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Safety of vaccine adjuvants: Focus on autoimmunity

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ABSTRACT

Questions have been recently raised regarding the safety of vaccine adjuvants, particularly in relation to autoimmunity or autoimmune disease(s)/disorder(s) (AID). The International Life Sciences Institute (ILSI) Health and Environmental Sciences Institute (HESI) formed a scientific committee and convened a 2-day workshop, consisting of technical experts from around the world representing academia, government regulatory agencies, and industry, to investigate and openly discuss the issues around adjuvant safety in vaccines. The types of adjuvants considered included oil-in-water emulsions and toll-like receptor (TLR) agonists. The state of science around the use of animal models and biomarkers for the evaluation and prediction of AID were also discussed. Following extensive literature reviews by the HESI committee, and presentations by experts at the workshop, several key points were identified, including the value of animal models used to study autoimmunity and AID toward studying novel vaccine adjuvants; whether there is scientific evidence indicating an intrinsic risk of autoimmunity and AID with adjuvants, or a higher risk resulting from the mechanism of action; and if there is compelling clinical data linking adjuvants and AID. The tripartite group of experts concluded that there is no compelling evidence supporting the association of vaccine adjuvants with autoimmunity signals. Additionally, it is recommended that future research on the potential effects of vaccine adjuvants on AID should consider carefully the experimental design in animal models particularly if they are to be used in any risk assessment, as an improper design and model could result in misleading information. Finally, studies on the mechanistic aspects and potential biomarkers related to adjuvants and autoimmunity phenomena could be developed.

1. Introduction

Vaccines play an important role in modern medicine in the prevention of diseases. Safety is paramount, as vaccines are often given prophylactically to healthy individuals. Most vaccines work under the basic premise that the immune system becomes primed from a possible future exposure upon vaccination, therefore, providing protection to an individual. In the case of highly purified subunit vaccines that lack intrinsic innate immune activators (natural adjuvants), various types of adjuvants are added during formulation to assist in a better education of the immune system, and thus, provide better protection for any future insult. Developing adjuvants is challenging, and adjuvants are under regulatory scrutiny as a result of theoretical and reported safety concerns [1], which include the risk of developing autoimmune diseases or AID, even if these concerns are controversial due to confounding factors that may attribute to the onset of AID. While research is constantly evolving to enhance adjuvant design [2], the scope of this manuscript focuses on two types of adjuvants in marketed vaccines: oil-in-water emulsions (e.g., squalene-based emulsions being used in influenza vaccines) and toll-like receptor (TRL) agonists (e.g., monophosphoryl lipid A (MPL)/aluminum salt combination in Hepatitis B and human papilloma virus (HPV) vaccines).

Possible safety concerns have arisen from studies in which adjuvants have induced AID in various animal models and from reports (primarily from one laboratory) that diverse compounds with "adjuvant" activity could be associated with silicosis, Gulf war syndrome (GWS), macrophagic myofasciitis (MMF), and post-vaccination phenomena [3]. The recent cases of narcolepsy observed during the 2009 pandemic influenza campaign with an AS03-adjuvanted vaccine [4,5] have further heightened awareness.

Autoimmunity and AID are complex and result from a combination of genetic, hormonal and/or environmental triggers [6]; thus, attributing causality is not easy. Certain adjuvants have specific receptor targets that strongly stimulate the immune system via pattern recognition receptors (PRRs), including TLRs, and stimulating

Abbreviations: AID, autoimmune disorders; AF, adjuvant formulations; ASIA, autoimmune/inflammatory syndrome induced by adjuvants; CFA, complete Freund's adjuvant; CIA, collagen-induced arthritis; DA, dark Agouti; DC, dendritic cells; EAE, experimental autoimmune encephalitis; EMA, European Medicines Agency; GWS, Gulf war syndrome; HESI, Health and Environmental Sciences Institute; HLA, human leukocyte antigen; HPV, human papilloma virus; Hsp, heat shock protein; IBD, inflammatory bowel disease; IFA, incomplete Freund's adjuvant; IFN, interferon; IL, interleukin; ILSI, International Life Sciences Institute; IMI, Innovative Medicines Initiative; MG, myasthenia gravis; miRNA, micro-ribonucleic acid; MMF, macrophagic myofasciitis; MoA, mechanism of action; MPL, monophosphoryl lipid A; mRNA, messenger ribonucleic acid; MS, multiple sclerosis; NOD, non-obese diabetic; O/W, oil-in-water; PAMPs, pathogen associated molecular patterns; PRRs, pattern recognition receptors; PY, person years; RA, rheumatoid arthritis; SjS, Sjögren's syndrome; SLE, systemic lupus erythematosus; snRNPs, small nuclear ribonucleic particles; TLRs, toll-like receptors; Tregs, regulatory T cells; UTR, untranslated region; W/O, water-in-oil; WOW, water-in-oil-in-water.

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such targets could theoretically increase the risk of initiation or progression of systemic AID [7].

As a consequence, questions are raised that need to be considered, such as: is the induction of AID in experimental settings due to exaggerated immune activation through the use of adjuvants? What is the relevance of animal data to humans? Do adjuvants induce autoimmunity and/or AID as a result of an exaggerated effect in a clinical setting, i.e., can some autoimmune diseases inherently be associated with adjuvants?

To address these questions, the International Life Sciences Institute (ILSI) Health and Environmental Sciences Institute (HESI) formed a multi-sector, international scientific committee, the Vaccines and Adjuvants Safety Project Committee (HESI committee), in 2011 to conduct a multi-faceted approach to assess the state of the science on adjuvants and AID. This approach included a survey of the current experimental and epidemiological literature and the convening of a Workshop on Adjuvants and Vaccines: Focus on Autoimmunity on October 18–19, 2012 in Amsterdam, The Netherlands. The workshop brought together 35 international scientists from academia, industry, and government to deliberate the relationship between adjuvants and vaccine safety [8].

2. Interplay between vaccines and the immune system is a delicate balance

The immune system is designed to recognize dangerous vs. safe antigens. It is a delicate system with many components that involve cells and signals that "turn on" and those that are responsible for "turning off" a response once the insult has cleared. Vaccines, and with the help of adjuvants, are designed to utilize this delicate balance in the immune system to drive protection for a host from viral, bacterial, or fungal infection. The physical–chemical properties of adjuvants and how they interact with the antigen(s), are important in defining how the immune system may respond, albeit to promote a protective humoral response or a response biased toward cell-mediated immunity. Antigen selection and adjuvant design are no longer empirical, and new generation adjuvants can specifically direct the desired immune responses [9]. Adjuvants, like oil-in-water or TLR agonists are designed to promote a more robust and/or tuned immune response to the antigen in the vaccine formulation in order to provide better protection. Oil-based emulsion adjuvants include MF59 (squalene), ASO3 (squalene, α-tocopherol), and Montanide (Seppic; various metabolizable and mineral oils). There are abundant animal study data on one example of this class of adjuvants, MF59 [10]. These data illustrate the complex molecular mechanisms associated with this class of adjuvants as illustrated in Fig. 1. MF59 both increases the uptake of antigens by antigen presenting cells (e.g., dendritic cells) and activates the innate immune response locally, providing the critical immunologically competent micro-environment for productive generation of B and T cell immunity. Importantly, MF59 does not activate the immune system systemically, and does not lead to detectable polyclonal immune activation even in the draining lymph node. Thus, a critical aspect of safe and highly effective adjuvants is one that can act locally at the site of injection in order to limit systemic effects, and therefore enhance its probability to be safe while still in an active form from an immune perspective.

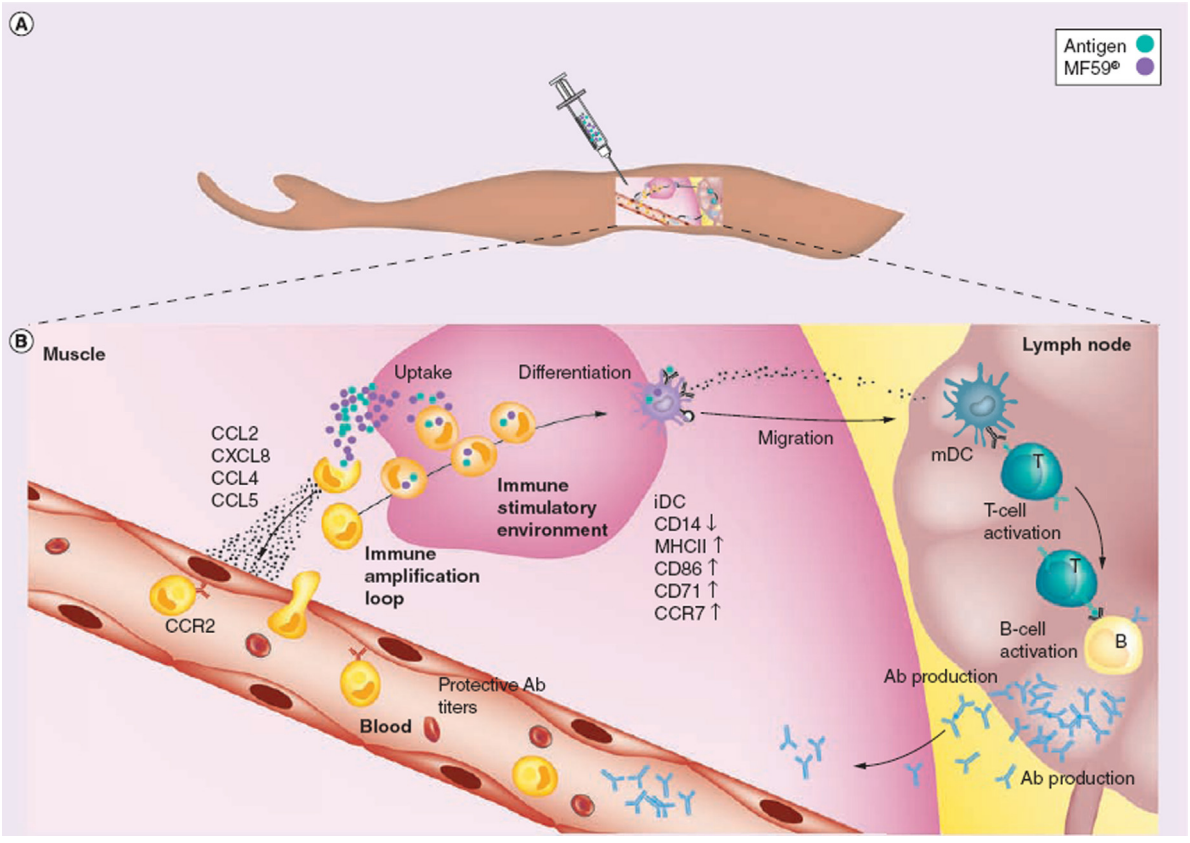

Fig. 1. Current understanding of MF59® adjuvant activity. (A) MF59 adjuvant in combination with a vaccine antigen is injected into the muscle. (B) In the muscle, tissue-resident monocytes, macrophages, and dendritic cells are activated and respond by inducing a mixture of chemokines (CCL2, CXCL8, CCL4, and CCL5), which results in a significant influx of phagocytic cells that take up the antigen and differentiate into antigen-presenting cells (dendritic cells). These cells are responsible for the efficient transport of antigen to the lymph nodes, where the immune response is triggered through the activation of T and B cells and antibody production. Ab: Antibody; iDC: Immature dendritic cell; mDC: Monocytic dendritic cell. Reprinted from Vaccine, 30, O'Hagan DT, Ott GS, De Gregorio E,and Seubert A. The mechanism of action of MF59–an innately attractive adjuvant formulation, 4341-8, copyright (2012), with permission from Elsevier [10].

TLRs, for their part, are a family of PRRs expressed predominantly by cells of the innate immune system that sense "danger" by recognizing specific exogenous pathogen associated molecular patterns (PAMPs) found on invading organisms, e.g., bacteria and viruses. Activation of TLRs drives the maturation of dendritic cells (DC) and other antigen presenting cells (APCs), activates naïve T cells, and increases immunogenicity. Many vaccines naturally contain PAMPs: for example, attenuated and killed vaccines contain viral or bacterial nucleic acids that activate various surface (Fig. 2) and/or endosomal (Fig. 3) TLRs [11], whole cell Gram-negative bacteria vaccine that express LPS (TLR4), and Gram-positive whole cell bacteria vaccine that express peptidoglycan (TLR2). This activation, either to surface or intracellular TLRs, would promote inflammatory cytokine production, which in theory could also be suggested as playing a pathogenic role in AID.

With an increased understanding of the immune system and vaccines, adjuvant formulations (AF) have been developed that combine different types of adjuvants into specific formulations that can localize and provide staggered antigen release (Signal 1) as well as provide a nonself and/or danger signal (Signal 2). Signal 1-facilitating adjuvants (mostly nonmicrobial) beneficially modify antigen-associated characteristics and delivery. Signal 2-facilitating adjuvants ("stranger", "danger", or endogenous immunopotentiators) beneficially modify host immune responsiveness during recognition of antigen by adaptive immune cells. Given the potential interplay between these adjuvants and the patient's immune system, these systems are being studied to assess both efficacy and risk. For example, the AS04-adjuvanted HPV-16/HPV-18 vaccine (Cervarix®), consists of a combination of TLR agonists, MPL, and aluminum salts. Cervarix® can be used to exemplify the mechanism of action (MoA) for AS04, and how it contributes to immunogenicity and efficacy in humans. MoA data showed that AS04 promotes an antibody response strictly dependent on spatial and temporal co-localization with the antigen [12]. It induces local nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activity and cytokine production within a few hours and days after immunization, respectively. This results in an increased number of activated antigen loaded dendritic cells and monocytes in the draining lymph node, which further increased the activation of antigen-specific T cells. These AS04-induced innate responses were primarily caused by MPL, whereas the aluminum salt prolonged the cytokine responses to MPL overall leading to an enhanced adaptive immune response to the antigens. In the case of Cervarix®, an integrated analysis of over 68,000 adolescents and young adults who received this vaccine platform demonstrated that there were low rates of autoimmune disorders, with no evidence of an increase in relative risk [12]. This study is a strong and powerful indication that formulated TLR4 agonists have, very likely, no impact on autoimmune disorders onset in humans after a 5-year follow-up at least.

3. AID is complex with many targets and contributing factors

All the various components of the immune system working in tandem can, however, malfunction. In case this system misdirects a response against self (by molecular mimicry or by blocking or counteracting peripheral tolerance), it can give rise to autoimmunity, which can be recognized through the presence of autoantibodies or autoreactive T cells. It can occur in both "healthy" individuals and in various disease states, and if it progresses from benign to pathogenic with the loss of control by the host, then AID is observed [13].

There have been over 80 different AID identified where the immune system inappropriately attacks itself via autoreactive antibodies or T cells, leading to destruction of tissues, abnormal growth of organ(s), and/or changes in function. AID can be either organ specific or systemic. Typical targets are blood vessels, connective tissues, endocrine glands, joints and muscles, red blood cells, or the skin. Common AID include systemic lupus erythematosus (SLE),

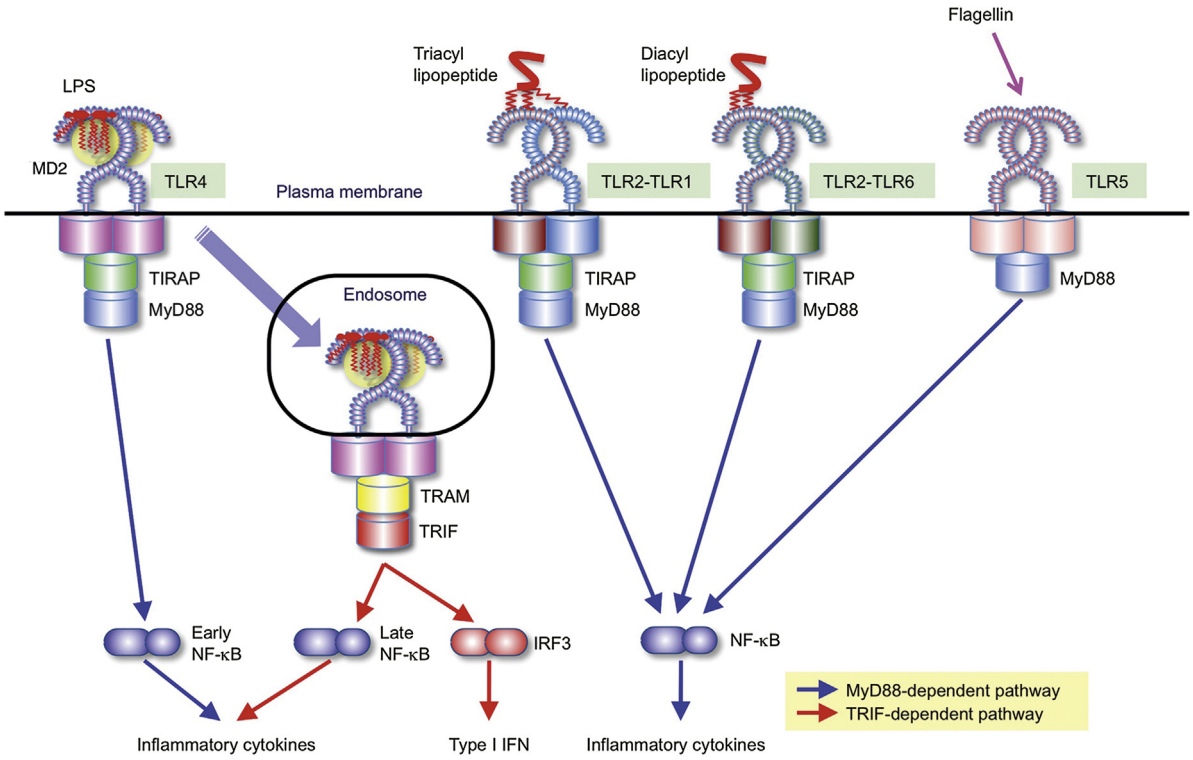

Fig. 2. PAMP recognition by cell surface TLRs and the subsequent signaling cascade. Reprinted by permission from Macmillan Publishers Ltd: Nature Immunology (Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on toll-like receptors. Nat Immunol, 11, 373–84), copyright (2010) [11].

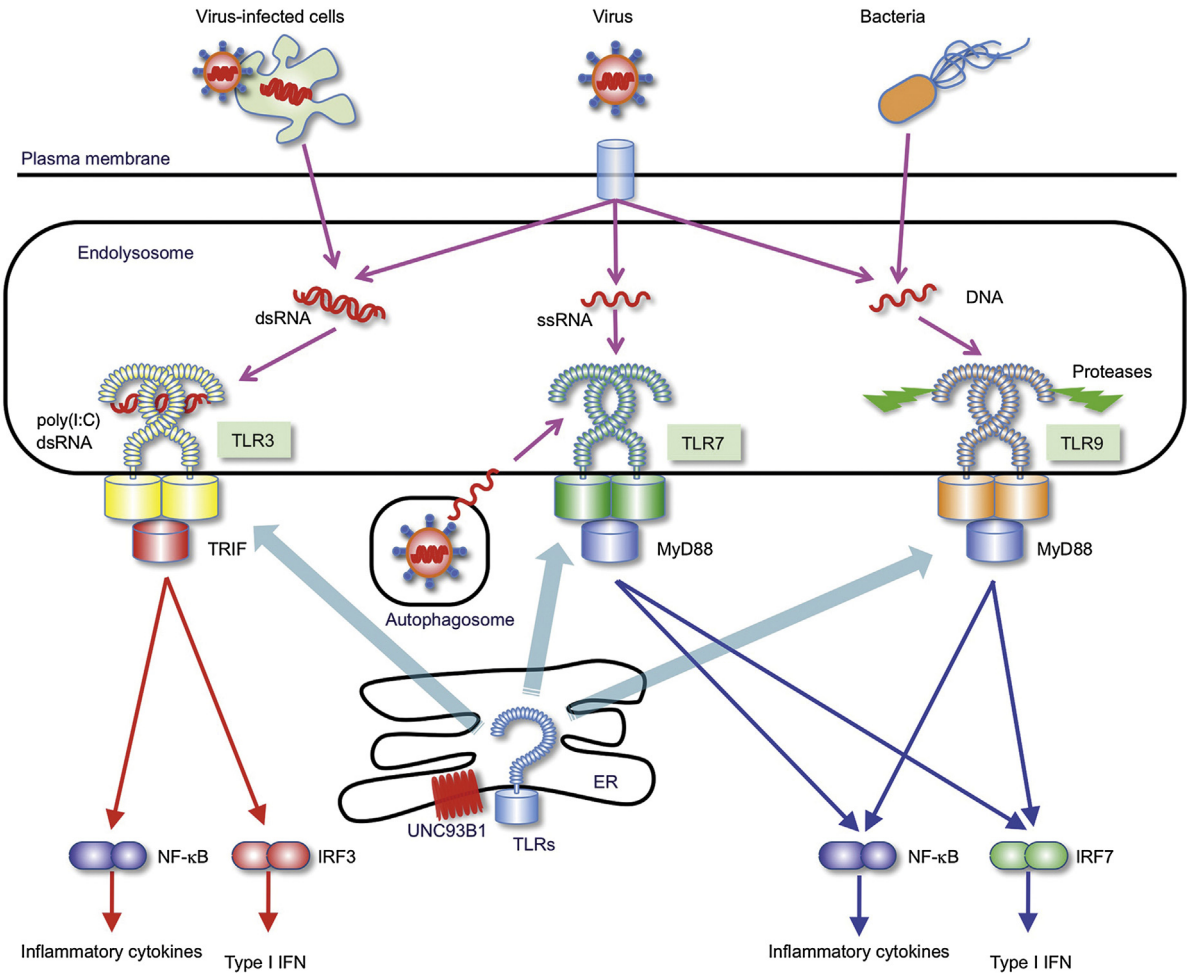

Fig. 3. PAMP recognition by intracellular TLRs and the subsequent signaling cascade. Reprinted by permission from Macmillan Publishers Ltd: Nature Immunology (Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on toll-like receptors. Nat Immunol, 11, 373–84), copyright (2010) [11].

type I (insulin-dependent) diabetes, Addison's disease, gluten-sensitive enteropathy (celiac disease), dermatomyositis, Grave's disease, Hashimoto's thyroiditis, multiple sclerosis (MS), myasthenia gravis (MG), pernicious anemia, reactive and rheumatoid arthritis (RA), and Sjögren's syndrome (SjS). There have been a small number of cases that have linked infectious diseases to AID (e.g., reactive arthritis, rheumatic fever, or vasculitis associated with hepatitis B virus infection). Background rates of AID are very low ranging from 0.8/100,000 person-years (PY) for autoimmune hemolytic anemia to 54.1/100,000 PY for thyroiditis–and vary by pre-specified criteria that included age, race, and gender [14]. For detailed classification of autoimmune etiology see Rose and Bona [15].

The risk of developing AID is complex, and multiple factors including genetic predisposition, environmental, and immune dysregulation may all play a role. These complex interrelationships should be considered when evaluating the potential causal relationships between autoimmune phenomena and/or disease and an adjuvant exposure.

Although association between vaccine adjuvant exposures and AIDs have been asserted in the literature and general media, there is no clear evidence of a causal association. For example, with some vaccinations, the most frequent assertions of AID (e.g., Guillain–Barré syndrome) relates to a relatively small number of patients, and in controlled studies of autoimmunity post immunization, there has been no evidence of an association found. For example, no evidence linking viral vaccines with type 1 diabetes, MS or inflammatory bowel disease (IBD) can be found [16].

4. Challenges in translation of preclinical models

4.1. Animal models

Animal models are an exploratory research tool, but is it possible to use them in a relevant manner to elucidate causes and mechanisms of AID? Could data from these models be used to support critical safety and efficacy assessments or drug/vaccine development decisions? Commonly studied animal models for evaluation of AID include experimental allergic encephalitis (EAE) for MS, collagen-induced arthritis (CIA) for RA, the (NZBxNZW) F1 mouse for SLE, and the non-obese diabetic (NOD) mouse for type I diabetes and SjS. There are also spontaneous (limited data) and inducible (CIA and EAE) nonhuman primate autoimmunity models. Animal models may provide insight into disease pathology because of certain physiological similarities with humans and their brief life span may allow the full disease onset to be studied within a short period of time. These animal models can be divided into two categories: models that are naturally susceptible to autoimmune disease (e.g., NOD) and those where AID is induced (e.g., CIA). The former allows for the testing of how adjuvants can accelerate or delay the onset of disease, while the later may test an adjuvant's ability to interfere with the induction/exacerbation mechanisms of AID. However, no

animal model can replicate the spectrum of a single human disease, and although helpful to study AID mechanisms, they may not be applicable to evaluate vaccine adjuvant-induced autoimmunity [17]. Immunological responses in animal models vary according to genetic influence and species-specific responses may be an obstacle in translating preclinical animal studies. In the case of adjuvant safety, it is critical to acknowledge important differences in dose, formulation, and route of administration between the products used to induce experimental autoimmune disorders in animals and the parallel scenarios for human exposure to adjuvants used in marketed vaccines.

The literature review by the HESI committee identified hundreds of papers showing various oil-based adjuvants that have been administered in animal models with either overt autoimmune phenotypes, or a propensity to develop AID [18,19]. While these models were generally established to look at the mechanisms behind autoimmunity and AID, rather than looking for any specific adjuvant effect, they do provide an indication of key experimental factors linked to adjuvant administration that could influence the potential for an autoimmune response. The autoimmunity models reviewed, e.g., arthritis, lupus, thyroiditis, glomerulonephritis, multiple sclerosis, diabetes, and pancreatitis, were developed primarily in small animals.

4.2. Formulations

Furthermore, differences in antigen release in vitro, immunogenicity in vivo, and tolerability between water-in-oil (w/o), water-in-oil-in-water (wow), and oil-in-water (o/w) emulsions are illustrated with examples from the literature [20]. Antigen release in vitro and immunogenicity in vivo correlated with o/w emulsions having the quickest in vitro release and the lowest immunogenicity, followed by wow, and w/o having the slowest antigen release and the highest immunogenicity. The oil used, droplet size, and ratio of oil to water, all affected the immunogenicity of antigen and the local tolerability. Considering the impact of physical/chemical properties of the various emulsion adjuvants on immunogenicity and tolerability, the human relevance of studies using pure squalene oil in rodents [21,22] draws into question as to whether the same conclusions seen in animals can be applied to humans.

4.3. Protocols

A closer look at protocols used to induce autoimmunity or AID in the animal models showed large doses were administered often via routes not relevant to human administration. Plus, in many cases, self-proteins are administered together with adjuvants in order to induce autoimmunity or AID [23]. Oil-in-water adjuvants in humans are dosed by the intramuscular route in a volume of 0.5 mL per vaccine dose. The oil and surfactant components of the adjuvants are in the range of 10–13 mg of the oil component (metabolizable squalene), and 2.5–5 mg of the surfactant components [24–26], per dose to humans with body weights from ~4 kg (infant) to 60 kg (adult). In rodent studies with complete Freund's (CFA) and incomplete Freund's (IFA) adjuvants, pristane (tetramethylpentadecane), mineral oils, and hydrocarbons (all non- or slowly metabolized), animals were dosed via intradermal injection or into the peritoneal cavity. In studies that showed pristane, IFA, and squalene oil induced arthritis in rats [21,22,27] and lupus associated autoantibodies in mice [28], with associated cytokines [29,30], the dose volumes ranged from 0.1 to 0.5 mL in mice and rats. For example, on a body weight basis, a dose of 0.5 mL to a 0.03 kg mouse is equivalent to injecting 200 mL into a 60 kg human. The differences in oils, formulations, dose levels, and route of administration, together with known and potential species differences in immune responses, means caution is needed in extrapolating the results in these animal studies to humans.

4.4. Focus on translation for TLR agonist adjuvants

Published studies on the link between TLR and AID illustrate the complexity of translating preclinical AID-related animal data to the patient. For many AID, there are conflicting data regarding the role (and identity) of TLRs, and data either indicating an exacerbation or amelioration of disease. Table 1 provides an overview of the various TLRs that may be involved in AID, and the similarities and differences between human and in animals. The variability between humans and animals is likely a reflection of the species-specific differences of TLRs in tissue and cellular distribution, expression, ligand recognition, and subsequent cascade pathways. From a preclinical perspective, studies often used either transgenic or induced disease models, including $TLR^{-/-}$ or myeloid differentiation primary response gene 88 $(MyD88)^{-/-}$ knockout animals [7,31–33]. Mouse gene knock-out studies show that some TLRs are essential for the development of autoimmunity in prone strains, although not necessarily causative. Likewise, administration of TLR agonists to animal models can exacerbate disease (e.g., imiquimod TLR7 can induce diabetes in NOD mice) [34]. However, data are inconsistent, and there are many examples where TLR agonists do not induce or exacerbate autoimmunity but can protect against autoimmunity. Few papers have addressed the differences in TLR distribution between animals and humans. The studies reviewed did not address the role that a TLR agonist may have on the disease when used at low doses with episodic administration that would be consistent with a vaccine adjuvant. This reiterates the previous point about translatable data based on dosing differences, and how this confounds the issue of relating to the clinic.

4.5. Biomarker discovery key to future AID research

While animal models are used to investigate the molecular mechanisms of autoimmunity, the identification of biomarkers may be useful in predicting and monitoring AID onset, progression, and its potential linkage to adjuvant exposure. Biomarkers are characteristics that are objectively measured and evaluated as indicators of normal and pathogenic biological processes [35]. One of the goals of research on AID-linked biomarkers is to identity markers that fluctuate with homeostasis rupture, tolerance breaking disease development and severity, and normalize following successful therapy [36].

A contemporary focus for AID is the role that regulatory T cells (Tregs) play in autoimmunity, self-tolerance, immune homeostasis, and suppression of immunity to pathogens/tumors. Tregs are capable of suppressing disease. For example, when a conserved Hsp70-epitope (B29) present in murine MHC Class II was transferred, it induced Hsp specific $CD4^+$ $CD25^+$ $Foxp3^+$ T cells and suppressed proteoglycan-induced arthritis (PGIA) in mice [37]. Transferred cells exhibited a stable phenotype and were found in joints and draining lymph nodes up to 2 months after transfer [37]. Furthermore, the relationship between TLR agonists and their ability to modulate immune function including Tregs response, highlight the potential this subset of cells may play in understanding the role between adjuvants and autoimmunity [38].

Another area of focus in biomarker discovery resides in micro-ribonucleic acids (microRNAs or miRNAs). They are small, noncoding RNAs, shown to be critical regulators of host genome expression at the post-transcriptional level. Recent developments in diverse miRNA profiling may enable the identification of specific miRNA as novel diagnostic and predictive markers for various diseases [39]. miRNAs bind to the 3′ untranslated region (UTR) of target messenger ribonucleic acid (mRNA) to inhibit translation or induce

Table 1
Summary of TLRs that have been suggested to play a role in various autoimmune diseases in clinical and nonclinical studies reported in the literature [31–36].

	Suggested involvement of TLRs in various autoimmune diseases									
	Diabetes		SLE		RA		EAE/MS		SjS	
	Human	Animal	Human	Animal	Human	Animal	Human	Animal	Human	Animal
TLR1										
TLR2	X	X			X	X	X	X	X	
TLR3	X	X			X				X	X
TLR4	X	X			X	X	X	X	X	
TLR7/8		X	X	X					X	
TLR9		X	X	X				X	X	
MyD88		X				X		X		

mRNA degradation and thereby downregulate genes. Various cells of the innate and adaptive immune system express distinct patterns of miRNAs and may regulate their functions. mRNA expression patterns are also altered in many diseases including cancer, AID, inflammatory disorders, infectious diseases and allergies. More specifically, miRNA dysregulation has been noted in SLE, rheumatoid arthritis, multiple sclerosis, scleroderma, etc. with expression patterns that may be influenced by adjuvants, and may hold potential as highly conserved and readily accessible biomarkers for the progression of AID [39].

Finally, within the tetramethylpentadecane (pristane)-induced lupus murine model of SLE, lies possible biomarkers and pathways for future studies of AID. The model is characterized by lupus-related autoantibodies, including the production of antinuclear antibodies, anti-dsDNA, small nuclear ribonucleic particles (snRNPs), Argonaute2/Su and ribosomal P autoantibodies and glomerulonephritis in non-autoimmune-prone strains of mice [40]. In this pristane model, lupus-like autoimmunity is dependent on interferon (IFN)-g, interleukin (IL)-6 and IL-12, and is also TLR7 and type I IFN receptor-dependent. Type I IFN has emerged as an important pathogenic pathway in several systemic AID. However, in the pristane model, type I IFN is mainly produced by Ly6c high-immature monocytes, as compared with humans, where it is produced by plasmacytoid dendritic cells [40]. Future work is, therefore, needed to decipher the importance of not only type I IFN but also in the cells that produce it to help guide work as a potential biomarker when studying AID.

4.6. Potential confounding factors in assessing adjuvant and AID linkages

Various factors are implicated in the pathogenesis of immune-mediated diseases. Direct influences such as activation of the innate immune system, by infectious agents, silicone, aluminum salts (i.e., those used as vaccine adjuvants) as well as indirect influences such as the length of time between an adjuvant exposure and before manifestation of symptoms, could all play a role. In recent years, four conditions: siliconosis, GWS, MMF, and post-vaccination adverse events have been hypothesized by some to be related to exposure to "immune activators". A shared/similar complex of signs and symptoms has been given the name Autoimmune/inflammatory Syndrome Induced by Adjuvants (ASIA) [3,41,42]. While ASIA is not recognized as an official diagnosis, and while there is no evidence of causality between ASIA and vaccines nor adjuvants, considering timeframe after initial exposure to "immune activators" could be applied as it is an important aspect to capture when assessing the scope and nature of potential causal factors leading to AID. When the time between an observed effect and exposure to a vaccine adjuvant spans the course of many years, this extended time period increases the variables potentially contributing to the onset of AID and makes it difficult to link to rare events, e.g., an AID, to a specific exposure. For example, some studies have suggested that exposure to components of silicone breast implants may lead to autoimmune/ASIA-like symptoms with a median latency period of 6 years [43], which has prompted the FDA to request longer-term follow-up studies. These examples highlight the importance of considering appropriate timeframes for safety monitoring after inoculation, and emphasizes yet another variable (time) that only further complicates the difficulty in determining the root cause in AID and if vaccine adjuvants can play a role.

Genetic predisposition also contributes to the complexity of determining a cause of AID. Narcolepsy is an immune mediated syndrome/AID, associated with a loss of hypocretin producing neurons in the hypothalamus, and is rarely apparent before adolescence. The results of the epidemiological research were shared confirming there was an increase in narcolepsy cases in the Swedish and Finnish cohort vaccinated with Pandemrix® against H1N1 back in 2009, particularly evident in young children [44]. Of these subjects, 47 post vaccination narcolepsy subjects were analyzed and shown to carry the narcolepsy related human leukocyte antigen (HLA) type DQB1*0602, indicating that those affected were genetically susceptible. Epidemiological data in four other EU countries did not show the same relationship between vaccination and narcolepsy [45]. Adding to the complexity of the issue, the group also looked at Canadian epidemiology data of individuals that had received Arepanrix® (GlaxoSmithKline, Canada), which contains H1N1 antigen produced in Canada rather than Europe, plus AS03. The background presence of the HLA type DQB1*0602 in the Canadian population is similar, albeit slightly lower at 20% compared to 26% in Finland. The Canadian epidemiology data showed no increase in narcolepsy and inhibition experiments of antibodies to H1N1 viral antigens showed differences in the antigenic epitopes between Arepanrix® and Pandemrix®. Since the workshop, other countries have investigated whether a link between vaccination and the onset of narcolepsy exists [44,46,47]. Some studies have found an increased incidence of narcolepsy in adolescence upon vaccination, and upon more in-depth analysis it appears that the narcolepsy develops mainly in those who possess the DQB1*0602 allele [47]. Furthermore, with a lack of an association observed outside of European countries, a hypothesis was proposed based on slight differences in the antigen formulations due to manufacturing process. This could result in immunologically important differences in the H1N1 antigen substance between Pandemrix® and Arepanrix®. The hypothesis proposed was that it is not the adjuvant per se that is responsible for the relationship, but rather detergents (polysorbate 80) present in Pandemrix® antigen but not in Arepanrix® that could modify the H1N1 viral antigen epitopes and their immunogenicity, and that molecular mimicry might be playing a role. The immunogenetic mechanism behind how these vaccines and their various components may have contributed to narcolepsy remain to be determined [48], and further studies of these differences may be needed to determine the cause behind this association for more informed future risk assessment.

5. Discussion

Vaccines are one of the most successful medical breakthroughs of modern day medicine with an extremely good safety record. However, the potential biological interplay between vaccine adjuvants and a patient's immune system has raised questions as to whether some incidences of AID can be causally linked to adjuvant exposures. Specifically, do some adjuvants have an intrinsic risk of autoimmune diseases or do they just stimulate the autoimmunity risk of certain antigens? The causes of AID are multifactorial (genetics, environment, age, etc.), and may be dependent/influenced by the type and magnitude of immune/inflammation response triggered under some circumstances in the host. Currently, many gaps in knowledge still exist and future work is needed to better understand and definitively address this question.

The discussions at the 2012 workshop, in synergy with the literature analysis conducted by the HESI committee, concluded that biologically active adjuvants could in theory increase the risk of autoimmunity by increasing the inflammation/immune response, but available pharmacovigilance and clinical databases provide no evidence of an increased risk of autoimmunity [49]. Although the number of marketed adjuvants is currently limited, there is no evidence as to whether a specific type of adjuvant would increase the risk of an AID.

Confounding aspects of potential genetic predisposition, as in the case of the Pandemrix®/narcolepsy data as well as variation in biological mechanism of action of adjuvants add to the complexity of this issue. Adjuvants are varied in their modus operandi, and/or physical properties, e.g., oil-based adjuvants, which act as a local depot, versus specific immunomodulators that target specific TLRs. Improved characterization of whether a specific adjuvant triggers a "broad immune reaction" or more restricted and defined pathways would aid in assessing biological mechanisms that could be causally linked to AID. Enhanced understanding of the native immune system function in reaction to specific adjuvant exposures, differences in mode of action, potency, dose relationships, models, routes, and temporality remain available opportunities to improve basic knowledge in this field. In addition to nonclinical studies, targeted post market analysis could assist in identifying potentially relevant mechanistic pathways or patient populations most at risk of AID.

From a regulatory perspective, it would be helpful to build consensus on key risk biological factors of AID and incorporate those into scientific justification for regulatory approval or dismissal of a vaccine. Examples of targeted risk management approaches in drug development include the use of a high throughput screening approach to select adjuvants targeting specific molecules that do not induce systemic activation and have a short half-life, or the selection of adjuvants known to be local acting and transient.

Available post-marketing data continues to show adjuvants have a good safety profile. New initiatives such as the IMI BIOVACSAFE project have been recently established to collect additional post-market data and scientific understanding related to the safety of vaccination. This program aims to contribute to the current knowledge base through clinical trials to define biomarkers, generation of databases and guidelines, etc. It is critical to remember that any potential relationships between vaccination and rare/very rare adverse events in humans can only be detected in large epidemiological studies following vaccination. However, even in large scale studies, the potential for multiple confounders and exposures over an extended temporal period make it challenging to scientifically establish causal linkages. While only one piece of a much larger puzzle, epidemiological data may help guide more sound decision making processes regarding vaccines by both researchers and indirectly by the general public. Along with routine post marketing pharmacovigilance of vaccines, large scale epidemiological studies may enhance the ability to adjust risk/benefit assessments to account for regional differences in disease epidemiology and associated morbidity/mortality, as well as differences in genetics, diet, nutrition, parasite burden, natural background rates of AID, etc. [50].

6. Conclusions

Autoimmune diseases are complex and multi-factorial disorders, and changes in autoreactive T and/or B cells homeostasis contribute to the development of autoimmunity. Such changes could, in theory, be induced by non-adjuvanted vaccines but might be even more frequent and plausible with adjuvanted vaccines; therefore, studying autoimmunity risk is important to be considered in the development of adjuvants. So far, there is no compelling clinical evidence that adjuvants are causally related to any autoimmune phenomena.

In animals, adjuvants (e.g., IFA and CFA), or components of adjuvants (e.g., oils) are being used to induce autoimmunity disease models in combination with auto-antigens or based on genetic sensitivity. The animal models used in autoimmunity research are not adequate to be used for risk assessment for potential AID for new adjuvanted vaccines in this respect. As shown above, there is no evidence of a link between adjuvanted vaccines and AID so far, but considering there are so many contributing factors in AID, it is challenging and complex to completely rule out a theoretical risk.

The future should focus on understanding the mode of action of adjuvants and mechanistic pathways of AID, which together may provide future biomarkers related to autoimmune diseases that may help provide better understanding and risk management.

Conflicts of interests statement

Jan Willem van der Laan declares no conflicts of interests, except for having received financial travel support from HESI for this project.

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