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Mechanisms of stimulation of the immune response by aluminum adjuvants

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Abstract

Aluminum adjuvants are widely used in human and veterinary vaccines. They are appropriate adjuvants for vaccines that confer protection by inducing antibodies via the induction of a type 2 immune response, but they do not induce cytotoxic T cell and cell-mediated immunity. The mechanisms by which aluminum adjuvants selectively enhance the immune response are poorly understood. Following exposure to interstitial fluid in vitro and in vivo, most antigens are rapidly desorbed from aluminum adjuvants, suggesting that sustained release of antigen from a depot does not significantly contribute to the adjuvant effect of aluminum compounds. However, the adsorption of antigens onto aluminum salts may result in a high local concentration of antigen at the injection site and enhance the uptake by antigen-presenting cells. Aluminum compounds can further enhance the immune response by direct or indirect stimulation of dendritic cells, activation of complement and by inducing the release of chemokines. The relative importance of these mechanisms remains to be determined.

Keywords: Aluminum adjuvants; Immune response; Antigen-presenting cells; Dendritic cells; Complement

1. Introduction

Aluminum salts have been used for over 70 years as adjuvants in vaccines. The adjuvant effect of aluminum salts was first reported in 1926 based on the observation that alum-precipitated diphtheria toxoid induced a better immune response than soluble diphtheria toxoid in guinea pigs [1]. Aluminum adjuvants in commercial vaccines have been characterized as aluminum oxyhydroxide (AlOOH, commonly referred to as aluminum hydroxide) and aluminum hydroxyphosphate ($Al(OH)_x(PO_4)_y$, commonly referred to as aluminum phosphate). Aluminum-containing vaccines are prepared by adsorption of antigens onto aluminum hydroxide or aluminum phosphate gels or by precipitation of antigens in a solution of alum ($KAl(SO_4)_2{\cdot}12H_2O$). Following precipitation, the alum-containing adjuvants are similar to aluminum phosphate adjuvants in composition and physicochemical characteristics [2,3].

Aluminum adjuvants are the only adjuvants allowed for use in human vaccines and are present in many veterinary vaccines. They have been administered to hundreds of millions of people with only rare reports of serious local reactions [4]. Indeed, adsorption and slow release of reactogenic vaccine components may reduce the incidence and severity of local and systemic reactions [5,6]. From an immunological standpoint, the main drawbacks of aluminum adjuvants are their weak or absent adjuvant effect with certain candidate vaccine antigens, the inability to induce cell-mediated and cytotoxic T cell responses and the tendency to induce IgE-mediated immune responses [4,7]. The adjuvant effect of aluminum compounds is generally more significant in primary than in secondary immune responses [8,9]. As will be discussed in more detail later, aluminum adjuvants augment the type 2 immune response without enhancing the type 1 immune response [10–12]. This makes aluminum adjuvants less suitable for vaccines against certain viruses and intracellular bacteria and parasites, for which antibodies alone provide insufficient protection. The induction of antigen-specific IgE responses may predispose susceptible individuals to allergic reactions against vaccine components.

Although aluminum adjuvants have been used for a long time, surprisingly little is known about the mechanisms by which they enhance the immune response. The two most commonly cited mechanisms are formation of an antigen depot and immunostimulation. A discussion of these mechanisms is the subject of this review.

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2. Depot effect

Glenny et al. suggested that precipitation of antigen with alum reduced the rate of antigen elimination from the injection site [13]. Injection sites were collected from guinea pigs 3 days after administration of alum-precipitated or soluble diphtheria toxoid. The injection sites were macerated and injected in naïve guinea pigs. The recipients of the material from the alum-precipitated diphtheria toxoid injected animals, but not the soluble diphtheria toxoid injected animals, developed an immune response. This suggested that the slow release of alum-precipitated antigens from the injection site resulted in prolonged exposure of the immune system to vaccine antigens and a better immune response. Subsequent experiments by Harrison demonstrated that some antigen is retained at the injection site for at least 7 weeks after injection of alum-precipitated diphtheria toxoid [14].

The depot effect was first challenged by Holt, who demonstrated that excision of the injection site after 7 or more days after injection of alum-precipitated diphtheria toxoid, did not interfere with the development of a humoral immune response to diphtheria toxoid [15]. Moreover, excision of a 14-day-old injection site, followed by maceration and re-injection into the same guinea pig, resulted in an enhanced immune response in comparison with animals in which the injection site was left intact suggesting that the antigen depot is not available to the immune system [15]. More recent experiments have documented the rapid release of HIV glycoprotein gp120 from aluminum hydroxide [16] and tetanus toxoid from aluminum phosphate [17] in vivo. About 80% of tetanus toxoid had disappeared from the injection site after 4 h [17]. This rapid desorption in vivo is most likely caused by the interaction of aluminum adjuvant with components of interstitial fluid including phosphate ions, citrate and proteins [18,19]. Indeed, mixing of model vaccines with sheep lymph fluid, which is similar in composition to interstitial fluid, resulted in nearly complete desorption of protein antigens within hours [20]. These experimental data suggest that sustained release of antigen from a depot site over days or weeks is unlikely to contribute to the adjuvant effect of aluminum compounds. This conclusion raises the question whether the adjuvant effect of aluminum salts is dependent on adsorption of vaccine antigens or whether simply co-injection of aluminum salts and antigens would be sufficient. The WHO recommends that >80% of diphtheria toxoid and tetanus toxoid antigens are adsorbed [21] and the United States Minimum Requirements state that >75% of diphtheria toxoid and tetanus toxoid need to be adsorbed [22]. This issue is difficult to address, because the degree of adsorption rapidly changes after injection into animals as a result of the interaction with interstitial fluid and there is no correlation between the degree of adsorption before and after exposure to interstitial fluid [20,23]. Adsorption may ensure a high localized concentration of antigen for a period of time that is sufficient to allow antigen uptake and activation of dendritic cells.

Recent studies have demonstrated that aluminum phosphate adjuvants enhance the immune response to DNA vaccines [24,25]. The aluminum adjuvant did not affect the expression of the DNA plasmid. It is unlikely that the newly synthesized and secreted antigens are adsorbed onto the aluminum salts in vivo, because many interstitial proteins have a high affinity for aluminum salts and prevent adsorption of antigens [19]. This suggests that adsorption is not always necessary for the adjuvant effect of aluminum, as long as the concentration of antigen is high enough to allow efficient antigen uptake by dendritic cells. Aluminum adjuvants probably enhance the response to DNA vaccines by a direct or indirect effect on antigen-presenting cells as discussed in Section 3.1.

3. Immunostimulation

3.1. Effect of aluminum adjuvants on antigen presentation

The initiation of the immune response occurs in the lymph nodes that drain the vaccination site. Dendritic cells play a critical role in the transport of antigen to the lymph node and in presenting and activating naïve antigen-specific T cells. They are present in immature form throughout the skin and mucosal tissues and at a lower density in skeletal muscle and other non-lymphoid tissues. The immature dendritic cells are highly efficient in capture and uptake of antigens by receptor-mediated endocytosis, pinocytosis and phagocytosis [26]. Following the uptake of antigens and in the presence of stimulatory signals, the dendritic cells migrate to the draining lymph node via the afferent lymph vessels. During their migration, the cells process the antigen and undergo a maturation process, resulting in mature dendritic cells that are highly efficient in presenting antigenic peptides to T cells. The mature dendritic cells activate the T cells by providing costimulatory signals and they can direct their differentiation into appropriate effector T cells.

Microbial molecules, such as lipopolysaccharide (endotoxin) and bacterial DNA provide a strong stimulus for the maturation of dendritic cells. Recognition of these molecules via toll-like receptors (TLRs) results in activation of the NF-κB signaling pathway and expression of costimulatory molecules and cytokines [27]. Protein antigens by themselves are poorly immunogenic because injection of these antigens provides only weak signals for dendritic cell maturation. An important role of adjuvants is to provide signals for the activation of dendritic cells. Given the efficient activation of dendritic cells by microbial molecules via TLRs, it is not surprising that many microbial products have proven to be potent adjuvants. It is unlikely that aluminum adjuvants act via a TLR, because aluminum hydroxide was an effective adjuvant in mice that lacked MyD-88, an adaptor

molecule in the TLR signaling pathway [28]. Nevertheless, there is some evidence that aluminum adjuvants can directly activate antigen-presenting cells. Human peripheral blood monocytes pulsed with aluminum hydroxide-adsorbed tetanus toxoid induced a much better proliferative response by autologous T cells than monocytes pulsed with soluble tetanus toxoid [29]. This correlated with an increased uptake of aluminum-adsorbed tetanus toxoid and induction of IL-1 secretion. Aluminum gel particles are $<10\,\mu m$ in diameter [3] and may be more efficiently taken up through phagocytosis than soluble antigen. Alternatively, direct activation of antigen-presenting cells by aluminum may result in more efficient uptake of soluble (desorbed) antigen. A recent study showed that aluminum hydroxide increased the expression of MHC II and several co-stimulatory molecules on peripheral blood monocytes accompanied by increased mRNA expression of IL-4 and a modest increase of IL-1, TNF and IL-6 [30]. Many monocytes appeared to differentiate into dendritic cells as indicated by their morphology and increased expression of CD83, a marker of mature dendritic cells. Neutralization of IL-4 and depletion of $CD4^+$ T lymphocytes abolished the increase of MHC II on the monocytic cells suggesting that the effect of aluminum on these cells was indirect.

The role of IL-1 in the adjuvant effect of aluminum has not been studied in detail. Only weak induction of IL-1β and undetectable IL-1α mRNA were observed in the draining lymph nodes following subcutaneous injection of aluminum hydroxide, but the injection sites were not studied [31]. TNF-α and IL-6 are other cytokines that are commonly released by activated monocytes and macrophages. The antibody response to aluminum hydroxide-adjuvanted antigen in mice was not diminished by a targeted mutation in TNFR-1, the major receptor for TNF-α or IL-6, indicating that the adjuvant effect of aluminum is not dependent on these cytokines [32].

Aluminum adjuvants may also indirectly activate antigen-presenting cells. Intramuscular injection of aluminum adjuvants causes tissue damage with necrosis of some skeletal muscle fibers [33]. Recent studies suggest that necrotic cells release yet to be identified molecules that activate dendritic cells [34,35].

3.2. Stimulation of type 2 immune responses by aluminum adjuvant

Activation of the immune system can lead to two types of responses [36,37]. The type 1 immune response is primarily a cell-mediated response characterized by the expression of IFN-γ and the type 2 immune response is an antibody-mediated response characterized by the expression of IL-4, IL-5 and IL-13. The control of the type of immune response is complex and includes genetic factors, antigen dose and the nature of the infectious agent.

Adjuvants exert a strong influence on the type of immune response. Many adjuvants, including complete Freund's adjuvant, saponin-containing adjuvants and immunostimulating complexes stimulate either a mixed or a type 1-biased immune response, whereas, aluminum adjuvants selectively stimulate a type 2 immune response [10–12].

The activation of naïve antigen-specific T cells does not occur at the site of vaccination, but in the draining lymph nodes. Dendritic cells transport antigen from the vaccination site to the lymph node where they present antigenic peptides to specific T cells. In addition, dendritic cells appear to be able to convey information from the injection site to the T cells in the draining lymph node and instruct $CD4^+$ T helper cells to differentiate into effector T_H1 or T_H2 cells. The molecular mechanisms by which dendritic cells accomplish this, are incompletely understood. IL-12 plays an important role in stimulating the differentiation of T_H1 cells as demonstrated by the failure of IL-12-deficient mice to generate a type 1 immune response [38]. IL-12 induces the expression of IFN-γ by T cells and NK cells and is primarily produced by dendritic cells and macrophages [39,40]. Lipopolysaccharide, bacterial DNA, double stranded (viral) RNA and cross-linking of CD40 molecules are potent signals for the production of IL-12 [41–43]. Dendritic cells that undergo maturation in the presence of these IL-12 inducing stimuli, skew $CD4^+$ T cell differentiation toward T_H1 cells. In the absence of IL-12, dendritic cells may induce T_H2 differentiation.

Several factors have been identified that inhibit the expression of IL-12 by dendritic cells. These include IL-10 [41], prostaglandin E2 [44] and the complement products iC3b and C5a [45–49]. As discussed in more detail below, it has been demonstrated that aluminum hydroxide can activate complement [50]. In addition, monocyte chemoattractant proteins (MCPs), a group of chemokines, inhibit the expression of IL-12 in human monocytes [49]. The importance of MCP-1 in the control of the immune response is suggested by the inability of MCP-1-deficient mice to generate a type 2 immune response [51]. Complement activation and selective induction of chemokines by aluminum adjuvants could contribute to the type 2-biased immune response.

IL-4 plays an important role in type 2 immune responses as it promotes type 2 and inhibits type 1 responses. Although dendritic cells can produce IL-4 [52], they generally do not appear to be a significant source of IL-4. Functional deletion of the IL-4 gene did not interfere with the adjuvant effect of aluminum hydroxide [53] and did not affect the differentiation of T_H2 cells as demonstrated by unchanged IL-5 secretion. However, in the absence of IL-4, aluminum adjuvant induced antigen-specific IgG2a antibodies and IFN-γ-secreting T cells indicating a type 1 immune response. Similar results were obtained with IL-4Rα-deficient and Stat-6-deficient mice [54] which demonstrates that both IL-4 and IL-13 are dispensable for the adjuvant effect of aluminum, but that they inhibit type 1 immune responses. This is consistent with the observation that IL-4 can suppress the secretion of IL-12 by dendritic cells [41].

3.3. Modifying the type 2 immune response induced by aluminum adjuvant

The preferential induction of a type 2 immune response and lack of a type 1 immune response may be appropriate for vaccines against extracellular pathogens, bacterial exotoxins and helminth parasites, but this is not a desirable property for vaccines against intracellular pathogens, such as viruses, mycobacteria and certain protozoa. Various strategies have been used to modify the formulation of aluminum adjuvants in order to change the induced immune response to a mixed types 1 and 2 immune response.

Co-administration of IL-12 with aluminum hydroxide and antigen in mice resulted in an increased antigen-specific antibody response in comparison with aluminum hydroxide and antigen alone [55]. Importantly, the mice that received aluminum hydroxide with IL-12 had a significant antigen-specific IgG2a response, consistent with a type 1 immune response, whereas, the mice that received aluminum only had no detectable antigen-specific IgG2a as expected. The addition of IL-12 also boosted the IgG1 response, demonstrating that both type 1 and type 2 immune responses were stimulated. The IL-12 was >98% adsorbed prior to injection into the mice and it was suggested that release of IL-12 over time may have increased its biological half-life [55].

Bacterial DNA is a strong activator of B cells, macrophages and dendritic cells. Bacterial DNA contains an increased number of CpG dinucleotides in comparison with mammalian DNA and these nucleotides are unmethylated. Synthetic oligonucleotides that contain ummethylated CpG motifs have similar immunostimulating effect as bacterial DNA [56]. CpG oligonucleotides are potent inducers of IL-12 and dendritic cell maturation [57] and stimulate a type 1 immune response. Co-administration of aluminum hydroxide with CpG oligonucleotides and antigen results in a marked increase of antigen-specific antibody responses of both IgG1 and IgG2a subclasses in comparison with either CpG oligonucleotides alone or aluminum hydroxide alone, indicating a strong synergistic effect [58]. Aluminum hydroxide has strong affinity for DNA [24], but it was not determined what percentage of the CpG oligonucleotides was adsorbed and whether adsorption was important for the synergistic adjuvant effect.

Algammulin is an adjuvant that is prepared by mixing of aluminum hydroxide with γ-inulin, a polysaccharide [59]. γ-Inulin is thought to enhance the immune response by activation of the alternative complement pathway, which might be expected to generate a type 2 immune response via suppression of IL-12 secretion as discussed above. However, the addition of γ-inulin to aluminum shifts the immune response towards a type 1 immune response [59].

3.4. Complement activation by aluminum adjuvants

Aluminum hydroxide activates the complement cascade [50]. The formation of complement product C3b was not inhibited by EDTA, suggesting that the activation of complement did not occur via the classical or alternative pathway. However, the activation was dependent on plasminogen. Complement factors play an important role in the regulation of B cell responses [60] and, as mentioned in Section 3.2, also affect the type 1 and type 2 balance of the immune response. B cells and follicular dendritic cells have two distinct receptors (CD21 and CD35, respectively) for C3 and C4 products. CD21 forms a complex with CD19 on B cells that facilitates signal transduction via the membrane immunoglobulin receptor. Indeed, immunization of mice with a fusion protein of lysozyme and C3d resulted in a markedly enhanced immune response [61]. The CD35 receptor on follicular dendritic cells binds immune complexes and retains these in undegraded form for several months. This may be important in the generation and maintenance of memory B cells. Thus, activation of complement by aluminum adjuvant may enhance the humoral immune response by targeting antigens to B cells and follicular dendritic cells. Monocytes and macrophages express receptors for the complement products iC3b and C5a. Signaling through these receptors inhibits the expression of IL-12 and this may play a role in the induction of type 2-biased immune responses by aluminum adjuvants [45–49].

4. Conclusions

Surprisingly little is known about the mechanisms by which aluminum adjuvants, which have been administered in millions of doses of vaccines, enhance the immune response to vaccine antigens. The depot mechanism, if defined as the slow sustained release of antigen over days or weeks, is unlikely in the light of recent work that has demonstrated a rapid desorption of antigen. However, adsorption of antigens to aluminum compounds may help to retain antigen at the injection site at a high concentration and for a sufficient period of time to allow uptake and activation of antigen-presenting cells. In addition, aluminum adjuvants activate antigen-presenting cells and complement, and induce chemokines as is evident from the local inflammatory response. How much each of these effects contribute to the adjuvant effect of aluminum compounds, remains to be determined. Knowledge of the mechanisms by which aluminum adjuvants selectively enhance the type 2 immune response may translate into newer adjuvants that combine the overall safety of aluminum adjuvants with improved efficacy.

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