Adjuvants in Allergy: State of the Art

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Opinion statement

Allergen-specific immunotherapy is administered through subcutaneous or sublingual routes to induce immunological tolerance to allergens. It is the only management option available for treating IgE-mediated allergy to seasonal and perennial allergens by disease modification, resulting in long-term remission. In the last few decades, research into the long-term efficacy and safety profiles of vaccines has led to the increased use of adjuvants in allergen immunotherapy. Through activation of innate immune pathways, adjuvants have been shown to improve the efficacy and safety profiles of allergen vaccines. This paper provides an overview of adjuvants, both in clinical and preclinical settings, currently in use for allergen immunotherapy.

Introduction

Vaccines have been used for centuries to treat and protect against illnesses. In fact, smallpox inoculation began as early as 1000 A.D. in China, although final eradication of the disease was the result of centuries of medical and technological improvements to Edward Jenner's Edward Jenner's 1796 success using cowpox material to create immunity to smallpox. Rabies vaccine in 1885 by Louis Pasteur was the next big contribution. In the 1930s, with advances in bacteriology, vaccines and antitoxins against tetanus, anthrax, diphtheria,

cholera, tuberculosis, and plague became available. In the 1950s, polio vaccine was created, and research was targeted at developing vaccines for other childhood infectious diseases [1]. Over the years, disease targets have evolved, and research has been focussed on conditions such as allergies and addiction to nicotine.

According to a 2008 World Health Organization estimate, 17 % of mortality in children worldwide was the result of vaccine-preventable diseases, including diphtheria, *Haemophilus influenzae* B, hepatitis B, measles, and meningitis.

Allergic disorders such as rhinitis, asthma, food allergy, allergic skin inflammation, and ocular allergy are major contributors to the economic healthcare burden. In recent decades, environmental and genetic predisposition has been linked to increased prevalence of allergies [2]. Since the term “allergy” was coined and first described in the 19th century, research and clinical advancement has played a major role in the development of innovative therapeutic approaches.

Allergen immunotherapy (AIT), available since 1911 as a result of pioneering research by Noon [3] and Freeman [4], has been a well-established therapeutic option over the past century, particularly in the treatment of allergic rhinitis [5]. While remarkable, however, advances in allergen immunotherapy have been limited by safety and efficacy concerns. As a workaround, adjuvants have been added to vaccines. These heterogeneous agents augment the adaptive immune response to proteins in vaccines and can direct the nature of the ensuing immune response to pro-

duce the most effective protection against a specific pathogen or allergen.

The potential roles of adjuvants in vaccines are many. In general, adjuvants are essential to induce an immune response when the use of live attenuated vaccines is not practicable. They may be used to augment immune responses in immunocompromised individuals such as the very young and very old. They also typically allow reduction of antigen load and number of doses administered.

Vaccines used in allergen immunotherapy are either natural or modified allergen extracts or second-generation preparations such as recombinant allergens, fusion proteins, peptide mixes, and plasmid DNA [6–8]. In contrast to natural extracts, which usually possess some intrinsic adjuvant activity, second-generation and chemically modified preparations (such as allergoids) typically have poor immunogenicity and require the use of an adjuvant [9, 10].

Use of adjuvants in vaccines

Adjuvants in vaccines direct the immune response by activating T and B lymphocytes and antigen-presenting cells (APCs). B lymphocyte activation follows cognate interaction with T helper cells and results in antigen-specific differentiation into memory B cells or plasma cells. Plasma cells are responsible for secretion of specific antibodies. T helper type 1 (Th1) cells activate cell-mediated immunity through the secretion of interferon gamma (IFN- γ) and other mediators, which in turn activate cytotoxic T lymphocyte (CTL) and natural killer (NK) cells. CTLs induce death of cells infected with intracellular pathogens, whereas NK cells instigate apoptosis in tumours and cells infected with viruses. Cytokine products of Th2 cells promote clonal expansion, affinity maturation, and class switching in B cells to generate plasma cells, which initiate humoral response. Neutralising antibody production by B cells is required for defence against extracellular pathogens, toxins, and helminths [11, 12].

Adjuvants stimulate the immune system through various mechanisms (Fig. 1). Some adjuvants enhance antigen persistence at the injection site and increase recruitment and activation of the antigen-presenting cells. Particulate adjuvants are capable of forming multimolecular aggregates which encourage uptake by APCs. Induction of innate immunity with predominant targeting of APCs is another recognized mechanism of action. Adjuvants are able to target PRR families, which signal through pathways to activate transcription factors, inducing the production of cytokines and chemokines.

Figure 1. Mechanisms of action of adjuvants in vaccines.

Certain adjuvants induce the innate immune response through activating inflammasome production [11, 12].

Mechanisms of allergen immunotherapy

CD4⁺ T lymphocytes play a prominent role in controlling the immune mechanisms involved in allergic inflammation [13, 14]. Secretion of the cytokines IL-4, IL-5 and IL-13 by allergen-specific Th2 T cells in clinically sensitised allergic individuals is a well-accepted phenomenon, although it does not fully explain why some atopic individuals manifest these responses yet never develop clinical symptoms of allergy. Only a complete understanding

of this phenomenon will allow a full appreciation of how natural “tolerance” of allergens is affected and how this can be mimicked by immune manipulation.

Setting aside this reservation, current concepts of the mechanism of clinical benefit of allergen immunotherapy are centered around four possible mechanisms:

- (a) Expansion of allergen-specific adaptive T regulatory cells producing anti-inflammatory cytokines such as IL-10 [13, 14];
- (b) Induction of the production by allergen-specific B cells of “blocking” IgG4 and IgA antibodies, which may impede binding of allergen to IgE bound to effector cells or, perhaps more significantly, allergen uptake and processing by antigen-presenting cells [15];
- (c) Reduction of the production of Th2 type cytokines by allergen-specific T cells, at least partly, by “skewing” to a more Th1 cytokine profile [16];
- (d) Direct effects, perhaps partly through increased production of inhibitory cytokines such as IL-10, on mast cell and basophil numbers and activation in the mucosa of the target organs of allergic disease [17, 18].

Adjuvants currently in use for vaccination and allergen immunotherapy

Mineral adjuvant molecules

Aluminium phosphate/hydroxide (alum) has been in use since the mid-1920s. Until recently it was the only adjuvant approved for human use by the U.S. Food and Drug Administration (FDA). It is a very widely used adjuvant for subcutaneous immunotherapy and other vaccines and is available only for subcutaneous administration. The antigen is adsorbed onto an aluminium hydrogel (typical quantities are 0.5 mg per injection). It has difficulty adsorbing small peptides and some recombinant vaccines, and it is ineffective for DNA-based vaccines.

Alum acts via induction of a strong Th2 response, through a depot effect, and by interaction with the innate immune system (Table 1).

Calcium phosphate has been in use for many years in paediatric diphtheria, pertussis, and tetanus (DTP) vaccine formulations. It is also popular for use in allergen immunotherapy vaccines. Unlike alum, it does not induce an IgE response. It acts through a depot effect, facilitating APC uptake [19].

Emulsions

Freund's complete (CFA) and incomplete (IFA) adjuvants are water or mineral oil emulsions with mannide mono-oleate emulsifiers. The complete adjuvant also contains heat-killed *Mycobacterium tuberculosis* or *Mycobacterium butyricum*. While both adjuvants are extremely effective in inducing both humoral and cell-mediated responses (Table 1), they can cause local irritation so severe that they are not approved for use in animals, let alone humans. Protein antigens in CFA cause strong Th1 and Th17 responses, for which the presence of the mycobacterial component is essential. Less toxic variants using squalene (biodegradable plant oil) are available

Table 1. Lists of common adjuvants, their routes of administration and their availability for clinical use

Adjuvant	Principal immune response stimulated	Route of administration	Status
Aluminium hydroxide	Th2 response	Subcutaneous	Clinical
Squaline oil-in-water	Th2 response	Subcutaneous	Clinical
Freund's complete adjuvant (CFA)	Th1 response	Subcutaneous	Clinical
Incomplete Freund's adjuvant (IFA)	Th2 response	Subcutaneous	
TLR4 - MPLA	Th1 response	Subcutaneous/intradermal/ sublingual	Clinical
TLR9- CpG ODN	Th1 response	Subcutaneous/intradermal/ sublingual	Clinical
Virosomes	Induction of IgG and IgA	Intradermal/subcutaneous	Clinical
ISCOMs	Th1 response	Subcutaneous	Preclinical

and widely used for prophylaxis of extracellular infections, as they induce high antibody titres to a wide range of protein allergens but are weaker at inducing cell-mediated immunity [20]. MF59 and AS03 are the common oil-in-water emulsions in use. These emulsions generate a mixed Th1 and Th2 phenotype. In addition, MF59 causes local stimulation and recruitment of dendritic cells and increases the uptake of antigens by the dendritic cells. It is currently being used in the seasonal influenza vaccine in Europe [21].

Virosomes

Virosomes incorporate viral fusion proteins such as neuraminidase and haemagglutinin into phospholipid liposomes. Virosomes mimic the infectivity of viruses, leading to presentation of internalised antigen on both class I and II MHC molecules. This results in IgA production and enhancement of mucosal immunity. Virosomes are efficient for the mucosal and intranasal delivery of DNA-based and proteinaceous vaccines [22, 23].

Monophosphoryl lipid A (MPLA)

This is a modified lipopolysaccharide extracted from the cell wall of gram-negative bacteria, particularly of the *Salmonella* species. It increases the activation of dendritic cells and T cells, inducing a shift in cytokine profile towards strong Th1 responses. The effect of MPLA is further enhanced by depot adjuvants [24]. MPLA acts on APC to skew allergen-induced T cell cytokine production [25].

Tyrosine

Tyrosine is a normal constituent of human plasma (10 mg/ml). In some allergen immunotherapy vaccines currently in use, tyrosine is employed as a "natural" adjuvant. Typically, the allergen is modified by glutaraldehyde treatment (allergoid) and then adsorbed onto L-tyrosine to produce modified allergen tyrosine adsorbate (MATA). Tyrosine enhances IgG but not IgE production, and probably also acts as a short-term depot adjuvant [26].

Oligonucleotides

Methylated deoxycytidine-deoxyguanosine (CpG) oligonucleotides are ligands for Toll-like receptor 9 (TLR9) found in intracellular vesicles of phagocytic cells. CpG oligonucleotides are recognised by innate immune receptors, inducing cellular and humoral responses to antigen with a Th1 bias. They can be used with microspheres [27].

Immune-stimulating complexes (ISCOMs)

These honeycomb complexes are composed of cholesterol, phospholipids and saponin. They have a strong negative charge and can trigger cellular, mucosal, and humoral responses [22].

Probiotics

Lactobacillus plantanum, *L. lactis*, and *Bifidobacterium* administered mucosally, together with allergens, induce Th1 and regulatory T cell differentiation. Probiotics have been used putatively to protect against childhood eczema as a result of induction of an allergen-specific Th1 response [28].

Other approaches to allergen immunotherapy

Adjuvants targeting pattern recognition receptors (PRRs)

Natural ligands or synthetic agonists for recognised PRRs have been in the limelight recently for possible use as adjuvants in various formulations. The following are some examples of PRR activating molecules currently under investigation for adjuvant activity:

- (a) Toll-like receptor 3 (TLR3) and RLR ligands: Double-stranded RNA (ds RNA) virus is a potent activator of innate immunity. Synthetic analogues of ds RNA have been employed as adjuvants in vaccines. These act either through activating TLR3 in cellular endosomes [29] or through RIG-I-like receptors (RLR), a family of cytosolic ribonucleic acid helicases. TLR3 activation induces IL-12 production and augments MHC class II and antigen presentation [30–36]. RLRs such as melanoma differentiation-associated gene 5 (MDA5) act, at least partly, through the activation of type I interferons [36, 37].
- (b) Toll-like receptor 4 (TLR4) ligand: Bacterial lipopolysaccharides with low toxicity — such as MPL, already described — have shown promise and are in use as adjuvants. These act mainly through the activation of TLR4 ligand [38].
- (c) Toll-like receptor 5 (TLR5) ligand: Molecular combination of bacterial flagellin with recombinant vaccine antigens generates fusion proteins, which are strong TLR5 agonists. These, in turn, promote the production of strong Th1 and Th2 T cell responses [39].
- (d) Toll-like receptor 7 and 8 ligands: ds RNA species are natural agonists for TLR7 and TLR8. TLR7/8 ligation activates multiple dendritic cell subsets, inducing the production of Th1 cytokines in cognate T cells,

which boost cell-mediated immunity, and antibody production by cognate B cells [40].

Nanoparticles as adjuvants

Biodegradable and non-biodegradable polymeric nanoparticles have been under scrutiny for potential use as adjuvants in allergen immunotherapy. These particulate delivery systems belong to a group of adjuvants that facilitate the uptake of antigen by the antigen-presenting cells (APC). These adjuvants may be used for both conventional and mucosal vaccination [41•].

Amalgamation of allergens and viral particles

Recombinant allergens coupled to bacteriophage QB-derived virus-like particles (VLPs) produce highly immunogenic vaccines that induce particularly strong B cell responses [42].

Conclusions

A greater appreciation of the possible therapeutic mechanisms involved in allergen immunotherapy is leading the way in improving the efficacy of vaccines using tailored adjuvants that harness both innate and acquired immune mechanisms. Nevertheless, it could be argued that the subject is still in its infancy and merits much more research in order to better induce relevant immune manipulation and simplify desensitisation regimens. Most research and development to date has been directed at adjuvants eliciting Th1 and regulatory CD4+ T cell responses to allergens. While alum, tyrosine, MPL, and CpGs have been tested and used in human allergen immunotherapy vaccines, their relative impact on clinical efficacy remains poorly understood. TLR ligands, bacterial toxins, and probiotics act by reducing allergic inflammation, as shown in murine models. These look very promising for future use in mucosal administration of allergen immunotherapy. Virus-like particles and mucoadhesive particulate adjuvants have shown promise in preclinical trials and are considered good candidates for future development of safer and more effective vaccines. Finally, investigation of other approaches to immune manipulation in the course of allergen immunotherapy, such as concomitant administration of corticosteroids and vitamin D to induce regulatory T cells, may hold further opportunities in the development of allergen vaccines.

Compliance with Ethics Guidelines

Conflict of Interest

Saima Alam declares no conflict of interest.

Joanna Lukawska declares no conflict of interest.

Christopher Corrigan has received honoraria payment for the development of educational presentations, and has had travel/accommodation expenses covered or reimbursed from Allergy Therapeutics.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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