

REVIEW ARTICLE

# Particulate Adjuvant and Innate Immunity: Past Achievements, Present Findings, and Future Prospects

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Particulates and crystals stimulate the immune system to induce inflammatory responses. Several nanometer- to micrometer-sized particulates, such as particle matter 2.5 (PM<sub>2.5</sub>), diesel particles, and sand dust, induce pulmonary inflammation and allergic asthma. Conversely, nanometer- to micrometer-sized crystal, sphere, and hydrogel forms of aluminum salts (referred to as “alum”) have been used as vaccine adjuvants to enhance antibody responses in animals and humans. Although most of these particulates induce type-2 immune responses *in vivo*, the molecular and immunological mechanisms of action as a vaccine adjuvant are poorly understood. In this review, recent advances in particulate adjuvant research from the standpoint of innate immune responses are discussed.

**Keywords** adjuvant, alum, innate immunity, particulates, vaccine

## INTRODUCTION: ADJUVANT AND INNATE IMMUNITY

Immune responses are categorized into two types: innate and adaptive. Innate immunity is mediated by macrophages and dendritic cells (DCs), which engulf and kill microbes. In contrast, adaptive immunity involves antigen-specific responses mediated by T cells, B cells, and memory cells. It had long been believed that the innate immune response functions as a temporal defense system against infection until the adaptive immune response can be elicited. However, recent studies have demonstrated that innate immunity is essential for the effective induction of adaptive immunity [1–3].

Vaccination mimics natural infection and induces pathogen-specific adaptive immunity effectively. Typically, vaccines contain two main components: antigens and adjuvants. An adjuvant is a substance that enhances antigen-specific (adaptive) immune responses when used in combination with a specific antigen. An adjuvant is thought to be an activator of innate immunity. In general, innate immune cells recognize pathogen-derived factors [e.g. pathogen-associated molecular patterns

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(PAMPs)], through pattern recognition receptors (PRRs) and induce inflammatory responses. There are four classes of PRRs: Toll-like receptors (TLRs), Nod-like receptors (NLRs), RIG-I-like receptors (RLRs), and C-type lectin receptors (CLRs) [4–7]. These receptors “sense” pathogen-derived factors and transduce activating signals into cells, triggering adaptive immunity against pathogens. Therefore, the ligands for PRRs, such as PAMPs and damage-associated molecular patterns (DAMPs), exhibit potent adjuvant properties that elicit adaptive immunity, and PRRs are considered to be receptors for adjuvants [1, 8].

However, the molecular and immunological mechanisms of many adjuvants used clinically (or those under development) have yet to be fully elucidated. For example, oil emulsions (e.g. Freund’s adjuvant and MF-59) and saponin-based adjuvants (e.g., QS-21 and ISCOM) exhibit strong adjuvant activities and could be promising candidates as adjuvants for new human vaccines [9, 10], yet no specific PRR(s) is identified.

An increasing number of particulates and nanoparticles have been reported to exhibit adjuvant activity. A well-known and widely used particulate adjuvant is aluminum salts, which is referred to as “alum” [11–13]. The mechanisms of induction of adaptive immunity by alum or a particulate adjuvant are also unclear, even though alum has been used as a human vaccine adjuvant for more than 80 years. The induction of adaptive immunity requires innate immunity. Hence, it has been proposed that particulates can activate innate cells, and that this action is accompanied by the induction of cytokines, chemokines, and other factors.

#### **PARTICULATES AND THE ADJUVANT EFFECT**

Several particulates are known to exhibit adjuvant effects in immune responses. Alum selectively stimulates humoral immune responses, especially type-2 helper (Th2) immune responses, which are characterized by the production of interleukin (IL)-4 and IL-5 and the induction of immunoglobulin (Ig) E and IgG1 [11–13]. (In the case of mice, IgG1 is categorized into Th2-dependent antibody, but the IgG isotype for human Th2 responses has not been clarified fully.) Similar to alum, crystalline silica (which causes a type of pulmonary fibrosis referred to as “silicosis”) induces Th2 responses and antigen-specific IgE and IgG1 [14]. It has been reported that synthesized particles, such as poly(lactic-co-glycolic acid) (PLGA), polystyrene particles, nickel oxide nanoparticles, and carbon nanotubes, induce humoral immunity, especially antigen-specific production of IgG1 and IgE [15–19]. Several particulate pollutants, such as diesel exhaust particles, have been shown to induce Th2 responses after intratracheal instillation and are thought to be the source of allergic diseases [20, 21]. In addition to artificial particulates, several crystals generated in the body induce inflammatory responses and possess adjuvant activity. Monosodium urate (MSU) crystals are formed if the concentration of uric acid released from damaged cells reaches saturation. MSU crystals act as DAMPs, and are the causative agent of gout. MSU crystals also act as Th2 adjuvants [22–26]. The biocrystalline substance hemozoin is a heme detoxification byproduct of malaria parasites. Hemozoin exhibits a potent adjuvant effect and induces humoral immune responses [27]. Chitin particles, which are biopolymers of N-acetyl-D-glucosamine found in fungi, helminthes, and insects, induce the accumulation of IL-4-producing eosinophils and basophils, and are associated with allergy [28]. In contrast to PAMPs such as lipopolysaccharide (LPS) and CpG oligodeoxynucleotides, almost all particulates preferentially elicit Th2 responses and the induction of IgE. Therefore, it has been hypothesized that the specific signals evoked by particulates in innate cells are involved in triggering adaptive (Th2) responses.

## PARTICLE SIZE AND IMMUNE RESPONSES

Particle size is thought to affect particulate-induced immune responses. Sharp et al. investigated the relationship between particle size and DC activation. They showed that the polystyrene particles measuring between 430 nm and 1  $\mu\text{m}$  activated DCs efficiently to produce IL-1 $\beta$  [15]. Hornung et al. demonstrated that the optimal size of silica crystals engulfed by macrophages was approximately 1  $\mu\text{m}$  [29]. Coban et al. investigated the adjuvanticity of hemozoin of different sizes. They reported that hemozoin particles measuring between 50 nm and 200 nm exhibited a stronger adjuvant effect compared with larger (2–20  $\mu\text{m}$ ) and smaller (<50 nm) particles [27, 30]. These results suggest that particles measuring between 200 nm and 1  $\mu\text{m}$  are the optimal size for phagocytosis and the stimulation of immune responses.

## DEPOT EFFECT

Antigen persistence and prolonged release, an effect referred to as the “depot effect” (first proposed by Glenny et al. in 1926), is believed to be responsible for the adjuvanticity of alum [31]. Harrison verified this hypothesis in 1935 by transferring the alum nodules from one guinea pig into a second guinea pig [32]. However, the depot effect has been questioned in several reports. Holt demonstrated that the antibody responses were normal if alum nodules were excised 2 weeks after immunization [33]. In particular, a recent report by Hutchison et al. demonstrated that the removal of the injection site 2 hours after the administration of antigen/alum had no effect on antigen-specific antibody and T-cell responses [34]. These studies suggest that the antigen depot does not play an important part in alum adjuvanticity, and that alum exhibits additional effects that account for its adjuvant properties.

## TH2 CYTOKINES AND IL-4-PRODUCING CELLS

Alum preferentially induces Th2 responses (which are characterized by the production of IgG1 and IgE) and IL-4 is a crucial factor for the induction of such Th2 responses. Alum and several other particulates induce the recruitment of IL-4-producing myeloid cells. Jordan et al. reported that alum induces IL-4-producing Gr-1<sup>+</sup> cells, and that these cells and IL-4 are required for the expansion of antigen-specific B cells *in vivo* [35]. Furthermore, Wang et al. demonstrated that alum-elicited Gr-1<sup>+</sup> cells are IL-4-producing eosinophils [36]. As stated above, chitin-induced IL-4-producing cells were eosinophils and basophils. Moreover, eosinophil recruitment is dependent on the leukotriene B<sub>4</sub> produced by macrophages [28]. However, it has been reported that the antigen-specific antibody responses are normal in several eosinophil-deficient mice (IL-5-deficient, GATA1 $\Delta$ , and Phil mice) compared with wild-type (WT) control mice after immunization with ovalbumin (OVA)-alum [37]. In addition, Ohnmacht et al. demonstrated that antigen-specific IgG1 and IgE responses were comparable in WT and basophil-deficient mice immunized with OVA and alum [38]. These studies suggest that IL-4-producing myeloid cells such as eosinophils and basophils do not participate in alum adjuvanticity or Th2 responses. Recently, it has been reported that CD1d-deficient [both type-I and -II natural killer T (NKT) cell-deficient]-mice, but not  $\text{J}\alpha 18$ -deficient (only type-I NKT cell-deficient)-mice exhibited reduced levels of antigen-specific IgG1 [39]. Type-II NKT cells appear to be required for alum-induced antigen-specific IgG1 responses in the regulation of IL-4-producing T cells.

There are several reports on IL-4 signaling and alum adjuvanticity [40, 41]. Brewer et al. reported on the involvement of IL-4 in the immunization of alum using IL-4-, IL-4R $\alpha$ -, and STAT6-deficient mice. These strains of mice did not induce the production of IgE and exhibited reduced levels of IgG1. However, T cells from IL-4R $\alpha$ - and STAT6-deficient mice produced normal or higher amounts of IL-4 and IL-5 in response to

a specific antigen. These results indicate that IL-4- and IL-13-mediated signaling is required for Th2-associated antibody production but is dispensable for alum-induced Th2 responses.

Recently, several reports focused on the importance of thymic stromal lymphopoi-  
etin (TSLP) on Th2 activation, and Al-Shami et al. demonstrated that TSLP receptor-  
deficient mice displayed reduced Th2 responses after immunization with OVA and  
alum [42]. However, allergen (without adjuvant)-induced Th2 responses were also re-  
duced in TSLP receptor-deficient or anti-TSLP antibody-treated mice [43, 44]. These  
results indicate that TSLP receptor-deficient mice are Th1 prone, and that reduced Th2  
responses are not specific to immunization with alum.

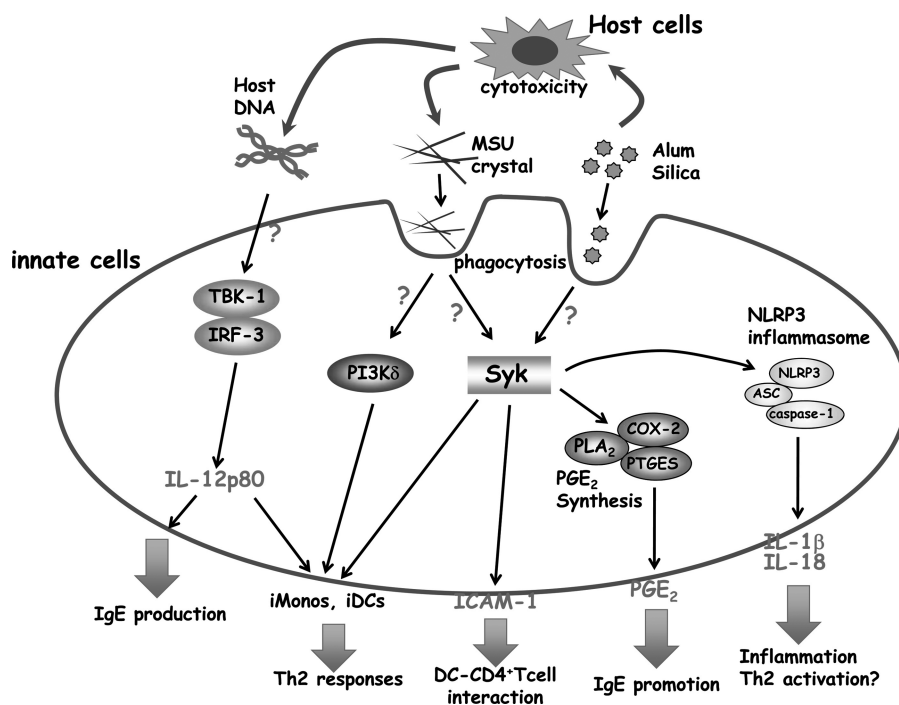
### **PARTICULATES AND MYD88 SIGNALING**

All TLR ligands are thought to be potent immune adjuvants through the activation  
of the adaptor molecules MyD88 and TRIF. Schnare et al. demonstrated that MyD88-  
deficient mice produced normal levels of OVA-specific IgG and IgE, but that elevated  
levels of total IgE were produced after immunization with OVA in alum [45]. The exces-  
sive amounts of total IgE appeared to be caused by the increased production of IL-13  
in MyD88-deficient T cells. Gavin et al. also reported alum adjuvanticity in mice defi-  
cient in MyD88 and TRIF, which lack TLR signaling. The antibody responses in these  
double-knockout (KO) mice were comparable with those in WT mice immunized with  
trinitrophenol (TNP)-hemocyanin in alum [46]. These results suggest that TLR signal-  
ing does not account for the action of alum and indicate that TLRs may act as negative  
regulators of IgE production. However, Da Silva et al. demonstrated that MyD88 path-  
way was required for alum-induced Th2 responses in asthma models [47]. The reason  
for these discrepant results is unclear. There might be differences in the alum (Imject  
alum, aluminum hydroxide, aluminum phosphate, or aluminum potassium sulfate)  
and OVA (endotoxin-free or not) used.

Conversely, hemozoin crystals seem to act as MyD88-dependent adjuvants in nat-  
ural and synthetic forms [27, 30]. The mechanism(s) underlying this dissimilarity be-  
tween alum and hemozoin particulates remains to be investigated.

### **NLRP3 INFLAMMASOME**

In 2008, several reports focused on the discovery that particulate adjuvants activate the  
NLRP3 inflammasome [29, 48]. The inflammasome is a PRR, and there are four classes  
of inflammasome: NLRP1, NLRP3, NLRC4, and AIM2 [5]. The NLRP3 inflammasome is  
one of the best characterized inflammasomes and is activated by particulates and crys-  
tals [15, 29, 48–53]. NLRP3 forms a multiprotein complex with apoptosis-associated  
speck-like protein containing a caspase recruitment domain (ASC) and caspase-1. The  
NLRP3 inflammasome promotes the secretion of inflammatory cytokines such as IL-  
1 $\beta$  and IL-18 as active mature forms cleaved by activated caspase-1. In addition to ac-  
tivation by PAMPs, several reports have demonstrated that particulates such as silica  
and alum stimulate macrophages and DCs to produce IL-1 $\beta$  and IL-18 through ac-  
tivation of the inflammasome, and that alum-induced antigen-specific IgG1 responses  
are significantly reduced in NLRP3-, ASC-, and caspase-1-deficient mice [48, 54]. Sim-  
ilar to alum, most particulate adjuvants are considered to have an adjuvant effect via  
inflammasome activation because silica, asbestos, PLGA, and MSU act as activators  
of the NLRP3 inflammasome. However, other reports have shown that the NLRP3 in-  
flammasome is not required for antibody production in response to vaccination using  
a particulate adjuvant, including alum [27, 37, 55, 56]. These contradictory reports on  
the role of the NLRP3 inflammasome may be because of different experimental con-  
ditions. Several studies used Imject alum [48, 54, 55], whereas other studies used alu-  
minum hydroxide [56]. Differences in genetic background of the animal used, such



**FIGURE 1.** Proposed mechanisms of particulate adjuvants (alum, MSU, silica) in innate immunity. Alum induces cell death, and the damaged host cells, such as macrophages and neutrophils, release genomic DNA and uric acid as DAMPs. The recognition mechanisms of genomic DNA are still unclear, but the TBK-1-IRF3 axis plays an important part in IgE production and iMono/iDC migration via the IL-12p80 production. The released uric acid forms MSU crystals, which are recognized by lipid sorting on DCs. The engulfed MSU crystals trigger the activation of Syk and PI3K $\delta$ , and induce inflammatory cells or a strong interaction between DCs and CD4<sup>+</sup> T cells. However, the released uric acid has not been shown to form crystals at the site of alum injection. Alum and silica stimulate macrophages and DCs to produce NLRP3 inflammasome-dependent IL-1 $\beta$  and IL-18. These cytokines contribute (at least in part) to acute inflammation and Th2 activation. Macrophages and DCs also induce PGE<sub>2</sub> in response to alum and silica via Syk activation. PGE<sub>2</sub> is involved in IgE production. iMonos: inflammatory monocytes; iDCs: inflammatory DCs.

as C57BL/6 [48, 54, 55] and mixed C57BL/6-129 [56], might contribute to the contrasting results. The involvement of inflammasome-dependent cytokines in alum adjuvanticity is an important issue. It has been demonstrated that IL-18 plays an important part in alum-mediated Th2 responses [57]. However, IL-1 and IL-18 signaling triggers MyD88-dependent signaling, and MyD88 signaling is dispensable for alum adjuvanticity (as described above). The NLRP3 inflammasome may participate in adjuvant activity through IL-1 $\beta$ - and IL-18-independent mechanisms, but the role of the NLRP3 inflammasome in the induction of adjuvant activity remains unclear (Figure 1).

### MSU AS A DAMP

Uric acid is a purine catabolite that is released from dying or stressed cells. Uric acid forms MSU crystals if the concentration of uric acid is saturated. Shi et al. demonstrated that uric acid and MSU crystals act as DAMPs and stimulate DCs to induce the maturation and activation of cells [58]. Interestingly, similar to alum, MSU crystals are known to activate Th2 responses preferentially [22–26]. Kool et al. demonstrated that uric acid is released in the peritoneal cavity after the injection of alum, and that antigen-specific T-cell responses were prevented after uricase treatment [22]. Alum is

known to induce cell death, and uric acid and MSU crystals induced by alum cytotoxicity appear to contribute to alum adjuvanticity (whether uric acid forms crystal *in vivo* in alum-injected sites is of considerable interest). In addition, this study demonstrated that uric acid-primed inflammatory monocytes and DCs have an important role in the activation of antigen-specific T cells [22]. However, this study reported that MyD88 signaling was required for this mechanism, which is a controversial proposal. Similar to alum and silica, MSU crystals have been reported to activate the NLRP3 inflammasome [50], and this finding is suspected to be linked to the adjuvant activity through the activation of the NLRP3 inflammasome. However, it has been reported that IL-1 $\beta$ , MyD88, and the NLRP3 inflammasome are dispensable for uric acid-dependent adjuvant activity, and that spleen tyrosine kinase (Syk) and PI3-kinase  $\delta$  in inflammatory monocytes and DCs are required for Th2 activation by uric acid (Figure 1) [25]. Syk is a nonreceptor tyrosine kinase and a key mediator of immunoreceptor signaling in immune cells. It has been demonstrated that Syk is involved in particulate-mediated innate cell activation [17, 51, 59]. The relationship between uric acid-induced Th2 activation and Syk is interesting. Although Syk is known to be activated by immunoreceptor tyrosine-based activation motif (ITAM)-bearing receptors [60], the underlying mechanisms of Syk activation by particulates is unclear.

Recently, several studies demonstrated the unique recognition mechanisms of particulates. Ng et al. analyzed the recognition of MSU crystals by DCs using atomic force microscopy. MSU crystals were shown to interact with DCs via receptor-independent mechanisms by directly engaging cell surface lipids (mainly cholesterol) [61]. The aggregation of lipid rafts triggers the recruitment and activation of Syk, and ultimately, Syk activates PI3-kinase, phagocytosis, and cytokine secretion (Figure 1) [61]. Flach et al. reported that alum also binds to the surface of DCs, leading to lipid sorting that is similar to MSU crystal-mediated activation of Syk and PI3-kinase. However, the uptake of alum is not required, and activated DCs interact with DC4<sup>+</sup> T cells via binding with intracellular adhesion molecule (ICAM)-1 and leukocyte function-associated antigen (LFA)-1 (Figure 1) [62]. Syk appears to be a key molecule for the activation of DCs via lipid sorting, but the mechanisms of Syk activation by MSU or alum are unclear. In general, Syk is known to be activated by Src family kinases such as Hck, Fgr, and Lyn, and ITAM-containing FcR $\gamma$  and DNAX-activating protein of 12 kDa (DAP12). However, DCs double-deficient in ITAM-containing FcR $\gamma$  and DAP12 or triple-deficient in Src family kinases (Hck<sup>-/-</sup>, Fgr<sup>-/-</sup>, and Lyn<sup>-/-</sup>) retain their function after activation by MSU crystals [61].

### LIPID MEDIATOR

Recently, we found that Th2-inducing particulate adjuvants have another unique mechanism for the activation of innate immune cells: alum and silica particulates stimulate macrophages to produce prostaglandins (PGs) in a similar way to the secretion of IL-1 $\beta$  and IL-18 via NLRP3 activation [17]. In addition to proinflammatory cytokines, lipid mediators such as PGs are involved in the induction of inflammatory responses. The well-characterized proinflammatory lipid mediator PGE<sub>2</sub> is a metabolite of arachidonic acid that is produced by various types of cells, including antigen-presenting cells [63]. Studies have shown that PGE<sub>2</sub> suppresses Th1 responses by elevating intracellular concentrations of cyclic adenosine monophosphate (cAMP) in DCs and macrophages, thereby inhibiting the production of IL-12 and interferon [64–66]. In addition, PGE<sub>2</sub> enhances IL-23 production by DCs and favors Th17 polarization [67, 68]. More recently, PGE<sub>2</sub> has been shown to facilitate the differentiation of Th1 cells in the presence of IL-12 and high doses of the co-stimulatory CD28 antibody via the activation of the PI3-kinase pathway [68]. Thus, PGE<sub>2</sub> exhibits various functions in the regulation of immune responses.

Silica and alum stimulate macrophages and DCs to produce IL-1 $\beta$ , IL-18, and PGE<sub>2</sub>. The PGE<sub>2</sub> production induced by silica and alum has been shown to be independent of the NLRP3 inflammasome because inflammasome-deficient (NLRP3<sup>-/-</sup>, ASC<sup>-/-</sup>, caspase-1<sup>-/-</sup>) macrophages produced normal levels of PGE<sub>2</sub> in response to silica and alum compared with WT counterparts. Treatment with a Syk inhibitor or the knock-down of Syk using small interfering RNA (siRNA) molecules markedly suppressed the production of PGE<sub>2</sub> after stimulation with silica and alum, demonstrating that Syk regulates particulate-induced PGE<sub>2</sub> production. In this case, the mechanisms of Syk activation by alum and silica are unclear. However, several reports (including those involving studies on MSU crystals) have demonstrated that particulates stimulate innate immune cells via Syk activation. Therefore, Syk may be a key molecule for particulate-induced immune responses (Figure 1). PGE<sub>2</sub> synthesis is regulated by cyclooxygenase (COX) and PGE synthase (PTGES), and COX-2 and PTGES (also known as mPGES-1) in particular have been reported to regulate stimulation-dependent PGE<sub>2</sub> production in macrophages [69]. PTGES-deficient macrophages do not produce detectable amounts of PGE<sub>2</sub> after stimulation with silica or alum. In addition, PTGES-deficient mice display reduced amounts of antigen-specific IgE after immunization with alum and silica. In contrast, the levels of antigen-specific IgG are normal in PTGES-deficient mice compared with WT mice. These results indicate that particulate-induced PGE<sub>2</sub> is involved in IgE production *in vivo* (Figure 1) [17]. Several reports have demonstrated that PGE<sub>2</sub> facilitates IgE production by the accumulation of increased levels of intracellular cAMP [70, 71]. Interestingly, neuropeptides such as vasoreactive intestinal polypeptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP) preferentially activate Th2 responses and increase intracellular cAMP levels in a receptor-dependent manner [72, 73]. In addition, the soluble extract of birch pollen consists of a lipid that is thought to be the causative agent of allergic asthma and to resemble the structure and function of PGE<sub>2</sub>. This lipid induces Th2 responses and intracellular cAMP accumulation in DCs [74, 75]. Agents for cAMP elevation may act as Th2 adjuvants.

Many particulates that exhibit adjuvant activity, such as MSU crystals, PLGA, chitin particles, nickel oxide, amorphous silica, and carbon nanotubes, stimulate macrophages to produce inflammasome-dependent IL-1 $\beta$  and inflammasome-independent PGE<sub>2</sub>. In addition, we have found that, similar to the release of uric acid, increased amounts of PGE<sub>2</sub> are released from damaged cells, suggesting that PGE<sub>2</sub> also works as a DAMP (Kuroda et al., unpublished data). These findings suggest that PGE<sub>2</sub> is a useful marker for the screening of particulate (Th2) adjuvants.

#### RELEASE OF NUCLEIC ACIDS FROM HOST CELLS

Activation of innate immunity by DAMPs appears to be a critical mechanism for adjuvant activity. Recently, it was reported that the DNA released from host cells mediates the adjuvant activity of alum [76]. In this study, alum induced the local accumulation of host DNA at the injection site during alum-induced cell death, and interestingly, treatment with DNase I decreased the antigen-specific antibody responses in mice immunized with OVA in alum. Purified genomic DNA mixed with OVA induced OVA-specific IgG1 and IgE responses as efficiently as the alum adjuvant. These results indicate that the alum-induced release of host DNA triggers initial innate immune responses. These responses are not dependent on TLRs, RLRs, or inflammasomes, and the mechanisms by which the host DNA triggers the immune response are unclear. However, interferon regulatory factor 3 (IRF3) and TANK-binding kinase 1 (TBK1) are required for the adjuvant activity of alum (Figure 1). It has been reported that antigen-specific IgE responses, but not IgG1 responses, are significantly reduced in IRF3-deficient and TBK1/tumor necrosis factor (TNF)-double-deficient mice.

TABLE 1. Summary of the effect of particulate (alum) adjuvant on immune system

	Proposed mechanisms	Adjuvant activity	References
Depot effect	Antigen persistence and prolonged release	• Depot effect is not required.	33), 34)
IL-4-producing cells and IL-4 signals	Th2 and IgE induction	• Eosinophils and basophils are not required. • IL-4 is required for IgE production, but not for Th2 cell differentiation.	37), 38) 40), 41)
MyD88 pathway and TLRs	Innate cell activation	• MyD88 and TLRs are not required.	42), 43)
NLRP3 Inflammasome	Activation of NLRP3 inflammasome and IL-1 $\beta$ , IL-18 release	• The involvement of inflammasome in adjuvant activity remain unclear.	27), 37), 44) 50)~52)
Uric acid (MSU crystal)	Released from damaged cells by alum Work as DAMPs	• Th2 induction by inflammatory monocytes and DCs via Syk and PI3 kinase activation.	25), 57)
Lipid mediator	Induced from macrophage and DCs by alum or silica	• PGE <sub>2</sub> is induced by Syk activation and promote IgE production.	17)
Nucleic acid (DNA)	Released from damaged cells by alum Work as DAMPs	• Th2 and IgE induction by inflammatory monocytes and DCs through the secretion of IL-12p80 and activation of TBK1-IRF3.	75)

Inflammatory DCs (derived from inflammatory monocytes) were identified as the cells responsible for the induction of Th2 responses. In addition, a reduced number of inflammatory DCs in the draining lymph nodes were observed in IRF3-deficient mice immunized with OVA in alum, and the transfer of WT inflammatory monocytes to IRF3-deficient mice increased Th2 cytokine and IgE production. This study also demonstrated that IL-12p80 (a p40 homodimer) is required for alum-induced migration of inflammatory monocytes, and that treatment with anti-IL-12p80 antibody partially attenuated the IgE responses in alum-treated WT mice (Figure 1). IL-12p80 is known to induce DC migration and to activate nuclear factor kappa-B (NF- $\kappa$ B) and p38 MAP kinase but not signal transducers and activator of transcription (STAT) proteins [77, 78]. Identifying the IL-12p80-producing cells involved in responses to alum or host DNA would be interesting.

IgG1 and IgE responses are uncoupled, i.e. the TBK1-IRF3 axis is required only for the IgE responses. It is believed that the Th2-related antibodies IgE and IgG1 are regulated by identical mechanisms. As described above, PGE<sub>2</sub> is only involved in IgE production, not IgG1 production. Although the mechanisms of the regulation of IgE and IgG1 production and the relationship between IRF3 and PGE<sub>2</sub> remain unclear, the investigation of these mechanisms may help to improve the adjuvants currently in use.

## FUTURE PROSPECTS AND CONCLUSION

A summary of the effects of particulate adjuvants is shown in Table 1. Particulate adjuvants (including alum) induce adaptive immunity. The development and modulation of adaptive immunity is regulated by innate immunity. However, the basis for the adjuvant activity of particulates and the mechanisms by which particulates activate



innate immunity are not fully understood. Alum has been used as a safe vaccine adjuvant in humans, but the limitations of alum include local reactions and the augmentation of IgE antibody responses [11, 79]. These limitations reflect the need for continuing research, and these limitations may be overcome by elucidation of the mechanisms of the effect of particulate adjuvants on immune responses.

Alum in combination with another adjuvant, an AS04, a combination of alum with monophosphoryl lipid A (MPL), has been licensed. In addition, a combination with potent Th1 stimulator such as IL-12 and CpG oligodeoxynucleotides shows a great promise, with improvement in alum-induced Th2 responses [11, 12, 80]. Other adjuvant combinations might be explored further. Thus, advances in adjuvant research could open new possibilities for the treatment of not only infectious diseases but also allergic inflammation and cancer.

### Declaration of Interest

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### ABBREVIATIONS

DC	dendritic cell
PAMP	pathogen-associated molecular pattern
PRR	pattern-recognition receptor
TLR	Toll-like receptor
NLR	Nod-like receptor
RLR	RIG-I-like receptor
CLR	C-type lectin receptor
DAMP	damage-associated molecular pattern
Th2	type-2 helper
IL	interleukin
Ig	immunoglobulin
PGLA	poly(lactic-co-glycolic acid)
MSU	monosodium urate
LPS	lipopolysaccharide
WT	wild-type
OVA	ovalbumin
NKT	natural killer T
TSLP	thymic stromal lymphopoietin
KO	knockout
TNP	trinitrophenol
ASC	apoptosis-associated speck-like protein containing a caspase recruitment domain
Syk	spleen tyrosine kinase
ITAM	immunoreceptor tyrosine-based activation motif
ICAM	intracellular adhesion molecule
LFA	leukocyte function-associated antigen
DAP	DNAX-activating protein of 12 kDa
PG	prostaglandin
cAMP	cyclic adenosine monophosphate
siRNA	small interfering RNA

VIP	vasoreactive intestinal polypeptide
PACAP	pituitary adenylate cyclase-activating polypeptide
COX	cyclooxygenase
PTGES	PGE synthase
IRF3	interferon regulatory factor 3
TBK1	TANK-binding kinase 1
TNF	tumor necrosis factor
NF- $\kappa$ B	nuclear factor kappa-B
STAT	signal transducers and activator of transcription
MPL	monophosphoryl lipid A

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