



Review

On vaccine's adjuvants and autoimmunity: Current evidence and future perspectives



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ABSTRACT

Adjuvants are compounds incorporated into vaccines to enhance immunogenicity and the development of these molecules has become an expanding field of research in the last decades. Adding an adjuvant to a vaccine antigen leads to several advantages, including dose sparing and the induction of a more rapid, broader and strong immune response. Several of these molecules have been approved, including aluminium salts, oil-in-water emulsions (MF59, AS03 and AF03), virosomes and AS04.

Adjuvants have recently been implicated in the new syndrome named "ASIA—Autoimmune/inflammatory Syndrome Induced by Adjuvants", which describes an umbrella of clinical conditions including post-vaccination adverse reactions.

Recent studies implicate a web of mechanisms in the development of vaccine adjuvant-induced autoimmune diseases, in particular, in those associated with aluminium-based compounds. Fewer and unsystematised data are instead available about other adjuvants, despite recent evidence indicating that vaccines with different adjuvants may also cause specific autoimmune adverse reactions possible towards different pathogenic mechanisms.

This topic is of importance as the specific mechanism of action of each single adjuvant may have different effects on the course of different diseases. Herein, we review the current evidence about the mechanism of action of currently employed adjuvants and discuss the mechanisms by which such components may trigger autoimmunity.

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1. Introduction

One of the brightest chapters of medical history is the development and the introduction of immunisation programmes [1]. The results generated by these interventions on human health and longevity changed profoundly the historical relationship between infectious diseases and human race [2–4]. The eradication of smallpox and the large reduction of cases of poliomyelitis and measles are some examples of the beneficial impact of immunisation programmes [2,3].

Classical vaccines rely on the use of whole killed or attenuated pathogens and many currently licenced vaccines are formulated with this technology [5]. Newer and current in-development vaccines are instead based on rationally designed and highly purified recombinant antigens characterised by an excellent safety profiles [5]. Because of their well-defined structure, such antigens may be less immunogenic than live attenuated or inactivated pathogen preparations, which intrinsically contain components capable of enhancing immunogenicity [6,7].

Vaccination with highly purified antigens typically results in the induction of a modest antibody and T cell response and requires multiple vaccinations to elicit sufficient antibody responses [6,7]. For this reason, a significant amount of efforts has been invested to identify components capable of ameliorating immune responses to be added to vaccines. These components are defined adjuvants and consist in well-defined molecules and/or formulations [6,8]. Adding an adjuvant to a vaccine antigen leads to practical advantages, including dose sparing and the induction of a more rapid, broader and strong immune response [8–10].

A general and over-simplified classification for vaccine adjuvants includes two broad groups, called delivery systems and immune potentiators [10]. Immune potentiators are often used in combination with the delivery systems and are thought to be able to shift the immune response towards a more Th1 (CD4+) cellular immune response [10]. Approved adjuvants include aluminium salts, oil-in-water emulsions (MF59, AS03 and AF03), virosomes and AS04 [8–10].

The development and the increasing diffusion of new vaccination and immunisation programmes have also raised concerns about the safety of adjuvants and their immunogenicity-enhancing effect in vaccines [11–18]. The term “ASIA—Autoimmune/inflammatory Syndrome Induced by Adjuvants” was coined in 2011 to describe the spectrum of immune-mediated diseases triggered by an adjuvant stimulus [11,13,15,16,19–22]. This syndrome comprehends an “umbrella” of clinical including post-vaccination phenomena caused by vaccine adjuvants [23–26].

The pathogenesis of the ASIA syndrome is founded on the hypothesis that an early exposure to an adjuvant may set in motion a chain of biological and immunological events that, in susceptible individuals, may ultimately lead to the development of autoimmune diseases [13,15,16,20,21,27].

Recent studies implicate a web of mechanisms in the development of vaccine adjuvant-induced autoimmune diseases, in particular, in those associated with aluminium-based compounds (Alum), which comprise a major bulk of contemporary adjuvants. Fewer and unsystematised data are instead available about other adjuvants, despite recent evidence indicating that vaccines with adjuvants different from alum may also cause specific autoimmune adverse reactions [28–32].

2. The rationale for this review

While adjuvants have been employed for over 80 years, the mechanism by which these components ameliorate immune responses has been generally under-studied and its importance has been under-appreciated for a long time [10]. The knowledge at the molecular and cellular levels of adjuvant-induced immune responses is a critical step in the developing of a more efficacious and safer generation of vaccines. This topic is of importance as the specific mechanism of action of each single adjuvant may have different effects on the course of different

diseases. As an example, some preclinical models highlighted the importance of Toll like receptor 4 (TLR4) activation in rheumatoid arthritis and systemic lupus erythematosus [33]. This datum may be of importance for adjuvants that interact directly with the TLR4 receptor, while it is unlikely to be of concern for adjuvants acting with different mechanism.

Herein we review the current evidence about the mechanism of action of currently employed adjuvants and discuss the mechanisms by which such compounds may trigger autoimmunity.

3. Mechanism of action of adjuvants

3.1. Alum

Aluminium hydroxide and other aluminium salts (Aluminium hydroxyl-phosphate sulphate, Aluminium potassium phosphate, and others), typically referred to as “Alum”, are the most widely used adjuvants in human and animal vaccines [34]. Alum elicits strong humoral immune responses primarily mediated by secreted antigen-specific antibodies [35,36], which are effective against diseases such as diphtheria, tetanus and hepatitis B, where neutralising antibodies to bacterial and viral antigens are required for protection [37]. In contrast, alum is a poor inducer of cell-mediated immune responses and is unsuitable for vaccines that require a strong cellular immune response [38].

The first evidence about the effect of alum as adjuvant was reported in 1926 by Glenny et al., who observed that the injection of diphtheria toxoid precipitated with potassium aluminium sulphate induced an antibody response in guinea pigs stronger than the one obtained with the toxin alone [39]. Authors also observed the formation of nodules at injection site [39], which were suspected to act as depot site for the antigens. This hypothesis was confirmed further by the observations of Harrison [40], who found that immune response could be transferred surgically, by extracting and injecting these nodules in a naïve animal. These observations suggested that the mechanism by which alum act as adjuvant was the formation of nodules.

These were the basis of the “depot theory”, which states that alum acts by forming nodules that slowly release antigen, thus providing both a priming and a boosting effect with the same inoculation [40].

Further analysis on alum nodule's structure questioned the depot theory: alum nodules were found to be composed of fibrinogen, but fibrinogen-deficient mice were found to develop a normal immune response to alum vaccines, thus indicating that nodules are not required for alum salts to act as adjuvants [41]. Hutchison et al., who found that the removal of alum depot has no effect on antigen-specific T- and B-cell responses, questioned the depot theory further [42].

Notwithstanding the claims against the depot theory, it remains to be understood whether the long term response (i.e. after 35 days from immunisation) is still due to a depot effect or if the depot mechanism may be of importance for driving local immune responses in draining lymph nodes following transport from the injection site [43].

A possible role for the NACHT, LRR and PYD domains—containing protein-3 (NLRP3) inflammasome was also proposed [44–46], as it had been reported that alum-induced immune responses were abrogated in NLRP3-deficient mice. Specifically, NLRP3-deficient mice were found to have an impaired cell recruitment, a reduced secretion of IL-1 β , expression of MHC class II and of the co-stimulatory molecule CD86. Additionally, these mice showed a decreased number of inflammatory monocytes carrying antigen into the draining lymph nodes and a reduced alum-driven antigen-specific IgG1 [44,47,48]. These data, however, were not further confirmed as Franchi et al. reported that the inflammasome is not required for the alum-dependent increase of antibody titres following intraperitoneal injection of human serum albumin [46].

Along with the “depot theory” and the inflammasome hypothesis, other theories have been put forward to explain the adjuvant effect of alum [43]. It has been shown that alum vaccination may lead to an

increased interleukin-4 production that bears the monocyte and neutrophil marker Gr1 [49] and that alum may trigger adjuvanticity by inducing uric acid release from dying cells [50].

Both these hypotheses, however, do not elucidate fully the role of alum, because it is not clear if the neutrophil marker Gr1 was the initial biomarker of a the response to alum [49] and because the amount of uric acid that was released after vaccination by cell death appears to be too low to induce the alum adjuvant effect [50]. An additional hypothesis suggests that uric acid, along with host DNA released following alum injection [50,51], may function as an endogenous danger signal, and trigger alarm and inflammation thus enhancing the immune response [52].

Another hypothesis that has been proposed to explain that alum adjuvant action is related to the activation of the Syk–PI3 kinase pathway [53–55]. Alum has been suggested to be sensed by plasma membrane lipids and to induce a receptor-independent activation of the Syk–PI3 kinase pathway [53]. Such activation has been described to inhibit IL-12p70 secretion and to promote PGE₂ production, thus shaping immunity by suppressing the TH1 phenotype [43,54,55].

Taken together these progresses in the understanding of the role of alum as adjuvant suggest that diverse and simultaneous signals are involved in driving an alum-dependent immune response activation (Fig. 1). Further analyses, as well as a better standardisation of laboratory practice [34], will hopefully lead to a full understanding of this topic.

3.2. Emulsion

Emulsions approved for clinical use, such as MF59, AF03 and AS03 [56–58], are liquid droplets dispersed throughout an immiscible liquid.

This class includes oil-in-water and water-in-oil emulsions. A number of members of this class, including Freund's incomplete adjuvant, are too toxic to be used in prophylactic vaccines.

Initial formulations were developed in the 1940s and relied on water-in-oil emulsions developed using non-degradable mineral oils. While effective, these combinations were poorly tolerated. Subsequent attempts to create more tolerable formulations allowed the production of the first approved emulsion adjuvant, MF59, an oil-in-water emulsion based on biodegradable oil [59]. The inclusion of emulsions has been proved to enable antigen dose sparing and to increase antigen-specific antibody titres. Furthermore, antibody response induced by emulsions appears to have a more balanced IgG1:IgG2a profile compared with alum [60]. The mechanism of action of MF59 and AS03 are now well characterised, while for AF03 a mechanism has not been described (Fig. 1).

3.2.1. MF59

MF59 consists of a two-phase system oil-in-water emulsion, which contains small (~160 nm in diameter) oil droplets stabilised by the addition of squalene [61].

According to recent analyses, the mechanism of action of MF59 is due to the combination of its components rather than that of a single one and only the entire emulsion acts as adjuvant [62,63]. Current evidence excludes a role for depot formation: studies monitoring the clearances of radiolabeled antigen and squalene in rabbits suggested that both the antigen and the adjuvant are rapidly removed from the injection site [60,64]. Additionally, the binding between antigen and adjuvant was found to be unnecessary for enhancing the immune

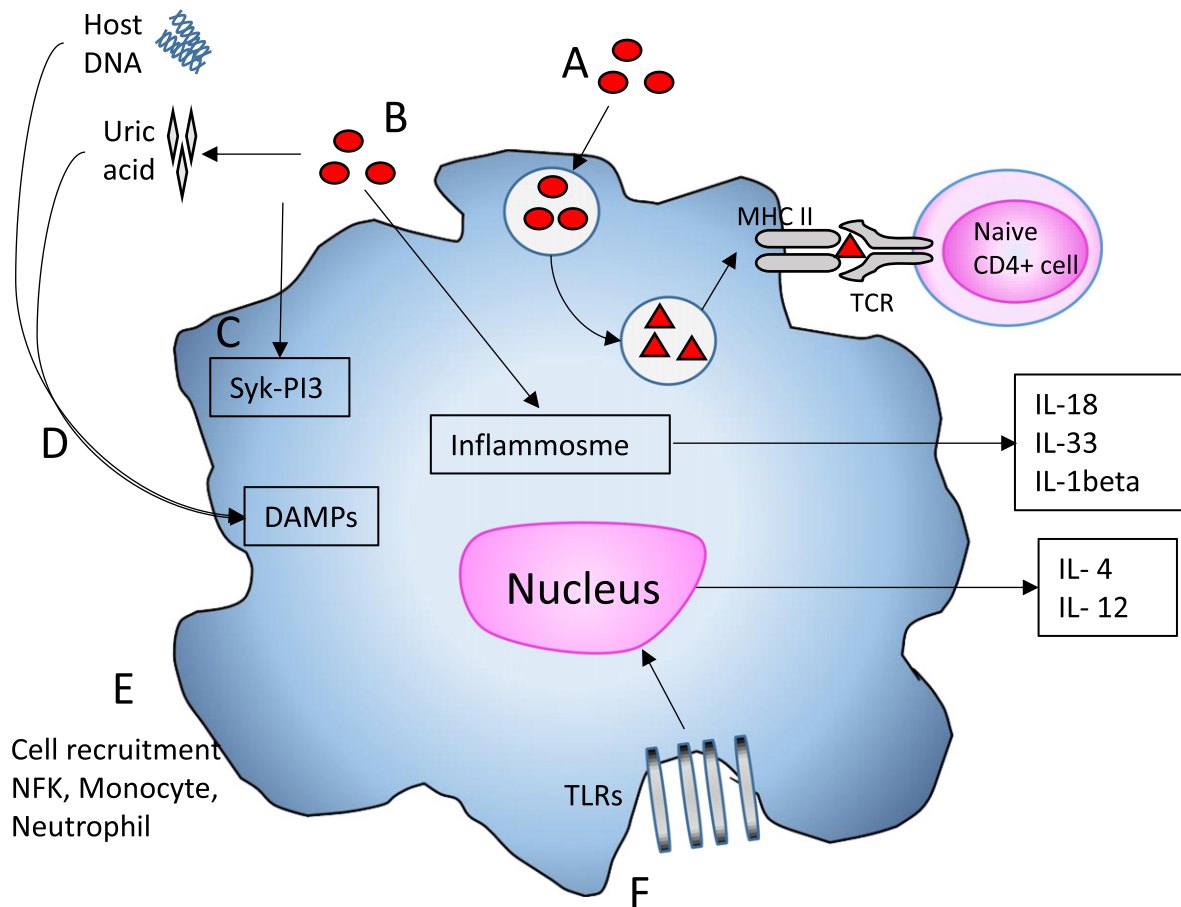


Fig. 1. Mechanism of action of adjuvants. A variety of mechanisms has been proposed to explain the adjuvant effect of current available adjuvants: A) enhanced antigen uptake; B) activation of the inflammasome; C) activation of the Syk–PI3 kinase signalling by uric acid crystals and alum; D) uric acid and host DNA released by necrotic cells are recognized by damage-associated molecular patterns (DAMPs); E) increased cell recruitment at injection site; and F) activation of toll like receptors.

response, thus ruling out the “depot theory” further [60]. Other analyses suggested that MF59 is transported mainly to the draining lymph nodes via a cellular carrier system and that this is the most important pathway in relation to the overall mechanism [65]. The cells containing antigen at the injection site were recognised as immature dendritic cells, thus suggesting that MF59 may act as ‘delivery system’ leading to enhanced antigen uptake [65]. By this hypothesis MF59 acts by creating an ‘immuno-competent environment’ at the injection site, as confirmed also by the fact that the adjuvant effect is maintained even if MF59 is administered up to 24 h before the antigen in the same site [65].

Further studies found a seven-fold increase of cells at the injection site two days after MF59 injection, which included mainly macrophages and minor populations of CD11c-positive dendritic cells [66].

This datum was confirmed further in mice knockout for CCR2, a major chemokine receptor largely present on monocytes, and in mice deficient for the molecule ICAM-1, which plays an essential role in cell to cell adhesion and extravasation of blood cells [66,67]. Interestingly, neither monocyte-derived dendritic cells, nor myeloid derived dendritic cells were activated directly by MF59 *in vitro*.

Monocytes, macrophages and granulocytes were instead stimulated by MF59, which determines the production of a series of chemokines capable of attracting monocytes and granulocytes further, thereby enhancing this feedback loop [67]. This datum was confirmed in a gene expression study, which also found muscle cells to be an additional target for MF59 [62]. MF59 induced a strong cellular recruitment in the injected muscles, including of granulocytes like neutrophils and eosinophils and potential antigen presenting cells (APCs), mostly monocytes, macrophages and dendritic cells [62]. All these newly recruited cells have the ability to take up both the antigen and adjuvant and transport them to draining lymph nodes [68,69].

At the molecular level, MF59 adjuvant action appears to be independent from NLRP3, and to depend on MyD88 and the apoptosis-associated speck-like protein containing CARD [70,71]. Both IL-4 and Stat-6, but not IFN- γ signalling have been proven to be necessary for MF59 adjuvant activity [72,73]. Further analyses will be required to identify the receptor or sensor for MF59 and the participating network of cell types.

3.2.2. AS03

AS03 is an Adjuvant System (AS) containing α -tocopherol and squalene in an oil-in-water emulsion. The mechanism of action of AS03 shares several characteristic with the MF59 adjuvant as both rely on a squalene-based oil-in-water emulsion [56]. The distinguishing trait of AS03 is the inclusion of α -tocopherol, which is the most bioavailable form of vitamin E. The contribution of this element in the AS03 adjuvant effect was investigated recently by comparing AS03 and an equivalent emulsion without α -tocopherol both *in vivo* and *in vitro* [56].

The study found that α -tocopherol enhanced the magnitude of the antigen-specific adaptive response, affected cytokine production, decreased the time of eosinophil and neutrophil migration to the draining lymph nodes and improved antigen-loading in monocytes [56]. Additionally, the induction of IL-6 in the draining lymph nodes was higher in the AS03 group, suggesting the functioning of α -tocopherol as an immune-modulator [56]. α -Tocopherol has also been found to be a strong activator of the transcription factor Nrf2 [74,75], which induces the expression of several target genes including catalytic active subunits of the constitutive proteasome [76].

3.3. Virosomes

Virosomes are spherical lipid vesicles that include the functional viral glycoproteins haemagglutinin and neuraminidase from influenza [77] and can be regarded as uni-lamellar liposomes carrying the spike proteins of influenza virus on their surface [78].

The suggested mechanism by which these adjuvants work involves direct interaction of virosome particles with APCs or, in some cases,

with B cells which, in turn, activate T cells. The influenza HA antigen targets the virosomes to APCs, which engulf it by endocytosis and present the antigens to T cells after proteolytic degradation. Pre-existing immunity against influenza may represent another important determinant for the immunostimulating effect of virosomes [77].

3.4. MPL and AS04

The immune system in vertebrates comprises innate and acquired immunity, both of which work cooperatively to protect hosts from infections. The innate immune system is designed to recognise a range of pathogens and relies on pattern recognition receptors (PRRs) to detect the pathogen-associated molecular patterns (PAMPs) [79] of bacteria, viruses, protozoa and fungi.

A major group of PRRs is that of the TLRs [80]. Each TLR recognises specific PAMPs, including bacterial lipopolysaccharide (LPS), flagellin, single strain RNA and double strain DNA [80–82]. One of the most studied members of this family, TLR4, is essential for signalling by LPS, which is a major component of the outer membrane of Gram-negative bacteria and shows potent immune-stimulatory activity [80,81,83].

LPS is a natural adjuvant and its derivative, monophosphoryl lipid A (MPL) retains adjuvant function without toxicity, due to preferential stimulation of the TLR4 and TRIF signalling pathways [84]. Because of these characteristics, LPS derivatives are likely to play an important role as adjuvants in current and being developed vaccines [84,85].

Early observations of the enhanced vaccine response obtained with Gram negative bacteria were followed by refinements in this approach using endotoxin, lipid A, MPL or synthetic analogues such as RC529 [86–88]. Additionally, MPL was approved in combination with alum (AS04) as adjuvant in two prophylactic vaccines [89].

The adjuvant mechanism of MPL and its analogues is a direct stimulation of the innate immune system, by activating NF- κ B transcriptional activity and the subsequent expression of pro-inflammatory cytokines, such as TNF- α and IL-6, APCs maturation and stimulation of Th cells [89] (Fig. 1). In a study, the induction of NF- κ B occurred within 5 h after the injection, thus indicating that MLP directly stimulates the TLR4-expressing cell populations within the muscle [89,90]. Infiltrating cells are likely to contribute to local immune response at later stages. NF- κ B induction was also reported in the draining lymph node at 5 h, thus indicating low levels of MPL at local lymph node, or an activation due to cytokines produced in the muscle [89]. Notably, the authors suggested that the spatial confinement of NF- κ B activity to the injection site and draining lymph node was a proof of a localised rather than a systemic immune response to AS04 injection [89]. Alum in AS04 appeared not to synergise with or inhibit MPL, but rather to prolong the cytokine responses to MPL at the injection site.

4. Mechanisms of adjuvants induced autoimmunity

A variety of mechanisms has been suggested as the means by which vaccines can initiate and/or exacerbate autoimmune diseases. The proposed mechanisms are very similar to the ones proposed for infectious agents (Fig. 2).

Autoimmune diseases are the by-product of the immune system recognising self-antigens as foreigners, leading to inflammation and tissues destruction [91–95].

It is accepted that several autoimmune diseases have a significant genetic background [96–99] and genetic variations may affect the risk of adverse reaction following vaccination [100].

Several examples of the effects of genetic variation on the safety and the efficacy of vaccines and adjuvants exist. Recently, Stanley et al. assessed markers predictive of the development of fever after smallpox vaccine. They identified 8 haplotypes in the 4 genes *IL1A*, *IL1B*, *IL1R1* and *IL18*, associated with an incremented or decremented risk for development of fever after smallpox vaccination [101]. Another analysis reported a possible correlation between a specific HLA-DR haplotype and the

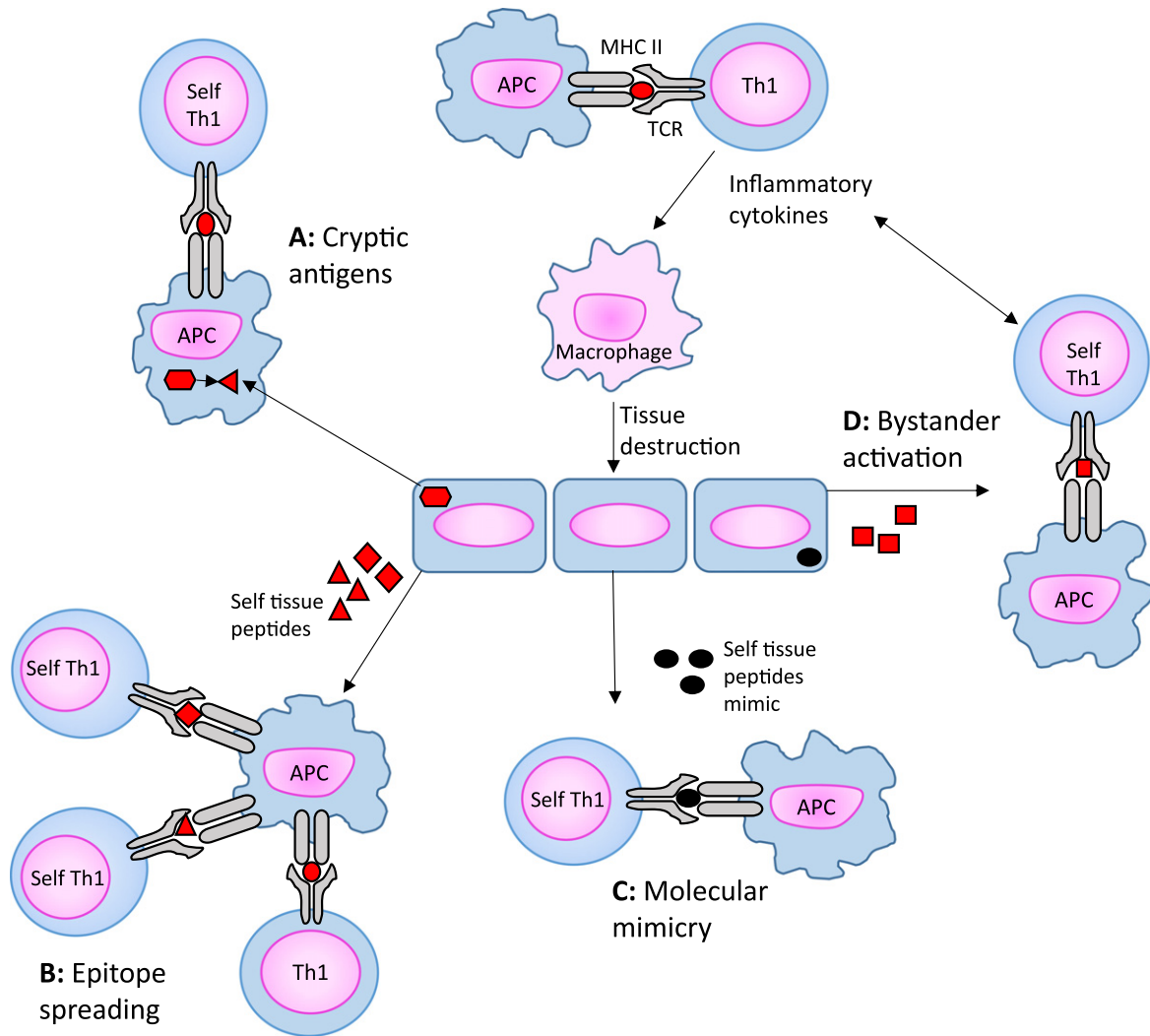


Fig. 2. Mechanism of autoimmunity. After normal immune response (upper panel), autoimmunity can occur through different mechanisms. A) Cryptic antigens involve a differential processing of self-peptides, which ultimately results in the activation of self-reactive Th1 cells. B) Epitope spreading mechanism theory is based on the continual damage and release of self-peptides during inflammation. This process results in a spreading of specificity of the immune response that ultimately includes also self-epitopes. C) Molecular mimicry: this mechanism is based on the similarity between a foreign and a self-epitopes, which results in the activation of cross-reactive T cells. D) Bystander activation is a non-specific activation of self-reactive Th1 cells through TCR dependent and independent mechanisms.

onset of vaccine-induced arthralgia [102]. Both these phenomena are major clinical criteria in the recently-defined ASIA syndrome [11,12], suggesting the basis for future determinations of this syndrome. The presence of a genetic background for ASIA syndrome has also been suggested recently due to specific clinical reports [103,104].

In predisposed individuals, adjuvants may determine autoimmunity or aggravate autoimmune diseases. This datum is consistent with both evidence from clinical case reports [30,103,105–110] and data from murine models where different adjuvants, such as alum and MF59 [111–115] were characterised by several mechanisms of action.

One of the most accepted mechanisms to explain the occurrence of autoimmunity following vaccination is molecular mimicry. This theory suggests that a foreign antigen shares sequence or structural similarities with self-antigens, thus activating T cells capable of recognising both the foreign and the self-epitope [116]. There is a variety of examples of bacterial infections initiating and exacerbating autoimmune diseases with this mechanism, including rheumatic fever and glomerulonephritis after *Streptococcus pyogenes* infections and Guillain-Barré syndrome induced by *Campylobacter jejuni* [116].

Molecular mimicry has been proposed as one of the main immunopathogenic mechanisms in post-vaccination CNS demyelination [117]. According to this hypothesis, the molecular similarity between the

proteins of the viruses used for the vaccination and CNS myelin components could result in the immune system recognising self-antigens as foreign ones [117].

Alongside molecular mimicry, other mechanisms have been proposed to explain the occurrence of autoimmune diseases after infection and some of these mechanisms may be important to explain correlations between autoimmunity and vaccines. Some of these mechanisms are antigen-specific, such as the above-mentioned molecular mimicry, while others are nonspecific and collectively known as “bystander activation” [118].

A series of mechanisms which involve tissue injury, cell death, oxidative stress, free radical production, and reparative changes, have been proposed to be determining a series of changes leading proteins that are usually recognised as self to be recognised as non-self [118,119]. These changes, which include altered expression, post-translational modifications, denaturation, misfolding, or mutations, ultimately results in autoimmunity phenomena [119]. Additionally, an inflammatory environment such as the one generated by infection may induce differential processing of released self-epitopes by APCs. This could result in proteins that are normally sequestered and shielded from immune recognition begin to be exposed to the immune system and thus acquire immunogenicity [119].

Alongside these antigen-specific mechanisms, other mechanisms independent from T Cell Receptor stimulations have been proposed. Virus infections lead to significant activation of APCs, which could potentially activate pre-primed auto-reactive T cells thus initiating an autoimmune disease. In addition to this mechanism, known as bystander activation of auto-reactive immune T cells, virus-specific T cells also might initiate bystander activation by killing the uninfected neighbouring cells through the release of tumour necrosis factor (TNF), TNF- β , lymphotoxin (LT), and nitric oxide (NO). This results in additional immunopathology at sites of infection, which in turn increase the release of cytokines in the inflammatory focus [120,121]. While this hypothesis is applicable to CD8+ T cells, evidence suggests that also CD4+ T cells that can recognise peptides in the context of class II molecules along with macrophages may directly kill uninfected cells in this bystander manner [122,123].

Adjuvants present in the vaccines can induce a non-specific activation of the immune system with a subsequent expansion of auto-reactive lymphocytes that may be accelerated further by defective regulatory cells/circuits, in genetically susceptible individuals [117].

While the above mentioned mechanisms are not specific for any single adjuvant, a very specific mechanism has been proposed to explain the role of AS03 in the development of narcolepsy [124]. Recent evidence from Northern Europe suggested that the influenza H1N1 vaccine contributed to the onset of narcolepsy among those 4 to 19 years old during the pandemic influenza in 2009–2010 [32,125]. Intriguingly, of the two different adjuvants that were used for this vaccine, namely AS03 and MF59, only AS03, which contains α -tocopherol, was associated with an increased number of narcolepsy cases [125].

Narcolepsy is a chronic sleep disorder characterised by the loss of hypocretin in the cerebrospinal fluid due to selective destruction of hypocretin-producing neurones in the perifornical hypothalamus [126]. A rapid increase of narcolepsy incidence was reported after the H1N1 vaccination campaign, especially in children and adolescents who received the AS03 adjuvanted H1N1 vaccine. Interesting, a genetic association of narcolepsy with HLA-DR2 and HLA-DQ1 in the major histocompatibility (MHC) region was described more than 25 years ago and further confirmed in other analyses [127]. Additionally, a large GWAS found an association between narcolepsy with cataplexy and T-cell receptor α chain gene (TCR α or TCRA) [128]. The increased risk of narcolepsy after AS03-adjuvanted H1N1 vaccination has questioned the role of H1N1 viral proteins or the AS03 adjuvant as environmental triggers in HLA-DQB1*06:02 positive people [129]. Such case highlights the importance of genetic background in predicting adverse drug reactions as in >99% of the cases are associated with the HLA-subtype DQB1*602, which differs in only nine residues in the β -chain from the wild type allele DQB1*601. These differences affect the peptide side chains in the P4 and P9 pockets, which bind the human hypocretin peptide, thus allowing larger hypocretin-specific fragments to be bound and presented with a higher affinity [126,130]. It was reported that α -tocopherol, the key component of AS03, could confer to the development of narcolepsy by activation of Nrf2 that finally leads to an elevated formation of longer hypocretin-derived fragments that can be presented by HLA-subtype DQB1*602 [124,131]. The case of α -tocopherol induced narcolepsy is emblematic of the pivotal role played by genetic predisposition in the development of vaccine adverse reactions [100,132,133]. Other intriguing genetic correlations have been reported for specific vaccines and adjuvants, including post vaccine fever [101], wheezing [134] and seizures [135].

5. Summary and conclusions

The recognition of ASIA syndrome as a nosographic entity represents an important step forward to the better understanding of a vast group of autoimmune diseases [11,26]. The idea that the adjuvant component of vaccines could enhance or trigger autoimmunity or autoimmune

diseases represents an intriguing observation that may explain a number of adverse reactions observed after vaccination.

However, several different adjuvants exist and each one has specific characteristics and a different mechanism of action that could affect both the immune response and the risk of adverse events. Our analysis highlights our current understanding of the mechanisms of action of available adjuvants and the mechanisms by which these components may determinate autoimmunity.

It should be emphasised that several factors including genetic predisposition [136–138], concomitant drugs [139,140], race and sex [141,142] affect vaccine response and influence the risk of adverse event following immunisation.

Additionally, serious adverse reactions to vaccination are very rare: we have recently estimated the incidence of ASIA syndrome after HPV vaccine to be 3.6 cases per 100,000 doses, and only a small amount of these cases was defined as “serious” [30].

Vaccines reported to be associated with ASIA syndrome include several adjuvants among which are AS04 [29,30,143], Alum [105,144], AS03 and MF59 [145–149]. This indicates that, despite the different mechanisms of action, all adjuvants may determine the ASIA syndrome. It remains to be defined whether the pathogenic pathway responsible for this syndrome is common to all adjuvants or specific pathways exists for different adjuvants [100].

Further analyses and a better understanding of the mechanism behind ASIA syndrome may allow determining which individual is at higher risk of adverse reaction due to vaccination because of his specific genetic background.

Conflict of interest

None.

Take-home messages

- Adjuvants are compounds incorporated into vaccines to enhance immunogenicity.
- Several of these molecules have been approved, including aluminium salts, oil-in-water emulsions (MF59, AS03 and AF03), virosomes and AS04.
- Adjuvants have recently been implicated in the new syndrome named “ASIA—Autoimmune/inflammatory Syndrome Induced by Adjuvants”.
- The pathogenesis of the ASIA syndrome is founded on the hypothesis that an exposure to an adjuvant may trigger the development of an autoimmune disease.
- Several different adjuvants exist and each one has specific characteristics and a different mechanism of action that could affect both the immune response and the risk of adverse events.
- All available adjuvants, despite their different mechanisms of action, have been implicated to the ASIA syndrome.
- Our analysis highlights our current understanding of the mechanisms of action of available adjuvants and the mechanisms by which these components may determinate autoimmunity.

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Vitamin D deficiency: the genetic bond

Vitamin D deficiency is a common finding in patients affected with autoimmune diseases and seems to affect the onset and the outcome of these conditions. Moreover, Vitamin D deficiency has been long feared since it can lead to a childhood disease, rickets. In rickets, a defective mineralization or calcification of bones before epiphyseal closure occurs, potentially lead to fractures and deformity. Beside reduced intake of Vitamin D, several factors may influence the development of rickets. Vitamin D receptor polymorphisms may indeed impair the activity of vitamin D, and a number of studies suggested an association between these variants and disease susceptibility. Mao S. and Huang S. have performed a meta-analysis on the polymorphisms of the Vitamin D receptor and the risk of rickets among Asians (Mao S, Huang S. *Arch Dis Child*. 2014 Mar;99(3):232–8). The authors included 16 studies focusing on VDR BsmI (rs1544410), TaqI (rs731236), FokI (rs2228570) and Apal (rs7975232) gene polymorphisms and the risk of rickets among Asians. They found that the B allele/BB genotype (BsmI site) was associated with the susceptibility of rickets ($p=0.017$ and 0.044 , respectively), and bb genotype was associated with lower risk of rickets ($p=0.033$). Similarly, the F allele/FF genotype (FokI site) was associated with the susceptibility of rickets, and ff genotype was associated with lower risk of rickets. AA genotype (Apal site) was associated with the onset of rickets ($p=0.044$). It is therefore evident that such polymorphisms may be partly responsible for rickets in Asian populations. Thus, it would be of interest to explore the role of these gene variants also in patients with autoimmune conditions, possibly linking genetically determined vitamin D deficiency and disease susceptibility or outcome. It is of note that Carvalho et al. (Carvalho C et al. *Lupus*. 2015 Feb 6) recently suggested an association between vitamin D receptor (VDR) gene polymorphisms and disease severity (chronic damage) in Portuguese patients with systemic lupus erythematosus.

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