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Special Feature

Vaccine adjuvants: Current state and future trends

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Summary The problem with pure recombinant or synthetic antigens used in modern day vaccines is that they are generally far less immunogenic than older style live or killed whole organism vaccines. This has created a major need for improved and more powerful adjuvants for use in these vaccines. With few exceptions, alum remains the sole adjuvant approved for human use in the majority of countries worldwide. Although alum is able to induce a good antibody (Th2) response, it has little capacity to stimulate cellular (Th1) immune responses which are so important for protection against many pathogens. In addition, alum has the potential to cause severe local and systemic side-effects including sterile abscesses, eosinophilia and myofascitis, although fortunately most of the more serious side-effects are relatively rare. There is also community concern regarding the possible role of aluminium in neurodegenerative diseases such as Alzheimer's disease. Consequently, there is a major unmet need for safer and more effective adjuvants suitable for human use. In particular, there is demand for safe and non-toxic adjuvants able to stimulate cellular (Th1) immunity. Other needs in light of new vaccine technologies are adjuvants suitable for use with mucosally-delivered vaccines, DNA vaccines, cancer and autoimmunity vaccines. Each of these areas are highly specialized with their own unique needs in respect of suitable adjuvant technology. This paper reviews the state of the art in the adjuvant field, explores future directions of adjuvant development and finally examines some of the impediments and barriers to development and registration of new human adjuvants.

Key words: adjuvants, immune response, mucosal immunity, vaccines.

Adjuvant origins

The goal of vaccination is the generation of a strong immune response to the administered antigen able to provide long-term protection against infection. To achieve this objective with killed as opposed to live attenuated vaccines, often requires the addition of an adjuvant.[1] Adjuvants are compounds that enhance the specific immune response against co-inoculated antigens. The word adjuvant comes from the Latin word *adjuvare*, which means to help or to enhance.[2] The concept of adjuvants arose in the 1920s from observations such as those of Ramon *et al.* who noted that horses that developed an abscess at the inoculation site of diphtheria toxoid generated higher specific antibody titres.[3,4] They subsequently found that an abscess generated by the injection of unrelated substances along with the diphtheria toxoid increased the immune response against the toxoid.[3,4] The adjuvant activity of aluminium compounds was demonstrated by Glenny *et al.* in 1926 with diphtheria toxoid absorbed to alum.[5] To this day, aluminium-based compounds (principally aluminium phosphate or hydroxide) remain the predominant human adjuvants.[6] In 1936, Freund developed an emulsion of water and mineral oil containing killed mycobacteria, thereby creating one of the most potent known adjuvants, Freund's complete adjuvant (FCA).[7,8] Despite being the gold standard adjuvant, FCA causes severe local reactions and is considered too toxic for human use. The oil in water emulsion without added mycobacteria is known as Freund's incomplete adjuvant (FIA) and, being less toxic, has been used in human vaccine formulations.[8] In the 1950s, Johnson *et al.* found that lipopolysaccharides (LPS) from Gram-negative bacteria exhibited adjuvant activity[9] and detoxified LPS or related compounds such as lipid A have since been used as adjuvants in human studies.[10] In 1974, Lederer *et al.* identified muramyldipeptide (MDP) as a mycobacterial component with adjuvant activity contained in FCA.[11] Bacterial components are often potent immune activators although commonly associated with toxicity, for example, bacterial DNA with immunostimulatory CpG motifs is one of the most potent cellular adjuvants.[12] Immunostimulatory CpG are unmethylated cytosine-guanine dinucleotides found in bacterial DNA but absent in mammalian DNA. Overall, several hundred natural and synthetic compounds have been identified to have adjuvant activity. Although a significant number are clearly more potent than alum, toxicity is perhaps the single most important impediment in introducing most such adjuvants to human use.[2]

Adjuvant roles

Adjuvants can be used for various purposes: (i) to enhance the immunogenicity of highly purified or recombinant antigens; (ii) to reduce the amount of antigen or the number of immunizations needed for protective immunity; (iii) to improve the efficacy of vaccines in newborns, the elderly or immunocompromised persons; or (iv) as antigen delivery systems for the uptake of antigens by the mucosa.[13–15]

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Adjuvant selection

Some of the features involved in adjuvant selection are: the antigen, the species to be vaccinated, the route of administration and the likelihood of side-effects.[16,17] Ideally, adjuvants should be stable with long shelf life, biodegradable, cheap to produce, not induce immune responses against themselves and promote an appropriate immune response (i.e. cellular or antibody immunity depending on requirements for protection).[18] There are marked differences in the efficacy of adjuvants depending on the administration route (e.g. between mucosal and parenteral routes). Hence new vectors, antigen delivery systems or adjuvant compounds need to take into account the characteristics of the proposed administration route.[19]

Adjuvant safety issues

The benefits flowing from adjuvant incorporation into any vaccine formulation have to be balanced with the risk of adverse reactions.[20,21] Adverse reactions to adjuvants can be classified as local or systemic. Important local reactions include pain, local inflammation, swelling, injection site necrosis, lymphadenopathy, granulomas, ulcers and the generation of sterile abscesses. Systemic reactions include nausea, fever, adjuvant arthritis, uveitis, eosinophilia, allergy, anaphylaxis, organ specific toxicity and immunotoxicity (i.e. the liberation of cytokines, immunosuppression or autoimmune diseases).[22,23] Unfortunately, potent adjuvant action is often correlated with increased toxicity, as exemplified by the case of FCA which although potent is too toxic for human use. Thus, one of the major challenges in adjuvant research is to gain potency while minimizing toxicity. The difficulty of achieving this objective is reflected in the fact that alum, despite being initially discovered over 80 years ago, remains the dominant human adjuvant in use today.

Adjuvant regulatory requirements

Regulations for the human use of adjuvants are far more rigorous than those applied to veterinary vaccines. In addition to preclinical studies on the adjuvant itself, the combined antigen-adjuvant formulation also needs to be subjected to toxicology prior to commencement of phase 1 clinical trials.[24] The toxicological evaluation is normally conducted in small animal species such as mice, rats or rabbits and should use the same administration route proposed for human use. The dose and frequency of vaccination for preclinical toxicology should be similar to or higher than the proposed human dose to maximize the ability to identify potential safety problems.[25] Preclinical studies may also help in selecting the optimum vaccine dose.[26]

Adjuvant classification

Adjuvants can be classified according to their source, mechanism of action or physicochemical properties.[2] Edelman[22] classified adjuvants into three groups: (i) active immunostimulants, being substances that increase the immune response to the antigen; (ii) carriers, being immunogenic proteins that provide T-cell help; and (iii) vehicle adjuvants, being oil emulsions or liposomes that serve as a matrix for antigens as well as stimulating the immune response. An alternative adjuvant classification divides adjuvants according to administration route, namely mucosal or parenteral. A third classification divides adjuvants into alum salts and other mineral adjuvants; tensoactive agents; bacterial derivatives; vehicles and slow release materials or cytokines.[17] A fourth more recently proposed system of classification divides adjuvants into the following groups: gel-based adjuvants, tensoactive agents, bacterial products, oil emulsions, particulated adjuvants, fusion proteins or lipopeptides.[27]

Adjuvant limitations

In spite of progress in the identification of mechanisms of adjuvant action, alum remains the dominant adjuvant for human vaccines. Although many other adjuvants have been proposed over the years, these have failed to be successful in humans largely because of toxicity, stability, bioavailability and cost. Because of effects of size, electric charge and hydrophobicity which regulate the incorporation of proteins into the adjuvant formulation, it is difficult to predict on an empirical basis which adjuvant will work most effectively with a particular protein or peptide. Moreover, epitope modifications may occur during formulation or conjugation. In the case of carrier proteins, a pre-existing immunity to the carrier protein is a major limitation.[27] Furthermore, each adjuvant generates a characteristic immune response profile. For example, the inability of alum-based adjuvants to induce Th1 antibody isotypes or cellular immune responses, and their poor adjuvant effect on polysaccharide antigens limit their applicability to many vaccines.[16]

Major adjuvant groups

Mineral salt adjuvants

Alum-based adjuvants

Since the experiments of Glenny *et al.*[5] alum salts, principally aluminium phosphate or hydroxide, have been the most widely used human adjuvants.[16] Unfortunately, alum salts are relatively weak adjuvants and rarely induce cellular immune responses.[28–30] Studies suggest alum salts work by causing the formation of an antigen depot at the inoculation site from where antigen is released slowly.[31] The trapping of soluble antigen in the alum gel may also increase the duration of antigen interaction with the immune system. Other mechanisms of action involve complement, eosinophil and macrophage, activation[32] and increased efficiency of antigen uptake by antigen presenting cells seen with particulate matter with a size under 10 μm.[33]

Whilst alum-based vaccines are generally well tolerated, granulomas are common when the subcutaneous or intradermal route is used rather than intramuscular injection.[34,35] Other specific limitations of alum adjuvants are increased IgE production, allergenicity[30,33,34,36–38] and neurotoxicity. Although under normal circumstances low doses of aluminium are excreted by the kidneys, under certain conditions such as reduced renal function, aluminium is accumulated in the body and becomes highly toxic. High aluminium levels in the body predominately affect the brain and bone tissues causing

fatal neurological syndrome and dialysis-associated dementia. Aluminium intoxication has also been associated with amyotrophic lateral sclerosis and Alzheimer's disease.

Other mineral salt adjuvants

The salts of calcium, iron and zirconium have also been used to adsorb antigens, although not to the extent of alum salts.[34] In particular, calcium phosphate has been used for diphtheria-tetanus-pertussis vaccines.[39,40] While having similar properties to alum salts, calcium phosphate has the advantage that it is a natural compound to the human body and is therefore exceptionally well tolerated. It has a reasonable capacity to adsorb antigens, induces high levels of IgG antibodies and does not increase IgE production. Neurological reactions to pertussis vaccines adsorbed to calcium phosphate are rare.[41]

Tensoactive adjuvants

Quil A is a saponin derived from an aqueous extract from the bark of *Quillaja saponaria*. Fractions purified from this extract by reverse phase chromatography, mainly QS-21, have been studied as alternatives to alum when strong cellular responses are required for a particular vaccine.[22,42,43] Saponins are tensoactive glycosides containing a hydrophobic nucleus of triterpenoid structure with carbohydrate chains linked to the nucleus.[42] Saponins induce a strong adjuvant effect to T-dependent as well as T-independent antigens. Saponins also induce strong cytotoxic CD8+ lymphocyte responses and potentiate the response to mucosal antigens.[42] Quil A has been used successfully for veterinary applications.[44] It is a natural product composed of more than 23 different saponins and is generally considered too toxic for human use. In addition to severe local reactions and granulomas, toxicity includes severe haemolysis reflecting the affinity of saponins for cholesterol present in erythrocyte membranes, resulting in membrane solubilization and haemolysis.[20,44–47] The Quil A-derived saponin QS-21, whilst less toxic than Quil A itself, has many of the same problems and may similarly prove unsuitable for most human uses other than therapeutic vaccines for life threatening illnesses such as HIV infection.[48]

Bacteria-derived adjuvants

Given their potent immunostimulatory capacity, bacteria-derived substances constitute a major potential source of adjuvants. Cell wall peptidoglycan or lipopolysaccharide of Gram-negative bacteria, enhance the immune response against co-administered antigens despite themselves not being very immunogenic. This adjuvant activity is mediated through activation of Toll-like receptors that mediate the danger signals activating the host immune defence system.[36] Different species of bacteria used as a source of adjuvants include *Mycobacterium* spp., *Corynebacterium parvum*, *C. granulosum*, *Bordetella pertussis and Neisseria meningitidis*. Unfortunately, as whole alive or killed microorganisms these are too toxic to be used as human adjuvants.[23] However, much of the adjunvanticity of these bacteria is mediated by N-acetyl muramyl-L-alanyl-D-isoglutamine, also called MDP. The adjuvanticity of MDP depends on the administration conditions.[11,49] In saline, it mainly enhances humoral immunity[20,50,51] whilst when incorporated into liposomes or mixed with glycerol it induces strong cellular immunity.[52] MDP activates many different cell types including macrophages, leucocytes, mastocytes, endothelial cells and fibroblasts inducing the secretion of cytokines such as IL-1, B-cell grow factor and fibroblast activating factor. MDP also induces an increase in the production of superoxides, prostaglandins and collagenase.[53] Different compounds derived from MDP include threonyl-MDP, a potent yet non-pyrogenic adjuvant.[23]

Another important group of compounds derived from the cell wall of Gram-negative bacteria are the lipopolysaccharides (LPS). They are potent B-cell mitogens, but also activate T cells to produce IFN-γ and TNF and thereby enhance cellular immune responses. The major structural element responsible for their toxicity and adjuvant effect is Lipid A. In low acid conditions, lipid A can be hydrolysed to obtain monophosphoryl lipid A, a compound which retains the adjuvant activity of Lipid A with reduced toxicity.[54] Another extract from bacterial walls is trehalose dimycolate (TDM), an adjuvant which simulates both humoral and cellular responses.[55] The demonstration that mycobacterial DNA had adjuvant activity, led to the discovery that the adjuvant activity correlated with a higher content of CpG motifs present in bacterial nucleic acids. DNA containing CpG motifs is one of the most potent cellular adjuvants.[12]

Adjuvant emulsions

This class includes oil in water or water in oil emulsions such as FIA, Montanide, Adjuvant 65, and Lipovant.[17] The mechanism of action of adjuvant emulsions includes the formation of a depot at the injection site, enabling the slow release of antigen and the stimulation of antibody producing plasma cells.[56] In general, these adjuvants are too toxic for routine human prophylactic vaccine use, although they may be suitable for use in terminal conditions such as cancer where there is a greater tolerance of side-effects. Frequent side-effects of emulsions include inflammatory reactions, granulomas and ulcers at the injection site. Various types of emulsions have been used, with different natural oils, in order to find more stable, potent and less toxic formulations. Adjuvant 65 offers the advantage over the mineral oil used in IFA[57–59] that it can be metabolized.[23] Different emulsions like oil in water[60] and water in oil in water[61] have been developed with the latter being as potent as IFA but more stable, less viscous and easier to administer with less resulting granulomas.[62,63] Montanide is a family of oil-based adjuvants that have been used in experimental vaccines in mice, rats, cats and dogs, using natural, recombinant and synthetic antigens. In humans, Montanide has been used in trial vaccines against HIV, malaria and breast cancer.[64]

Liposome adjuvants

Liposomes are synthetic spheres consisting of lipid layers that can encapsulate antigens and act as both a vaccine delivery vehicle and adjuvant. Liposomes have been used widely in experimental vaccines. The potency of liposomes depends on the number of lipid layers,[65] electric charge,[66] composition[67] and method of preparation.[67–69] They enhance both humoral and cellular immunity to protein and polysaccharide antigens.[66,69]

Liposomes help extend the half-life of antigens in blood ensuring a higher antigen exposure to antigen presenting cells after vaccination.[70] Stability, manufacturing and quality assurance problems seem to have been major factors behind the fact that as yet no adjuvant based on liposomes has been registered for human use.

Polymeric microsphere adjuvants

Among particulated and polymeric systems, poly (DL-lactide-coglycolide) microspheres have been extensively studied. These are biocompatible and biodegradable microspheres able to incorporate different antigens. One of the advantages of this system is the capacity to manipulate the degradation kinetics of the microspheres by varying the relative concentration of their components, thereby controlling the time of antigen release.[71,72]

Cytokines as adjuvants

Cytokines are included in the modern classification of adjuvants. IFN-γ is a pleiotropic cytokine able to enhance cellular immune responses through a variety of mechanisms.[73] Granulocyte-macrophage colony stimulating factor (GM-CSF) enhances the primary immune response by activating and recruiting antigen presenting cells.[74] The practical application of GM-CSF as an adjuvant has been limited by the requirement for multiple doses, toxicity and the immunogenicity of heterologous cytokines.[17] Cytokines are particularly seen to have potential in DNA vaccines where the cytokine can be expressed by the same vector as the antigen.

Carbohydrate adjuvants

Inulin-derived adjuvants

Several complex carbohydrates of natural origin stimulate cells from the immune and reticulo-endothelial system.[75] The main source of these polysaccharides have been plants and fungus. Gamma inulin, a carbohydrate derived from plant roots of the Compositae family, is a potent humoral and cellular immune adjuvant. Gamma inulin is a potent alternate complement pathway activator increasing production of activated C3 and thereby activating macrophages.[76] Gamma inulin is effective at boosting cellular immune responses without the toxicity exhibited by other adjuvants such as FCA. Gamma inulin can be combined with a variety of other adjuvant components, for example, aluminium hydroxide, to produce a range of tailor made adjuvants with varying degrees of Th1 and Th2 activity. For example, Algammulin is a combination of γ-inulin and aluminium hydroxide. Algammulin exhibits a higher ratio of Th2 to Th1 activity than γ-inulin alone, its overall effect being equivalent to alum despite having a lower overall alum content.[77,78] Inulin-based adjuvants have successfully been tested in multiple animal models in combination with such antigens as diphtheria and tetanus toxoid, respiratory syncytial virus, the E7 protein from the Human Papilloma Virus, Herpes Virus 2 glycoprotein D, Hepatitis B surface antigen, influenza haemagglutinin, Haemophilus influenzae antigens and antigens from *Plasmodium falciparum*. Major advantages of inulin-derived adjuvants are that they induce both Th1 and Th2 immune responses, unlike alum do not induce IgE, and are not associated with any significant local or systemic toxicity.[79] Inulin is metabolisable into simple sugars fructose and glucose. Inulin does not, therefore, suffer from concerns regarding long-term accumulation and toxicity that are associated with metal-based compounds such as alum.

Other carbohydrate adjuvants

Polysaccharides based on glucose and mannose which have adjuvant action include glucans, dextrans, lentinans, glucomannans and galactomannans. Levans and xylans,[80] (82) also have immuno-enhancing activity. Macrophages have glucan and mannan receptors, activation of which stimulates phagocytosis and cytokine secretion plus release of leukotrienes and prostaglandins. Polysaccharides have been used for immune stimulation in patients with cancer.[81] *In vitro*, mannan activates monocytes and macrophages to secrete IFN, TNF, GM-CSF, IL-1 and IL-6.[82] Acemannan, a natural polysaccharide extracted as a mucilaginous gel of the *Aloe barbadensis*, stimulates generation of cytotoxic T lymphocytes (CTL)[83] and the cytotoxic activity of NK cells.[84] Recently, acemannan has been shown to enhance the immune response to nasally administered Hepatitis B surface antigen (HBsAg), generating similar levels of IgG antibody titres in sera compared to the immune response generated by an intramuscular alum-based HBsAg control vaccine.[85]

Adjuvant formulations

New adjuvant formulations have resulted from the mixture of different adjuvants in the same formulation. As a general rule, two or more adjuvants with different mechanisms of action are combined to enhance the potency and type of the immune response to the vaccine antigen. For example, alum salts can be formulated in combination with other adjuvants such as Lipid A to increase immunogenicity. Similarly algammulin which is the combination of γ-inulin plus alum has increased absorptive capacity and increased ability to stimulate Th2 responses.[77] Saponins such as Quil A have also been used as a part of immunostimulatory complexes (ISCOMS).[42] ISCOMS are virus like particles of 30–40 nm and dodecahedric structure, composed by Quil A, lipids and cholesterol. Antigens can be inserted in the membrane or encapsulated. A wide variety of proteins have been inserted in these cage-like structures.[86–88] ISCOMS can be used through the oral, respiratory and vaginal routes.[89] ISCOMS are particularly effective in activating cellular immunity and cytotoxic T cells[42] but often have problems with stability and toxicity.

Mucosal adjuvants

The development of adjuvants for mucosal immunization is an important current area of vaccine development. The quality of mucosal adjuvants needs to take into account the ability to stimulate the uptake of antigen through the various mucosal routes, and its ability to enhance the immunogenicity of mucosally-delivered antigen. Different results can be obtained for the same adjuvant when administered by a parenteral or mucosal route. Alum salts, the most widely used parenteral adjuvants, are ineffective when administered by the oral or

nasal route.[90] The mucosa is a door of entry for many pathogens. Although it is very difficult to generate mucosal antibodies through parenteral vaccination, it is possible to obtain mucosal as well as parenteral immunity by inoculating antigen by the mucosal route.[91] For pathogens colonizing mucosal surfaces or those having a mucosal route of entry, protection correlates well with a strong local mucosal response.[92] For mucosal immunization, several adjuvant strategies involve binding or coating with specific ligands to deliver the antigens to specialized epithelial cells (M cells). It is also important to correctly match the physicochemical characteristics of the antigen-like size, electric charge, and hydrophobicity to let the antigen cross mucosal barriers.[19] After optimization of these characteristics, the selected adjuvant may also enhance the immune response by mechanisms already described: adsorption and depot effect, cytokine induction, complement activation, recruiting of different cell populations, the delivery to different APC, the regulation of the expression via MHC class I or class II and the stimulation of the production of different subtypes of antibodies.[93]

Bacterial derivatives

Some well known parenteral adjuvants, like MDP, monophosphoryl lipid A (MPL) and LPS, also act as mucosal adjuvants. Compounds like bacterial toxins of *Vibrio cholerae* (CT) and *Escherichia coli* (HLT) and their respective toxoids are particularly useful mucosal adjuvants.[94–96] Although CT remains one of the most potent known mucosal adjuvants, it suffers from high toxicity and also induces a strong immune response against itself. The strong adjuvanticity of CT and HLT may be explained by their ability to increase antigen presentation by B cell, B-cell differentiation to IgA secreting cells, interaction with T cells and increase cytokine production.[97] The B subunit of CT is less toxic and strategies have been taken to mutate the gene coding for CT in order to detoxify the cholera toxin.

Synthetic or inactivated antigen delivery systems

This group of mucosal adjuvants includes different synthetic polymeric particles composed by biodegradable poly(DL-lactide-co-glycolide) (DL-PLG), cellulose acetate, iminocarbonates, proteinoid microspheres, polyanhydrides, dextrans, as well as particles produced from natural materials like alginates, gelatine and plant seeds. Other natural compounds like liposomes, virosomes and ISCOMS can also be included in this group.[98] Particle size is one of the major factors involved in the mucosal delivery. It has been shown that particles over 10 μm are not adsorbed by the intestinal mucosa.[99] Lower size particles can be taken up by Peyer's patches, and those lower than 1 μm can penetrate to lymph nodes and the liver, and reach the circulatory system.[100,101] Liposomes, cochleates and microparticles can bind mucosal surfaces by hydrophobic interactions, but their entry to M cells is not efficient because they are rapidly trapped in mucosal gels and most of them are not able to reach the mucosa. Macromolecules or particles conjugated or covered by ligands such as cholera toxin b chain (CTB) are limited by their need to gain accessibility to specific receptors.[102] The balance between hydrophobicity-hydrophilicity for the antigen delivery systems can be modified to obtain a modulation in the immune response.[100] The use of ligands linked to particles can result in the specific adherence to M cells, but only in a size range restricted by the glycocalix. Particles of 1 μm or higher require targeting of ligands to M cells.[103]

Living antigen mucosal delivery systems

Some pathogenic bacteria have the ability to overcome the difficulties of non-living systems in being uptaken easily by specific M cell receptors. One example is attenuated *Salmonella typhi* ty21a, which has a lectin-like interaction with polysaccharide receptors on M cells. Also *V. cholerae and* poliovirus strains can be used for oral immunization of heterologous antigens. Genetically modified strains of these microorganisms have been used as a carrier of heterologous antigens.[104] The biology of these living vectors introduces new challenges. *V. cholerae* vaccine strains without the genes coding for their toxins remain toxic.[104] Pre-existing immune responses in a high number of subjects previously immunized naturally or by vaccination constitutes a major drawback for this strategy.

Cytokines

High doses of IFN-α abrogate oral tolerance.[105] Similar results have been obtained with IL-12.[106] This suggests that orally administered cytokines may be able to be used as mucosal adjuvants to overcome systemic immune unresponsiveness, for example that seen in chronic Hepatitis B infection.

Adjuvants for DNA immunization

When naked DNA immunization commenced in the 1990s, it was supposed that this kind of immunogen would not need adjuvants. It is now clear that novel strategies are required to enhance the potency of DNA-based vaccine candidates. The strategy of co-inoculating plasmids coding for different cytokines or costimulatory factors to enhance the immune response generated by the vaccine plasmid has been used successfully.[107] Co-inoculation of the plasmid expressing B7-2 along with a DNA vaccine candidate from HIV-1, increased the cellular immune response specific for HIV-1. Also a plasmid expressing GM-CSF boosted the humoral immune response to protein G from rabies virus when two plasmids coding for each protein were co-inoculated.[73] IL-12 expressing plasmid co-inoculated along with another plasmid coding for an HIV-1 protein enhanced the cell mediated immunity specific for VIH-1.[108]

DNA vaccines and particulate adjuvant systems

Polymers and particulate systems have been used in the field of DNA immunization. Polylactic microspheres, polycarbonates and polystyrene particles about 1 μm in size have been used mucosally and parenterally, resulting in better results compared to free DNA administration.[109] The use of mannans covering polymers of N t-butyl N′ tetradecylamin-propionamidine (diC14 amidine), have been used as an immunoenhancing strategy for DNA vaccination. The main effects caused by the co-administration of these structures with DNA is the increase in DTH and CTL responses.

DNA vaccine immunomodulators of cancers have been used in DNA immunization. Ubenimex (UBX) increased humoral and cellular responses to DNA vaccination.[110] The

immune response against DNA encoded antigens has been evaluated nasally and parenterally using immunomodulators already used with protein antigens like MPL and saponins.[111] The response found after intramuscular inoculation of DNA in PBS have been explained in part by the 'danger signal' offered by the DNA itself. Immunostimulatory sequences from procariotic DNA are able to induce several cytokines like IL-12, TNF and IL-6, and thereby have an adjuvant action.[112]

Cancer vaccine adjuvants

There is increasing excitement regarding the potential for anti-cancer vaccines to slow or even eradicate some tumours.[113,114] These vaccines utilize either complete tumour cells, tumour antigens or tumour growth factor receptors combined with powerful adjuvants. These vaccines, being based on self molecules are generally of very low immunogenicity thereby leading to the requirement for potent adjuvants for effect. Approaches taken include the use of Montanide adjuvants, very small size proteoliposomes (VSSP) obtained form the external membrane of *Neisseria meningitidis*[115] or the use of peptides adjuvated with GM-CSF.[116]

Conclusions

Despite an explosion of knowledge regarding immune function over recent decades, we remain almost totally reliant for human adjuvants on aluminium-based compounds whose activity was first discovered over 80 years ago. Recent advances in vaccine development and, in particular, the increasing use of recombinant subunit and synthetic vaccines makes the need for improved adjuvants all the more acute. Although there are glimmers of hope that new adjuvants may rectify some of the deficiencies of aluminium-based adjuvants, there remains a concern that many of these promising adjuvants will never be approved for human use for logistical or commercial rather than scientific reasons. Clearly there are some major barriers other than just a lack of scientific knowledge of adjuvants that are standing in the way of availability of new adjuvants. First and foremost, unacceptable side-effects and toxicity preclude the use of many candidate adjuvants and this is particularly true for prophylactic paediatric vaccines where safety issues are paramount. Second, the regulatory bar has been raised significantly since the days when alum was first introduced as a human adjuvant. Indeed, it is likely that if alum hadn't been in use all these years and was first put forward to regulatory bodies for approval today, it would be refused registration on the basis of safety concerns. Third, it is not possible for adjuvants to be approved as products in their own right as they can only be registered as part of a vaccine combination. It is possible that many good adjuvant candidates have failed to reach the registration phase, not because there were any problems with the adjuvants themselves, but because the vaccine combination was not effective or had toxicity. This could be seen as analogous to throwing out the baby with the bath water. Fourth, having invested considerable funds in the development of a new vaccine antigen, few companies are prepared to risk this investment by conducting clinical trial program of candidate antigens with a new and unproven adjuvant as this could bring the whole development program unstuck if there turned out to be problems with the adjuvant. Fifth, most vaccine companies choose to keep their proprietary adjuvant data secret and therefore until such time as they themselves wish to register a vaccine product based on their adjuvants, then they will not share their knowledge of these adjuvants with others. Finally, the cost of developing a new product such as an adjuvant is now prohibitive. Whilst it might be possible to justify an investment of several hundred million dollars on a new vaccine given the prospect of recovering this investment from vaccine sales, the same does not hold true for adjuvant development costs, for which there is no easy source of cost recovery. For all the above logistical and commercial reasons there is a continuing major unmet need for safe and non-toxic adjuvants, particularly for adjuvants capable of strongly boosting cellular immune responses which are not associated with undue toxicity. Despite many advances of immunology, this key objective remains the 'holy grail' of vaccinology. Hence, the importance of major public institutions such as the NIH and WHO and charities with interests in vaccine development such as the Gates Foundation to fund adjuvant research and development programs as part of their general support for vaccine development.

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