccine to the proper concentration prior to administration. This is usually sterile saline or sterile water (www.fda.gov).

Surfactants/emulsifiers

Surfactants or emulsifiers are wetting agents that alter the surface tension of a liquid and lower the tension between two liquids likes detergent. An example is polysorbate 80 (tween 80) which is often used in foods such as ice cream. It is made from sorbitol (sugar alcohol) and oleic Acid (omega fatty acid).

Animal products

Animal products are commonly used in the manufacture of vaccines and are necessary for growing the vaccine pathogens. Commonly used animal cell lines and other material used in vaccines are derived from chicken, cow, pig, sheep and fish. These are to either provide nutrient to support growth of the pathogen, or cell lines in which the pathogen may grow. Albumin is a common plasma protein. The VERO cell line is one of the only animal cell lines licensed for human use and has been derived from African Green Monkey kidneys. As this cell line is of primate origin, it is susceptible to several human viruses including poliovirus (Dowdle *et al* 2003). For some pathogens which are only infective in humans such as rubella, it is often necessary to grow them on a culture of human cells. During vaccine manufacture the vaccine pathogen will be purified from the

growth media. In some cases there may be minute traces of this material remaining in the vaccine.

Human derived products

Vaccines are biological products, with both organic (sometimes including blood products and animal products) and inorganic materials used in their manufacture. The vaccine strain of rubella was derived from a fetus infected with rubella in 1965. The virus was then cultured in a cell line that was taken from fetal tissue. Mature human tissue has cells only capable of dividing a few times before dying whereas fetal cells can continue to divide and be propagated indefinitely. Rubella is a human disease and requires human cells to replicate. Early attempts to use animal cell lines were unsuccessful. There has been no further (new) fetal tissue used in the production of Rubella vaccine since the original development of the vaccine in 1965 (Pulendran and Ahmed, 2006).

Salts

The most commonly used salt in vaccines formulation is sodium chloride, sodium phosphate, succinic acid and sodium borate. The concentrations of the salts used in any given formulation are based on isotonicity, pH, and other stabilizers being used in the formulation. A typical range is from 5-20 mM salt concentration. These concentration are selected to reduce pain on injection and to accord rapid normalization with physiological fluid surfactants used in MF59 emulsion include tween 80 and sorbitan trioleate. Various excipients included in US licensed vaccine are given in **Table 4**.

Table 4. Various excipients included in US licensed vaccine

Excipients	Use	Vaccine
Aluminum hydroxide	Adjuvant	Anthrax (biothrax), Dtap (certiva, infanri.v, acel-inaune), Dt (massachusetts), Td (massachusetts), Hib (ped- vaxhib), Hib-hepatitis b (connvax), Hepatitis a (harris, vaqta), Hepatitis b (engerix-b, recombivax-hb), Lyme disease (lvnerix)
Aluminum Phosphate	Adjuvant	DTaP, DTwP (Massachusetts, BioPort), DT (Wyeth-Lederlc), Td (Massachusetts, Wyeth-Lederle), Pneurnococcal (Prevnar), Rabies (Bio-Rab)
Aluminum potassium sulfate	Adjuvant	DTaP (Tripedia). DTwP (Aventis Pas (eur), DT (Aventis Pasteur). Td (Aventis Pasteur)
Amino acids	Growth medium	Hepatitis A (Havrix), Typhoid oral (Vivotif)
Ammonium sulfate	Protein fractionation	Hib (Act-HIB)
Amphotericin B	Anti-bacterial	Rabies RabAvert)
Ascorbic acid	Antioxidant	Typhoid oral (Vvotif)
Bactopeptone	Growth medium	Influenza (varies seasonally)
Beta-propiolactone	Viral inactivator	Influenza (Fluvirin), Rabies (Imovax RabAvert)
Benzethonium chloride	Preservative	Anthrax (BioThrax)
Bovine albumin or serum	Growth medium, protein stabilizer	Hepatitis A (Havrix, Vaqta), Poliovirus attenuated, Rabies (Inumax, RabAvert), Vaccinia (DryVax), Varicella (Varivax)
Brilliant green	Dye	Vaccinia (Dn,Vax)
Chlortetracycline	Anti-bacterial	Rabies (RabAvert), Vaccinia (Dn'Vax)
DNA	Manufacturing residue	Hepatitis A (Vaqta)
Ethylenediamine-tetraacetic acid sodium (EDTA)	Preservative	Rabies (RabAvert), Varicella (Varivax)
Egg protein	Growth medium	Influenza, Yellow fever (YF-Vas)

Fetuin (a bovine serum protein)	Affinity ligand for chromatography	DTaP (Certiva)
Formaldehyde. Formalin	Anti-microbial, preservative	Anthrax (BioThrax), DTaP (all brands), DTwP (all brands), DTwPHib (Tetrammne), DT (all brands), Td (all brands), Hepatitis A (Havrix, Vagta), Hib (ActHIB), Influenza (Fhuogen, FluShield, Fluzone), Japanese encephalitis (JE-Vax), Poliovirus inactivated
Gelatin	Stabilizer in freeze-drying. Solvent	DTaP (Acel-Imune. Tripedia), Influenza (Fluzone), Japanese encephalitis (JE-Vax), Measles (Attenuvax), Mumps (Mumpsvax), Rubella (Meruvax If), MMR (MMR-II), Rabies (RabAvert), Typhoid oral (Vivotif), Varicella
Gentamicin	Anti-bacterial	Influenza (F1uShield)
Glycerin	Solvent	Vaccinia (DrvVax)
Glycine	Protein stabilizer	DTaP (Acel-Imune), DTwP-Hib (Teta none), DT (most brands), Td (most brands)
Human serum albumin Rabies (Imovax)	Growth medium	Rabies (Imovax)
Hydrochloric acid	Adjust pH	DTaP (most brands), DT (mostbrands)
Hydrogen peroxide	Toxin detoxifier	DTaP (Certiva)
Kanamycin	Anti-bacterial	Lyme disease (LvnteRix
Lactose	Stabilizer in freeze drying. Filling	BCG (Tice). Hib (some packages), eningococcal (Menomune), Typhoid ral (Vivotif)
Magnesium stearate	Lubricant for capsule filling	Typhoid oral (Vivotif)
Monosodium glutamate	Stabilizer	Varicella (Varivax)
Mouse serum protein	Growth medium	Japanese encephalitis (JE-Vax)
MRC-5 cellular protein	Growth medium	Hepatitis A (Havrix, Vaqta), Rabies (Imovax, RabAvert), Varicella (Varivax)
Neomycin	Anti-bacterial	Influenza (Fluvirin), Measles (Attenuvax), Mumps (Mumpsvax), Rubella (Meruvax II), MMR (MMR-II), Poliovirus attenuated (Orinnine), Poliovirus inactivated (Ipol), Rabies (Imovax, RabAvert), Vaccinia (DryVax)
Ovalbumin	Growth medium	Rabies (RabAvert)
Phenol	Preservative. anti-bacterial	Pneumococcal (Pneunurvax-23), Typhoid inactivated (Tvphim Vi), Vacinia (DryVax)
Phenol red	pH indicator, dye	Poliovirus attenuated
2-Phenoxy Ethanol	Preservative	DTaP (Infanrix), Hepatitis A (Havrix), Lyme disease (LvmeRix), Poliovirus inactivated (Ipol)
Phosphate buffers	pH adjust	DTaP (all brands), DT (most brands), Hib (Act-Hib), Hepatitis A (Harrix), Hepatitis B (Engerix-B), 5rLyme disease (LvineRix), Poliovirus inactivated (lpol), Rabies (BioRab), Typhoid inactivated (Tvphim Vi), Varicella (Varivax)
Polydimethylsilozone	Anti-foaming agent	Anti-foaming agent
Polymyxin B	Anti-bacterial	Influenza (Fluvirin)
Polysorbate 20	Surfactant	Hepatitis A (Havrix)
Polysorbate 80	Surfactant	DTaP (Infanrix. Tripedia), Influenza (Fluogen)

Potassium glutamate	Stabilizer	Rabies (RabAvert)
Silicon	Anti-foaming agent	Lynie disease (LwneRix)
Sodium acetate	Adjust pH	DT (most brands), Td (most brands)
Sodium bisulfate	Preservative	Influenza (Fluogen)
Sodium borate	Adjust pH	Hepatitis A (Vaqta), Hib-Hepatitis B (Conn-ax)
Sodium chloride	Adjust tonicity	Most vaccines including Anthrax, BCG, Cholera, DTaP, DTwP, DTwP-Hib, DT, Td, Hepatitis A, Hepatitis B, Hib. Influenza, Lyme disease, Pneumo-coccal, Polio inactivated, Rabies, Typhoid inactivated, Varicella, Yellow fever
Sodium hydroxide	Adjust pH	DT (most brands), Td (most brands)
Sorbitol	Stabilizer, solvent	Measles (Attenuvax), Mumps (Muntpsvax), Rubella (Meruva.t 11), MMR (MMR-I/), Polio attenuated, Yellow fever (YF-Vax)
Streptomycin	Antibacterial	Influenza (Fluogen), Poliovirus attenuated (Orinute), Poliovirus inactivated (Ipol), Vaccinia (DryVax [dihydro streptomycin])
Sucrose	Stabilizer in freeze-drying	Hib (Act-HIB), Typhoid oral (Vivotif), Varicella (Varivax)
Thimersal	Preservative in some multidose containers	DTaP (some containers), DTwP (most containers), DT (most brands), Td (most brands), Hepatitis B (some packages), Hib (some packages), Influenza (all brands), Japanese encephalitis (JEVax), Meningococcal (Menomune), Pneumococcal (Pnu-/Lnune 23), Rabies (BioRab)
Tri(n)butylphosphate	Viral inactivater	Influenza (FluShield)
Vitamins unspecified	Growth medium	(Rabies Unimax)
Yeast protein	Growth medium	Hepatitis B (Engerix-B. Reconnbirax HB), Hib (HibTiter), Hib-Hepatitis B (Conn rax)

Residuals from the manufacturing process

Residual substances are those substances used in the course of manufacture of the vaccine but which are not included in the finished vaccine product. There may be residues of these substances in miniscule amounts (Laurent *et al* 2007). They often come from the manufacturing processes of either culturing the organism or of inactivating the live organism or toxin, ready for use in the vaccine. Bovine serum: serum (the clear liquid that contains protein and separates from blood on clotting), originating from cows, used as a growth medium for some cell cultures. a) Neomycin: an antibiotic used in vaccine production b) Streptomycin: an antibiotic used in vaccine production c) Polymyxin B: an antibiotic used in vaccine production d) Formaldehyde: commonly used fixative and antibacterial agent, used to inactivate the active vaccine component (*e.g.* polio virus in oral polio vaccine).

Excipients used to improve stability of vaccine

Certain additives, salts and bulking agents may be added primarily to improve vaccine stability upon storage. Stabilizers inhibit chemical reactions and prevent components separating or sticking to the vial during transport and storage (Treanor *et al* 2006). Examples of stabilizers include sugars such as lactose and sucrose, amino acids such as glycine and monosodium glutamate (salts of amino acids), albumin. Relevant physiochemical and/or biological properties, based on the characteristics of the adjuvant, should be employed in assessing the stability of the adjuvant during storage. Stability

indicating parameters may include structure and antigen adsorption/binding characteristics.

Analytical assay and quality control of excipients for vaccine formulation

Any excipients for a vaccine formulation is treated as a component of a parenteral formulation and must adhere to strict FDA requirement of compliance and regulation of this material. Most of the excipients used in vaccine formulation (except adjutants) are also used in many other parenteral formulations and thus have a long stability and tolerability profile (Dellepiane *et al* 2000). Many components like adjutants have guidelines for the purity, monomer rational and concentration. Ideally purity greater than 98% is considered as the minimal criterion. The selection of concentration is based on extensive preclinical evaluation to show minimum reactogenicity and enhanced immunogenicity. Typical assay used for quantifing the excipients is based on reversed phase high performance liquid chromatography, sodium dodecyl sulfate polyacrylamide gel electrophoresis, spectrophotometric analysis and colorimetric analysis (Gotschlich *et al* 1972).

Selection and types of vaccines for next generation

Next generation vaccine formulation will comprise several antigens that will include glycoconjugates, recombinant proteins, plasmids, oligonucleotide, peptides and additional adjuvant molecule for enhanced immunogenicity (Fernandez *et al* 2000). This complex formulation will need a rational selection of stabilizers, preservatives and buffers.

Most paramount in this selection is that the stability of all components of vaccines should be such that the potency of final formulation is maintained. Enhanced shelf life is second parameter that would dictate formulation development in vaccines. New vaccine modalities such as DNA vaccines are currently being explored using charge PLG microparticles as delivery system (O'Hagan and Rappuoli, 2004). Next generation vaccine formulation will comprise of several antigens that will include the glycoconjugates, recombinant proteins, plasmids, oligonucleotide, peptides and the additional adjuvant molecules for enhanced immunogenicity (Lagos *et al* 1998). This complex formulation will need a rational selection of stabilizers, preservatives and buffers. Most paramount in this selection is that the stability of all components of vaccines should be such that the potency of final formulation is maintained.

Enhanced shelf life is second parameter that would dictate formulation development in vaccines. New vaccine modalities such as DNA vaccines are currently being explored using charge PLG microparticles as delivery system (dela Crutz *et al* 2007).This and same novel delivery technologies will be essential component some of next generation vaccines.

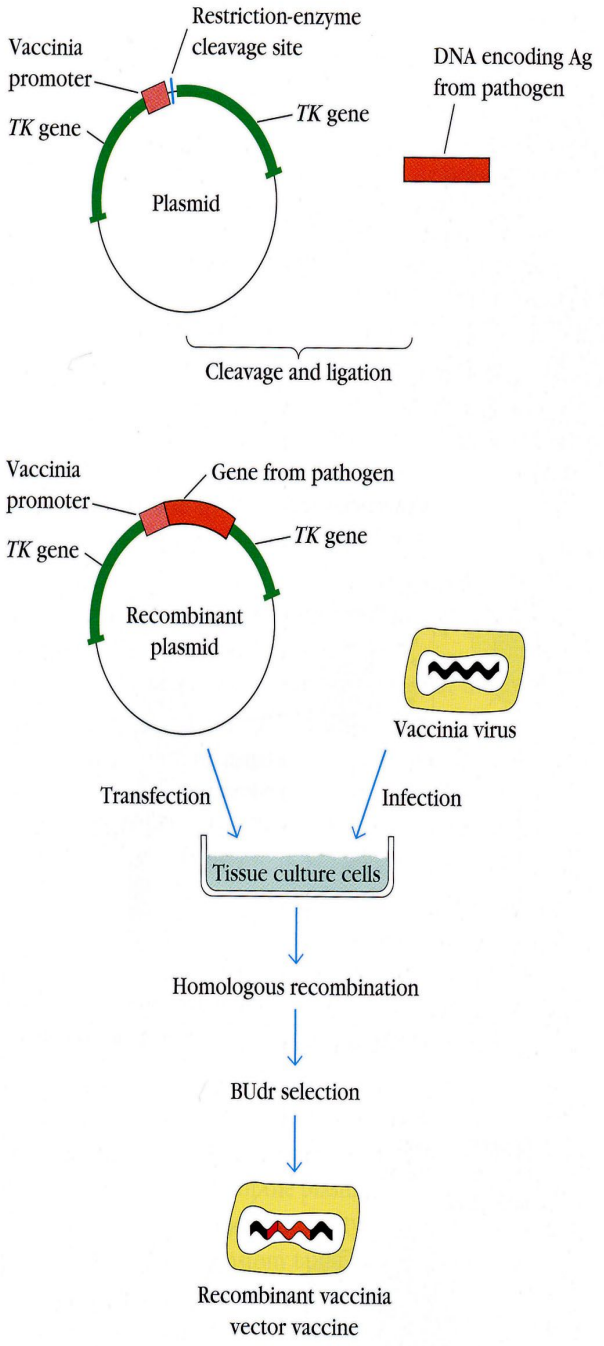

Figure 1. Formulation of recombinant/vector vaccine

Classification of vaccines for human use

Recombinant vectored vaccines:

Genes of a pathogen can be introduced into attenuated bacteria or viruses (ex yellow fever expressing West Nile antigens). Pox viruses often used as vectors (fowl canary and cowpox vaccinia virus) viruses, adenoviruses prime boost strategies (**Figure 1**).

DNA Vaccines:

a) Plasmid DNA injected into muscle, encoded protein antigen expressed b) Induces AMI and CMI^{+} memory c) Single plasmid can be tailored to make variety of vaccines d) Refrigeration not needed for handling and storage of plasmid DNA e) Can be administered on microscopic gold beads with gene gun.

CONCLUSION

Vaccines are biological medicinal products which are effective in providing protection against a large number of infectious diseases. In general, it would be desirable if the guidelines for vaccine formulation and development could be harmonized all over the world to avoid abusiveness. This would shorten the time to market as well as the cost for developing new vaccines production technologies. This is especially of value if the new vaccine is also used in developing countries. Immunization is one of the best ways to improve health by using adjuvant and other excipients. In the past, vaccines developed against diseases afflicting rich countries. *eg.* measles and polio have been widely and effectively used in developing countries. Though these diseases kill millions of people, the communities affected cannot afford to buy vaccines at a price those factors force researchers to discover next generation vaccine for better prospectus in future. Vaccines require dedicated production facilities that include physical and chemical barriers to protect workers from pathogen exposure and finely regulated temperature and ventilation to keep the biologics viable while stored. Moreover, it requires good quality of adjuvant and excipients for patient safety and hygienic. Also, because the product is injected, the purity standard has to be much higher than for a pill. Therefore, new approaches for financing in order to stimulate the industry to increase its efforts in vaccine research and development should be created.

REFERENCES

Atmar RL, Keitel WA, Patel SM, Katz JM, She D, El Sahly H, Pompey J, Cate TR, Couch RB. Safety and immunogenicity of nonadjuvanted and MF59-adjuvanted influenza A/H9 N2 vaccine preparations. *Clin. Infect. Dis*. 2006;43(9): 1135-42. [DOI: 10.1086/508174]

Bernstein D, Cardin R, Strasser J, Chalk C, Farley N, Fairman J. Juvimmune is a novel vaccine adjuvant that enhances protection of mice from lethal HSV-2 infection following immunization. In: Infectious Diseases Society of America 45th Annual Meeting, San Diego, CA; 2007: presentation 1147.

Brewer JM. (How) do aluminium adjuvants work? *Immun. Lett.* 2006;102(1):10-5. [DOI: 10.1016/j.imlet.2005.08.002]

Burdin N, Guy B, Moingeon P. Immunological foundations to the quest for new vaccine adjuvants. *BioDrugs* 2004;18(2):79-93.

dela Cruz T, Milley R, Hillebrand A. Conjugation of Immunostimulatory DNA to Conserved Viral Antigens as a Universal Influenza Vaccine. In: Infectious Diseases Society of America 45th Annual Meeting, San Diego, CA; 2007: presentation 1145.

Dellepiane N, Griffiths E, Milstien JB. New challenges in assuring vaccine quality. *Bull. World Health Organ.* 2000;78(2):155-62.

Dowdle WR, De Gourville E, Kew OM, Pallansch MA, Wood DJ. Polio eradication: the OPV paradox. *Rev. Med. Virol.* 2003;13(5):277-91. [DOI: 10.1002/rmv.401]

Frech SA, Kenney RT, Spyr CA, Lazar H, Viret J-F, Herzog C, Gluck R, Glenn GM. Improved immune responses to influenza vaccination in the elderly using an immunostimulant patch. *Vaccine* 2005;23(7):946-50. [DOI: 10.1016/j.vaccine.2004.06.036]

Garcon N, Chomez P, Van Mechelen M. GlaxoSmithKline Adjuvant Systems in vaccines: concepts, achievements and perspectives. *Expert Rev. Vaccines* 2007;6(5):723-39. [DOI: 10.1586/14760584.6.5.723]

Gotschlich EC, Rey M, Triau R, Sparks KJ. Quantitative determination of the human immune immune response to immunization with meningococcal vaccines. *J. Clin. Invest*. 1972;51(1):89-96. [DOI: 10.1172/JCI106801]

Guy B. The perfect mix: recent progress in adjuvant research. *Nat. Rev. Microbiol*. 2007;5(7):505-17. [DOI: 10.138/nrmicro1681]

Janeway Jr. CA, Medzhitov R. Innate immune recognition. *Annu. Rev. Immunol*. 2002;20:197-216. [DOI: 10.1146/annurev.immunol.20.083001.084359]

Lagos R, Valenzuela MT, Levine OS, Losonsky GA, Erazo A, Wasserman SS, Levine MM. Economisation of vaccination against Haemophilus influenzae type b: a randomised trial of immunogenicity of fractional-dose and two-dose regimens. *Lancet* 1998;351(9114):1472-6. [DOI: 10.1016/S0140-6736(97)07456-4]

Lang J, Wood SC. Development of orphan vaccines: an industry perspective. *Emerg Infect. Dis.* 1999;5(6):749-56.

Laurent PE, Bonnet S, Alchas P, Regolini P, Mikszta JA, Pettis R, Harvey NG. Evaluation of the clinical performance of a new intradermal vaccine administration technique and associated delivery system. *Vaccine* 2007;25(52):8833-42. [DOI: 10.1016/j.vaccine.2007.10.020]

Lindblad EB. Aluminium adjuvants--in retrospect and prospect. *Vaccine* 2004;22(27-28):3658-68. [DOI: 10.1016/j.vaccine.2004.03.032]

Luke CJ, Subbarao K. Vaccines for pandemic influenza. *Emerg. Infect. Dis*. 2006;12(1):66-72.

Mbawuike I, Zang Y, Couch RB. Humoral and cell-mediated immune responses of humans to inactivated influenza vaccine with or without QS21 adjuvant. *Vaccine* 2007; 25(17):3263-9. [DOI: 10.1016/j.vaccine.2007.01.073]

Neuzil KM, Jackson LA, Nelson J, Klimov A, Cox N, Bridges CB, Dunn J, DeStefano F, Shay D. Immunogenicity and reactogenicity of 1 versus 2 doses of trivalent inactivated influenza vaccine in vaccine-naive 5-8-year-old children. *J. Infect. Dis*. 2006;194(8):1032-9. [DOI: 10.1086/507309]

Nicholson KG, Colegate AE, Podda A, Stephenson I, Wood J, Ypma E, Zambon MC. Safety and antigenicity of non-adjuvanted and MF59-adjuvanted influenza A/Duck/Singapore/97 (H5N3) vaccine: a randomised trial of two potential vaccines against H5N1 influenza. *Lancet* 2001; 357(9272):1937-43. [DOI: 10.1016/S0140-6736(00)05066-2]

O'Hagan DT, Rappuoli R. Novel approaches to vaccine delivery. *Pharm Res*. 2004;21(9):1519-30. [DOI: 10.1023/B:PHAM.0000041443.17935.33]

Pashine A, Valiante NM, Ulmer JB. Targeting the innate immune response with improved vaccine adjuvants. *Nat. Med.* 2005;11(4):S63-8. [DOI: 10.1038/n m1210]

Petrovsky N, Aguilar JC. Vaccine adjuvants: current state and future trends. *Immunol. Cell Biol*. 2004; 82(5):488-96. [DOI: 10.1111/j.0818-9641.2004.01272]

Podda A, Del Giudice G. MF59-adjuvanted vaccines: increased immunogenicity with an optimal safety profile. *Expert. Rev. Vaccines* 2003;2(2):197-203. [DOI: 10.1586/14760584.2.2.197]

Pulendran B, Ahmed R. Translating innate immunity into immunological memory: Implications for vaccine development. *Cell* 2006;124(4):849-63. [DOI: 10.1016/j.cel l.2006.02.019]

Stephenson I, Bugarini R, Nicholson KG, podda A, Wood JM, Zambon MC, Katz JM. Cross-reactivity to highly pathogenic avian influenza H5N1 viruses after vaccination with nonadjuvanted and MF59-adjuvanted influenza A/Duck/Singapore/97 (H5N3) vaccine: a potential priming strategy. *J. Infect. Dis*. 2005;191(8): 1210-5. [DOI: 10.1086/428948]

Treanor JJ, Campbell JD, Zangwill KM, Rowe T, Wolff M. Safety and immunogenicity of an inactivated subvirion influenza A (H5N1) vaccine. *N. Engl. J. Med*. 2006; 354 (13):1343-51.

Vogel FR, Hem SL. Immunologic Adjuvants. In: Plotkin SA, Orenstein WA, eds. Vaccines. 4th ed. Philadelphia, PA: Saunders; 2004: 69-79.

*****