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Review Article

Immunological adjuvants in allergy vaccines: Past, present and future

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Abstract

Hundreds of compounds have been tested over the years in a search for adjuvants to incorporate with antigens or allergens to enhance the immune response. Despite this, aluminum salts have been the only adjuvants that have been both registered for clinical application and used on a large scale until recently. Salts of aluminum, such as aluminum hydroxide, have been used as general immunologic adjuvants for several decades. Some allergen vaccines used for the treatment of allergy are still formulated with aluminum-based adjuvants. These formulations have generally proved efficacious and have a good safety profile compared with simple aqueous extracts. However, there is reported sensitivity and toxicity associated with use of aluminum. In addition, aluminum salts are known to be potent stimulators of T helper (h) 2 cell activity. Because Th2 activity directs towards an allergic response, aluminum salts are potentially counterproductive when used as adjuvants in the immunologic treatment of type 1 hypersensitivity. Many soluble and insoluble molecules have been reported to have adjuvant activity in experimental systems. Some of these have been used clinically, but side effects, such as local granuloma formation, have led to their withdrawal from clinical use. Newer depot-type adjuvants, such as insoluble calcium salts, tyrosine (now registered) and coupled alginates, may eliminate some of the potential problems of aluminum salts and are currently used in some allergy vaccines but have not as yet formed a complete replacement. Liposomes, iscoms and biodegradable microspheres are now being considered for clinical use as adjuvants for both oral and parenteral routes. Soluble adjuvants that are capable of directing the immune response in a more selective way are currently in development for use in allergy vaccines. One of these, the Th1-directing adjuvant monophosphoryl lipid A (MPL®; Corixa, Seattle, WA, USA), is now in clinical use in allergy vaccines formulated with the depot adjuvant L-tyrosine. Other ways of stimulating a Th1 response using immunostimulatory DNA sequences (immunostimulatory DNA sequences (ISS) or CpG motifs) as 'built-in' adjuvants are being studied. Further interesting adjuvants reported in the literature, such as Montanide ISA 720, SAF-m, RC-529 and QS21, may also be applicable to allergy vaccination.

Key words: adjuvant, allergy, aluminum salts, immunotherapy, monophosphoryl lipid A, T helper 1 cells, T helper 2 cells.

Introduction

It is now 70 years since Glenny[1] showed that alum-precipitated diphtheria toxoid was more immunogenic in laboratory animals than an aqueous presentation of toxoid. Since then, various aluminum compounds have been used as immunologic adjuvants in vaccines, such as those used to protect against diphtheria, tetanus, typhoid and pertussis, and they are widely used substances for this purpose. Until recently, aluminum compounds were the only adjuvants permitted in vaccines for the prophylaxis of infectious disease and toxemia.

The use of these aluminum compounds was extended to allergy vaccines by Sledge[2] and alum salts have been the mainstay as far as depot adjuvants have been concerned for a long time. Some allergy companies continue

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to provide certain product ranges of alum-adsorbed allergens for allergy vaccination (AV) and there is substantial evidence that these products can be effective in reducing allergic symptoms in appropriately diagnosed and treated patients.[3] The extensive use of alum as an adjuvant in allergy and prophylactic vaccines over many years has led to a large accumulation of information on its benefits and some of its drawbacks compared with the limited knowledge available of other adjuvants. Hence, alum features prominently in the present review. A number of problems, including tolerance, have been associated with the use of alum. Although the levels of aluminum permitted in injections are limited (e.g. by the *European Pharmacopoeia*) to 1.25 mg per dose,[4] the induction of sensitivity to aluminum has still been reported in some patients. There is an increasing requirement for standardization of final product forms used for AV. However, meaningful quality assurance measurements of the potency of these vaccines are problematic owing to difficulties in the recovery of allergens adsorbed to the alum. Alum is known to be an efficient T helper (h) 2 cell inducer, which may potentially exacerbate rather than alleviate the allergic condition, although in practice the evidence that alum reduces the efficacy of treatment is weak.

It may be time now to consider replacing alum compounds with other more selective molecules, possibly of biological origin. This publication also describes some of the adjuvants that were used previously but are no longer permitted, newer depot formulations and particulate adjuvants being researched and other adjuvants that are already in use for the treatment of type 1 hypersensitivity. In particular, the review discusses some of the more modern approaches towards the selective stimulation of key components of the immune system (e.g. Th1 cells) by soluble adjuvants, such as monophosphoryl lipid A (MPL), currently used in a novel allergy vaccine, and studies with immunostimulatory DNA sequences (ISS) coupled with major allergens.

Rationale for the inclusion of aluminum salts in vaccines

The salts of aluminum that are used most frequently to adjuvant allergy and other vaccines are aluminum potassium sulfate (alum), aluminum hydroxide and aluminum phosphate. These salts are often generically referred to as 'alum' and that convention is used in this report.

Alum is a component of AV formulations, first as a depot formulation, enhancing the induction of an immune response and allowing a reduction in the dose of allergen and fewer injections compared with aqueous regimes. Second, the slow-release characteristics of the preparation reduce the immediate and potentially dangerous reaction of injected allergens with IgE antibodies on mast cells and basophils. These are the rationales for the use of depot adjuvants generally.

It is clear that alum can enhance the induction of a primary response to the included allergen or antigen. It is probable that the slow-release characteristics of the preparation contribute to this effect[5] but, in addition, the particulate nature of alum adsorbates is likely to enhance the induction of an immune response. It was demonstrated by Mannhalter *et al.*,[6] using tetanus toxoid, that a very much greater amount of antigen was associated with macrophages when the tetanus toxoid was adsorbed to aluminum hydroxide rather than presented in a soluble form. They concluded that in the early phase of the immune response alum probably exerts its effect at the level of the antigen-presenting cell (APC). This enhancement is not so apparent when alum-adsorbed antigen is used to induce a secondary immune response.[5] IgE-mediated allergy is characterized by a well-established immune response to the offending allergens, so specific immunologically based treatment is also targeted towards effecting some change during a secondary type stimulus. Therefore, the use of alum at this stage for its immunostimulatory properties alone may not be appropriate for AV.

Sensitivity to aluminum

Alum-adsorbed allergens have been reported to induce local adverse events during AV. Of course, allergens themselves may induce a local reaction particularly when long-term depot-type adjuvants are used. Local reactions sometimes include both painless and painful local swellings and abscess formation, which are short lived or, rarely, more long-lived fibroses. These foci may sometimes reactivate after several years despite there being no measurable aluminum in biopsies of the sites.[7]

Sensitivity can be induced to aluminum itself from the use of AV containing alum salts as adjuvants. Clemmensen and Knudson[8] confirmed this by patch testing, which demonstrated that sensitivity (probably delayed) could be induced by AV with an alum-adsorbed grass pollen extract. Two similar cases were reported by Castelain *et al.*,[9] who described patients with pruritis of the arms following AV with alum-adsorbed allergens. The

patients also had persistent nodules, which were shown to contain large amounts of aluminum.

In a further study of alum-adjuvanted AV,[10] children were observed to develop persistent excoriated papules and subsequent patch tests to $AlCl_3$ were positive in nine of 13 cases. The authors concluded that aluminum is most likely to sensitize when injected in preparations used for immunization rather than when taken in through other routes. Frost *et al*.[11] performed a follow-up study in 202 children monitoring 1–3 years after cessation of treatment with alum-adsorbed allergens. They found that 13 children had severely pruiginous treatment-resistant nodules. The nodules in six of these cases were examined further and shown to consist of germinal centres, five having aluminum crystals scattered both between the cells and within the phagosomes of macrophages. Patch testing showed sensitivity to aluminum in four of six cases.

Although induction of sensitivity to aluminum is relatively rare, it should be considered as a possible adverse event following alum-adjuvanted AV. Obviously, treatment involving maintenance therapy or successive courses could increase the likelihood of induction of aluminum sensitivity.

Other possible toxic effects of aluminum

Other potentially damaging effects of alum should be considered before treatment.

The uptake of aluminum into soft tissue, such as spleen and liver in patients with renal failure, may further an entry into the reticuloendothelial system and, so, cause a possible immunosuppressive effect. This has been highlighted as a situation that should be prevented by avoiding an increased loading of aluminum[12] in certain patients.

Vogelbruch *et al*.[13] showed that poor injection technique, in which aluminum salts were localized at an intradermal site, could lead to persistent intradermal granuloma formation with consecutive dermal necrosis. It was concluded that this was not likely to be an allergic reaction to aluminum.

The possible implication of aluminum for induction of Alzheimer's disease should also be considered.[14]

Immune enhancement by aluminum-containing adjuvants

There is no doubt that alum adjuvants are capable of enhancing the specific immune response to an adsorbed antigen. Therefore, alum is used regularly in the immunization of experimental animals, in particular to induce IgE antibody responses in mice and rats through its propensity to stimulate preferentially Th2 cell activity.[15] This infers that alum is an inappropriate adjuvant to use for the treatment of IgE-based allergy, where it may be expected to make the condition worse. However, if, as suggested above, alum has very little effect on the secondary response, then its Th2 cell-inducing properties may be of no consequence in AV. There is some contrary evidence that the addition of alum to both the primary immunization and a booster injections of pertussis vaccine, itself a Th2 adjuvant, can induce IgE antibody to the antigen.[16] There is no firm evidence that alum-adsorbed allergens exacerbate the symptoms of allergy when used for AV; in fact, it is accepted by many that an adequate hyposensitizing effect can be achieved with such products.

Alternatives

Aluminum salts have stood the test of time as depot adjuvants for use in AV vaccines. Their long use has enabled a vast body of knowledge to be accumulated regarding their properties and, not surprisingly, both beneficial and occasionally adverse effects have been reported. Although side effects occurred in relatively few patients, and their use should be avoided in some circumstances, their ability to provide generally a safer and more efficacious therapeutic effect than an equivalent aqueous formulation is accepted by many specialists. Their IgE-inducing properties in animals have cast some doubt on their use, although no definitive proof is available that this can exacerbate the allergic state in humans. However, other more appropriate adjuvants are now available for use in allergy vaccines and others should be considered for the future.[15]

It is of interest that it is in the allergy field that most progress has been made in the introduction of newer forms of adjuvant with several different molecules, albeit mainly those that form depot formulations, currently used in allergy vaccines.

The naturally occuring amino acid L-tyrosine, which acts as an adsorbent for allergen extracts, has been used successfully for some years as a depot form for AV.[17] Allergy vaccines prepared this way have been registered with a number of regulatory authorities. This amino acid is relatively insoluble at neutral pH and, therefore, allergens or, indeed, antigens can be coprecipitated with the

tyrosine or adsorbed onto preinsolubilized tyrosine. Such a product has the clear advantage over alum salts because it is metabolizable, with a half-life at the site of injection of approximately 48 h. It is of particular interest that tyrosine stimulates the induction of an IgG isotype pattern in mice more consistent with Th1 cell stimulation compared with the Th2-like effects seen when alum is used as an adjuvant. Some more hydrophobic derivatives of tyrosine have been shown to have even more powerful adjuvant effects in experimental systems, but have not as yet been developed further.[18]

Calcium salts, in particular calcium phosphate, are also available for the same purpose. Clinical results have indicated the usefulness of AV vaccines adjuvanted this way and a good safety profile is apparent.[19]

One of the most interesting approaches has been to couple allergens to larger molecules. Alginate is an example of one of these molecules and a soluble and successful range of AV products based on this principle has been available for several years.[20]

Failures of the past

A comprehensive search through the relevant patent literature reveals many hundreds of soluble and insoluble materials with immune-enhancing properties as demonstrated in *in vivo* and *in vitro* systems. A complete appraisal of all these possibilities is outside the scope of the present review; however, it is worth quoting some examples of the approaches that failed for various reasons.

Depot adjuvants based on Freunds adjuvant[21] have been used in experimental systems and at various times have been introduced into the clinic. The principle is one in which allergens are usually prepared in an aqueous phase and an emulsion made by the addition of oil and an emulsifying agent. The source of the oil and emulsifying agent has usually directed whether the product is clinically acceptable. For example, mineral oils have been rejected, whereas vegetable oils, such as peanut oil (Adjuvant 65),[22] were considered more acceptable some time ago. Vaccines made this way have often had a good immunogenic profile, were efficacious and helped to prevent treatment-induced anaphylactic side effects when used for AV of allergic patients. However, local lesions have, until now, prevented their introduction on a prolonged routine basis.

Various soluble molecules, in particular those derived originally from bacterial cell wall sources, such as muramyl dipeptide[23] and its many analogs, were also believed to have great promise as adjuvants, but the potential toxic effects have never been separated satisfactorily from the desirable immune-stimulatory properties.

Other molecules with innate adjuvant properties, such as macrophage-stimulating activity, have actually been coupled to the antigen or allergen of interest in an attempt to limit these biological properties solely to enhancement of the immune response to that antigen or allergen. For example *N*-formyl-methionyl-leucyl-phenylalanine was coupled covalently to allergens and shown to have potentially useful effects in experimental animal systems.[24] Unfortunately, none of these promising developments has ever been progressed satisfactorily to enable them to be used regularly in humans.

Adjuvants of the future

More exciting products that can entrap allergens, such as liposomes, iscoms and biodegradable microspheres,[25] are in development with the possibility that they may induce Th1 cell activity. It has been suggested that such particles could be useful for both oral and parenteral AV. Control of particle size and constituents added during manufacture can impart properties relevant for stimulation of the common mucosal immune system via M cells in the intestine or other sites or provide a formulation that is readily attractive to APC. Reproducibility of manufacturing processes has slowed their progression to regular clinical use.

Despite the earlier disappointments in the developments of emulsions, it is known that a number of such products with an improved safety profile over the earlier examples are now under study for use as a means of adjuvanting vaccines against pathogenic organisms. Squalenes (metabolizable carbohydrates related to cholesterol) are showing promise. The oil-in-water preparation MF59 has been evaluated in vaccines for various pathogens (e.g. in HIV vaccine clinical studies).[26] The chemically related adjuvant SAF-m has been investigated in clinical trials for the immunotherapy of melanoma.[27] The water-in-oil adjuvant Montanide ISA720 is another metabolizable oil that has been clinically evaluated when incorporated with recombinant malarial antigens.[28] No doubt, it will not be long before some of these will be also studied for their potential to enhance the efficacy and safety profiles of allergy vaccines.

Saponins (triterpenoid glycosides) have been used exclusively for some time as veterinary adjuvants, without progression to human use due to toxic properties, such as

hemolytic effects on red blood cells. However, the safety aspects have now improved significantly as a result of purification measures[29] and a purified saponin QS21 has been evaluated in both infectious disease and cancer vaccines.[30]

Allergy is now believed to be mediated by an imbalance between allergen-specific Th1 and Th2 cells, with an excess of Th2 cell activity.[31] Cytokines from Th2 cells, such as interleukin (IL)-4 and IL-13 are believed to lead to a raised IgE levels[32] and IL-5 and others to the eosinophilia and related late-phase response of allergy.[33] Intuitively, therefore, adjuvants that preferentially support Th1 responses to associated antigens would be candidates as adjuvants to correct the T cell balance and, thus, would have potential utility in allergy vaccination.

One of the most fascinating recent developments has sprung from the observation during investigations of immunization of mice with antigen DNA that certain bacterial immunostimulatory DNA sequences (ISS or CpG motifs) can act as Th1 adjuvants, as demonstrated by antibody and cytokine induction.[34] Following gene immunotherapy with plasmid DNA (pDNA) encoding β-galactosidase, $CD4^+$ T cells were stimulated with antigen *in vitro*, resulting in IgG2a induction and production of interferon (IFN)-γ, but no IL-4 or IL-5 were released. In the same experiment, IgE and IgG1 were induced by β-galactosidase in alum and, in contrast, IL-4 and IL-5 were released and no IFN-γ was detected. The Th1 response was found to be resulting from a non-coding sequence (ISS) in the backbone of the pDNA. Advantage has been taken of this finding by coupling a non-coding ISS directly with single recombinant allergens, such as Amb a 1,[35] or by coadministration in the case of Cry j 1 and Cry j 2.[36] DNA vaccination of mice with plasmid DNA coding for Cry j 1 has also been seen to produce a Th1 response in mice.[37] Although experimental data suggest that these are appropriate forms for AV, the practicalities associated with the number of separate products required and the cost of obtaining full data for registrations of each may be prohibitive.

A new generation of soluble adjuvants, derived from detoxified bacterial cell wall components and originally designed for use with bacterial, viral and oncology vaccines, is currently under study. These compounds have the potential to enhance the status of allergen-specific immunotherapy because of their capacity to induce the production of a more Th1 cell-like rather than Th2 cell-like profile of cytokines.[38] One of these, monophosphoryl lipid A (MPL®; Corixa, Seattle, WA, USA), has been included as an adjuvant in allergy vaccines.[39] MPL® is a lipopolysaccharide extracted from the cell wall of *Salmonella minnesota*. It is detoxified by acid and organic/alkali treatment and highly purified by high-pressure liquid chromatography. The pure well-characterized product is composed of analogous molecular species (congeners) having different fatty acid side chains. The novel adjuvant is a component of a melanoma vaccine, Melacine® (Corixa, Seattle, WA, USA), registered in Canada[40] and has been studied in infectious disease vaccines.[41–43] MPL® has been shown to activate APC, probably through TLR4 (toll receptor for lipopolysaccharide). It enhances phagocytosis, upregulates major histocompatibility complex (MHC) class II molecules on APC and causes release of IL-12, IL-1, tumor necrosis factor-α and granulocyte–macrophage colony stimulating factor from APC. Interferon-γ and IL-2 are released from Th1 cells probably indirectly via the effects of IL-12.[44]

Allergy vaccines have been developed containing both birch, grass, *Parietaria* and olive pollen extracts with reduced IgE-binding activity (allergoids), which are co-adsorbed with MPL® to insoluble tyrosine. It is known that analogous products to treat allergy to ragweed and Japanese cedar pollen and to house dust mite are under development. The combination of MPL® adjuvant and tyrosine has been shown, in animals, to be synergistic in terms of antibody and Th1 cell induction.[45] Cytokine changes consistent with a Th2/Th1 reorientation have been reported in clinical trials. In particular, an increase in INF-γ and a reduction in the seasonally induced rise normally seen in IL-4 and IL-5 release from allergen stimulated T cell clones were observed after therapy.[46] Lymphocyte proliferation tests have shown an immediate rise in total lymphocyte responses after treatment, but only with respect to allergen-specific stimulation, followed later to a fall below the baseline,[47] possibly due to an increase of IL-10 expression. Specific IgG antibody was raised and the normal specific IgE antibody rise expected following the first years treatment with other allergy vaccines did not occur. It is of note that the seasonal rise in specific IgE seen in placebo-treated patients was eliminated by the active product.[39] A very strong Th1 response, particularly associated with Gram-negative bacteria and viruses, is sometimes associated with delayed hypersensitivity. No local 24 h delayed hypersensitivity reactions developed during the injection regimen and there were no systemic or respiratory side effects consistent with a type IV hypersensitivity response. Adverse

events reported were qualitatively and quantitatively no greater than those seen with more conventional allergy vaccines. The enhanced efficacy obtained in placebo-controlled clinical studies facilitated a reduction in the number of injections required for treatment from the many often used with current vaccines to only four pre-seasonal treatments.[39] This approach is of value in terms of increased patient and doctor compliance and brings obvious pharmacoeconomic advantages. Allergy vaccines containing MPL® are now available in some European countries.

Sublingual immunotherapy (SLIT) is growing in popularity as a treatment for respiratory allergy[48] due to the apparent lack of side effects and patient compliance. Although the immunologic mechanisms responsible for the beneficial effects are still not clear, the possibility of local adjuvants being used to enhance the efficacy of treatment is already being considered and the search is on for materials with the relevant properties. MPL® has already shown a potential in experimental animals to promote a Th1-like serum antibody profile and IgA antibody in the secretions to an associated antigen delivered by the mucosal route.[44] One may argue over the definition of adjuvants, but there are other novel ways of potentially enhancing the immune response to SLIT. Penetration enhancers (e.g. fatty acids, bile salts, surfactants) are under investigation,[49] as are bioadhesives (e.g. chitosan, carbopols), which promote absorption to mucosal surfaces.[50] The use of either of these two strategies could elevate the very low uptake of antigen that is achieved by existing SLIT but, of course, the safety and tolerance aspects would have to be very carefully evaluated. If any of the above approaches to treatment become proven, then the whole area of allergy vaccination may be extended from its current niche position to a first-choice therapy in well-diagnosed patients.

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