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REVIEW ARTICLE

Comparative Safety of Vaccine Adjuvants: A Summary of Current Evidence and Future Needs

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Abstract Use of highly pure antigens to improve vaccine safety has led to reduced vaccine immunogenicity and efficacy. This has led to the need to use adjuvants to improve vaccine immunogenicity. The ideal adjuvant should maximize vaccine immunogenicity without compromising tolerability or safety. Unfortunately, adjuvant research has lagged behind other vaccine areas such as antigen discovery, with the consequence that only a very limited number of adjuvants based on aluminium salts, monophosphoryl lipid A and oil emulsions are currently approved for human use. Recent strategic initiatives to support adjuvant development by the National Institutes of Health should translate into greater adjuvant choices in the future. Mechanistic studies have been valuable for better understanding of adjuvant action, but mechanisms of adjuvant toxicity are less well understood. The inflammatory or danger-signal model of adjuvant action implies that increased vaccine reactogenicity is the inevitable price for improved immunogenicity. Hence, adjuvant reactogenicity may be avoidable only if it is possible to separate inflammation from adjuvant action. The biggest remaining challenge in the adjuvant field is to decipher the potential relationship between adjuvants and rare vaccine adverse reactions, such as narcolepsy, macrophagic myofasciitis or Alzheimer's disease. While existing adjuvants based on aluminium salts have a strong safety record, there are ongoing needs for new adjuvants and more intensive research into adjuvants and their effects.

Key Points

The existing human vaccine adjuvants have a high level of safety.

The relationship between specific adjuvants and rare adverse reactions, such as narcolepsy or macrophagic myofasciitis, remains to be resolved.

More research is needed into adjuvants and how they work.

1 Introduction

Traditional vaccines, such as whole-cell pertussis vaccines [1] or whole-virus influenza vaccines [2], are highly immunogenic, albeit at the price of significant local and systemic reactogenicity. To reduce reactogenicity, modern approaches incorporate split, subunit or recombinant antigens from which reactogenic contaminants such as lipopolysaccharide, DNA and RNA are removed. As highlighted by acellular pertussis vaccines, the improved safety of subunit vaccines comes at the price of reduced immunogenicity [1]. The move to subunit vaccines has also resulted, in some cases, in a shift from a balanced T helper (T_h)-1 and T_h2 vaccine response to a more T_h2-biased response [1]. While reversion to whole-cell vaccine approaches could improve immunogenicity [3], it would

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also recreate excess reactogenicity. Incomplete virus splitting during manufacture was found to be responsible for excess hospitalizations for febrile convulsions caused by a recently withdrawn paediatric inactivated influenza vaccine, thereby highlighting this trade-off [4]. Thus, there is a close relationship between vaccine immunogenicity and reactogenicity arising from contaminants such as lipopolysaccharide, DNA and RNA contained in whole-cell vaccines, which act as both inbuilt adjuvants and reactogens [5]. Both properties reflect the ability of these contaminants to induce inflammation via activation of innate immune receptors, with the consequence that their adjuvant action and reactogenicity are inseparable, with dose-limiting reactions including local swelling and pain plus systemic fever and malaise [6]. Adjuvant reactogenicity can thereby be regarded as a dose-dependent phenomenon reflecting local tissue damage and systemic inflammation induced by activation of innate immune receptors [7]. Should an adjuvant induce excess reactogenicity in a vaccine and the problem be unresolvable by lowering the dose of the reactogenic component, then the combined adjuvanted vaccine formulation could be regarded as potentially unsafe, although even this is context dependent; for example, the withdrawn paediatric influenza vaccine mentioned above was still regarded as safe and remained approved for use in older individuals not at risk of febrile convulsions [4], thereby highlighting the extreme complexity of vaccine safety assessment.

An even greater challenge than adjuvant safety assessment, which focuses on the chance of immediate adverse effects (pain, swelling, fever), is the assessment of adjuvant risk, which refers to the relative possibility of development of any adjuvant-associated problem over the life of the individual being immunized. The most challenging aspect of assessment of adjuvant risk is determination of the basis of reported associations between use of vaccines containing specific adjuvants and development of rare autoimmune or chronic degenerative disorders—for example, associations between use of squalene emulsion–adjuvanted vaccines and narcolepsy [8] or Gulf War syndrome [9] or between use of aluminium adjuvants and the chronic granulomatous inflammation macrophagic myofasciitis (MMF) [10] or Alzheimer's disease [11]. Such assessments are made exceedingly difficult by the paucity of data, the inability to perform controlled studies in humans to prove causation and the potentially extremely long time period between immunization and onset of symptoms. Hence, causation in the vast majority of such cases has never been established, leaving uncertainty as to whether any of these associations might be real or are just linked by chance. There is thus a great need for better research tools with which to probe the nature of such associations. This review focuses on current adjuvants that have at least reached the stage of human clinical trial testing to identify what is known and what is still to be learned about all aspects of adjuvant safety.

2 Literature Search Methods

Articles were identified in PubMed, using the keyword terms 'vaccine adjuvant safety' and 'vaccine adjuvant toxicity', with a focus on articles published in the last 10 years. Only human adjuvants for which there were published clinical trial data were included.

3 Adjuvant-Associated Local Toxicity

Local adjuvant-associated side effects range from mild injection site pain, tenderness, redness, inflammation and swelling at one end of the spectrum, to formation of granulomas, sterile abscesses, lymphadenopathy and chronic skin ulceration at the other end (reviewed in reference [6]). Local vaccine side effects may reflect direct chemical irritation due to a non-physiological pH, osmolarity, salt concentrations or direct cell toxicity. Such local irritant effects are typically associated with immediate and severe injection site pain, followed by an inflammatory response triggered by the tissue damage. Examples of adjuvants that induce local reactogenicity include saponins (e.g. Quil A, QS21, immune-stimulatory complexes [ISCOMs], Iscomatrix®) and oil emulsions (e.g. complete Freund's adjuvant [CFA], incomplete Freund's adjuvant [IFA], Montanide®, MF59, AS03) [7]. Immediate reactions are likely to reflect irritation and inflammation induced by the adjuvant component itself but, if delayed by 24–48 h, may reflect an excessive delayed-type hypersensitivity (DTH) response against a vaccine component in an already primed individual [12]. Local reactogenicity is not life threatening but could still lead to significant morbidity—for example, at worst, a sterile abscess needing surgical drainage or skin ulceration requiring skin grafting. Some local reactions, such as severe pain, while not directly damaging to physical health, may still have a strong negative impact on the public's perception of the risk–benefits of immunization and hence should be avoided on these grounds.

4 Adjuvant-Associated Systemic Toxicity

Systemic reactogenicity includes symptoms such as fever, headache, malaise, nausea, diarrhoea, arthralgia, myalgia and lethargy. These largely reflect adjuvant-associated innate immune activation and downstream inflammation.

Adjuvants that strongly activate innate immune receptors—for example, adjuvants based on pathogen-associated molecular patterns (PAMPs)—may thereby be most prone to systemic reactogenicity. This includes toll-like receptor (TLR) ligands, such as monophosphoryl lipid A (MPL), flagellin, lipoarabinomannan, peptidoglycan or acylated lipoprotein (reviewed in reference [7]). Systemic reactogenicity is also an issue for adjuvants that induce local tissue damage (e.g. oil emulsions and saponins), as this results in release of endogenous damage-associated molecular patterns (DAMPs) that activate innate immune receptors and induce inflammation [13]. Typically, such inflammation-associated adjuvant reactogenicity would be expected to settle once the innate immune response subsides, but may potentially last for up to several weeks post-immunization.

At the serious end of the systemic toxicity spectrum is the potential for rare immunological toxicities resulting from aberrant immune activation driven by the adjuvant. This includes problems such as immune bias—for example, the eosinophilia, allergic reactions and anaphylaxis caused by T_h2 bias imparted by aluminium adjuvants [14]. It also includes the potential for adjuvants to induce chronic immune activation and inflammation that does not settle post-immunization. An example would be the syndrome of MMF, whereby long-lasting tissue depots of an aluminium adjuvant have been linked to symptoms of chronic fatigue syndrome [15], although, as discussed later, this association has been questioned by bodies such as the World Health Organization (WHO) Global Advisory Committee on Vaccine Safety (GACVS) [16].

Finally, there is the risk that an adjuvant may either act as the trigger or increase the likelihood of a vaccine causing an autoimmune disease. An example is the ability of inflammatory oil emulsion adjuvants to induce adjuvant arthritis in genetically susceptible animal models [17]. Adjuvant-associated immune dysregulation and the potential to cause autoimmune disorders represent the most widely debated aspect of adjuvant risk assessment. Spontaneous autoimmune conditions affect only a small number of genetically susceptible individuals in the general population [18]. Hence, even if a vaccine/adjuvant combination was thought to cause autoimmune disease, this would be very hard to prove, particularly if everyone in the population had received the vaccine.

Also included within the spectrum of potential adjuvant systemic toxicity is the potential for chronic organ toxicity of the compounds themselves. For example, aluminium or oil emulsions can form long-term tissue depots, and this has been postulated to cause chronic toxic effects. However, detection of chronic toxicity and determination of any causal relationship can be extremely difficult, if not impossible, because of the long delays between disease onset and environmental exposure—for example, immunization—which may have occurred decades later.

5 Making Sense of Adjuvant-Associated Adverse Events

There have been periodic reports highlighting potential temporal associations between immunization with adjuvanted vaccines and the occurrence of adverse events. Needless to say, an association may not represent causation, which needs to be established in each individual case. Examples of such associations include reports of MMF in patients previously receiving vaccines containing an aluminium adjuvant [15], and narcolepsy in children immunized with pandemic influenza vaccine containing a squalene emulsion adjuvant [19, 20]. Notably, the incidence of reported adverse events within the context of the total immunized population is often extremely small. Thus, the vaccine-attributable risk of developing narcolepsy was estimated at 1:16,000 vaccinated Finnish 4- to 19-year-olds [19], but, if expressed as a ratio of the total immunized Finnish population irrespective of age, it would be closer to 1:100,000. Although the prevalence of MMF is not known, the Henri Mondor Hospital, which identified and specializes in this syndrome, reported that 600 cases were diagnosed over a 10-year period [21], but this needs to be put into the perspective of the total French population, numbering over 64 million. Hence, the media and anti-vaccine lobby groups are often biased towards reporting and focusing on rare vaccine adverse effects while generally ignoring the extremely large denominator of the total immunized population from which such cases are drawn.

The problem of rare vaccine adverse events from a regulatory perspective is that it is often extremely difficult, if not impossible, to ever establish proof of causality. Hence, the best that can be done is to assess whether causation is plausible or not, using knowledge of a particular vaccine's or adjuvant's mechanism of actions. Even in situations where causation is held to be probable, such as in the case of the specific pandemic influenza vaccine and childhood narcolepsy, it is still not possible to identify the responsible component(s) of the vaccine, such as the relative contribution of the antigen or adjuvant, if present. While animal models might seem to be the best solution for testing causation of adverse reactions, direct extrapolation from such models is difficult, with no guarantee that they accurately reflect the human context. Hence, all vaccine adjuvant safety assessments are subjective in nature. This indicates an urgent need for more research into methods to better assess adjuvant safety and to investigate rare adverse events that may possibly be vaccine and/or adjuvant related. To better understand these adjuvant safety issues, it is

useful to individually examine each of the adjuvants for which human data are available.

6 Aluminium Adjuvants

After almost a century, aluminium salts maintain their dominance as adjuvants in human vaccines. This reflects the fact that aluminium adjuvants are extremely effective at enhancing antibody responses, are well tolerated, do not cause pyrexia and have the strongest safety record of any human adjuvants [7]. Hence, aluminium adjuvants remain the gold standard against which all new adjuvants need to be compared, and any new adjuvant must prove that it provides better protection, tolerability or safety, or preferably all of these, when compared with an aluminium adjuvant. This has proved extremely hard to achieve, explaining aluminium's ongoing dominance. Aluminium's action was initially thought to be due to local antigen depot effects, but the situation is now recognized as more complex, with NALP3-mediated inflammasome activation, interleukin (IL)-1 production, cell necrosis, DNA release, and activation of DAMP and PAMP receptors all proposed to contribute to its action [22–24]. Other metal salts (including iron and beryllium [25, 26]) that also induce lysosomal rupture and phagocyte cell death share alum's adjuvant activity [27, 28], suggesting that induction of cell death is a common feature of adjuvants based on metal salts [29]. The propensity to kill phagocytes may help explain alum's inability to induce robust cellular immunity, as live antigen-presenting cells are required for efficient antigen cross-presentation to CD8 T cells [30]. Aluminium adjuvants suffer from a number of minor toxicities, which are potentially explained by their mechanism of action. For example, aluminium induces injection site pain and tenderness [31], which may reflect cell necrosis and induction of inflammasome activation and IL-1 production [32]. Aluminium salts' propensity to induce cell death and inflammasome activation could also explain why some subjects develop persistent lumps and granulomas at the injection site [31]. Aluminium adjuvants also induce contact dermatitis to aluminium in a fraction of immunized subjects [33]. Aluminium adjuvant-containing vaccines can cause post-immunization headache, arthralgia and myalgia, which could reflect alum's propensity to induce IL-1, with IL-1 administration to human subjects reproducing these symptoms [34]. On the positive side, aluminium adjuvants rarely cause severe local reactions and are not normally associated with systemic inflammatory problems, such as pyrexia.

A potential issue is aluminium adjuvants' propensity to induce T_h2 immune bias with increased eosinophil and immunoglobulin (Ig) E production, thereby increasing the risk of allergy and anaphylaxis [14, 35–37]. This phenomenon can be reproduced in a murine ovalbumin sensitization model, where sensitization by repeated immunization with ovalbumin plus an aluminium adjuvant induces susceptibility to allergic asthma and lethal anaphylaxis upon subsequent ovalbumin re-exposure. Aluminium adjuvant-associated allergic sensitization can be prevented in IL-4 receptor knockout mice or by administration of interferon (IFN)-γ [38] or CpG-containing oligonucleotides (CpG) [39], indicating that the allergy sensitization is due to aluminium adjuvants' excessive T_h2 bias. T_h2 immune bias may be a particular problem in children who are already genetically biased towards excessive T_h2 immune responses and allergies [40]. Excess T_h2 bias is a particular problem for vaccines against viruses such as respiratory syncytial virus (RSV) or severe acute respiratory syndrome (SARS) coronavirus, where aluminium-adjuvanted vaccines have been shown to increase the risk of lung eosinophilic immunopathology upon virus infection [41, 42]. This mechanism is thought to have contributed to the deaths of children administered an experimental formalin-inactivated aluminium-adjuvanted RSV vaccine after they became infected by RSV [43]. In a mouse model, SARS lung eosinophilic immunopathology could be prevented if animals were immunized with a SARS antigen in combination with a non-T_h2 polarizing delta inulin adjuvant in place of the aluminium adjuvant [42]. This suggests that adjuvants that do not share the T_h2 bias of aluminium would be safer for use with vaccines against pathogens such as RSV or SARS, where an excessive T_h2 bias could otherwise result in detrimental immune responses in response to viral infection.

In cats, dogs and ferrets, aluminium adjuvants cause local chronic granulomatous lesions, which can progress to malignant fibrosarcomas [44]. Why similar tumours are not seen in aluminium-immunized humans is not known. However, aluminium adjuvants in humans have been reported to cause MMF [15, 45]. Symptoms of MMF syndrome include myalgia, arthralgia, marked asthenia, muscle weakness and fever [15, 45]. Abnormal findings in MMF patients include elevated creatine kinase levels and an elevated erythrocyte sedimentation rate plus a myopathic electromyograph. Muscle biopsies from MMF patients have shown infiltration by sheets of macrophages with granular periodic-acid-Schiff-positive content and with aluminium deposits being demonstrated in the lesions by energy dispersive X-ray microanalysis [46]. The syndrome is hypothesized to be due to the persistence of vaccine-derived aluminium tissue deposits, resulting in a perpetual cycle of macrophage ingestion of alum, intracellular lysosomal rupture, phagocyte death and ingestion of alum-containing dead phagocytes by newly recruited macrophages, leading to a chronic inflammatory reaction

[46], although the link between the muscular MMF lesion and the described MMF symptoms remains a contentious area of debate [16]. Some MMF patients have been reported to demonstrate neurological manifestations resembling multiple sclerosis [47]. Since the original description of MMF in 1993, more than 600 cases have been diagnosed in France [21], with sporadic case reports from other countries [10]. These numbers need to be put into the perspective of the total immunized French population, which likely numbers over 64 million. Currently, the only treatment is surgical resection of the aluminium at the original muscle injection site. Interestingly, the symptoms of MMF closely resemble those of Muckle–Wells syndrome, which is caused by inherited mutations that result in constitutive inflammasome activation [48]. As aluminium adjuvants are now known to also induce inflammasome activation [32], it is possible to speculate that MMF might occur in individuals who are also susceptible to chronic inflammasome activation. If so, MMF could essentially represent a low-grade acquired form of Muckle–Wells syndrome—a plausible mechanism, given aluminium's known molecular actions. While the GACVS accepts that MMF is a lesion containing aluminium salts identified by histopathological examination found at the site of previous vaccination with an aluminium-containing vaccine, it has concluded "that there is no evidence to suggest a resulting clinical illness or disease" [16]. The GACVS recommended that to further understand MMF, additional research studies need to be undertaken to evaluate clinical, epidemiological, immunological and basic science aspects of this disease [16].

Antiphospholipid syndrome (APS) is an autoimmune disorder manifested by elevated titres of antiphospholipid antibodies, arterial and venous thromboembolic events, recurrent spontaneous abortions and thrombocytopenia [49]. Tetanus toxoid hyper-immunization is able to reproduce APS in mice, which correlates with the induction of cross-reactive low-affinity anti-β(2) glycoprotein I [anti-β(2)GPI] antibodies [50]. In C57BL/6 mice, tetanus toxoid hyper-immunization with aluminium adjuvants but not glycerol resulted in an increase in low-affinity anti-β(2)GPI IgG antibodies and a decrease in maternal fecundity consistent with the aluminium adjuvant being a critical component in this model of APS [50]. To what extent aluminium-adjuvanted tetanus vaccines might contribute to the rare human cases of APS is not known.

High aluminium levels in the body predominantly affect the brain and bone tissues, causing a fatal neurological syndrome and dialysis-associated dementia [51]. Cerebral aluminium accumulation has also been observed in Alzheimer's disease [52]. Aluminium exposure through paediatric parenteral nutrition has been shown to impair bone mineralization and to delay neurological development [11]. While low doses of aluminium are renally excreted, under conditions of reduced renal function, aluminium can accumulate in the body and become toxic. Furthermore, environmental aluminium loads are greater than in the past, to which the additional load of a multiplicity of alum-based vaccines must be added [53]. Research using aluminium oxyhydroxide particles labelled with fluorescent functionalized nanodiamonds confirmed that 21 days post-immunization, the brains of mice contained, on average, 15 solid aluminium particles, and parallel studies in vitro confirmed that aluminium adjuvant was toxic to neuronal cell cultures [54]. This is consistent with mouse studies showing neurotoxic effects—including neural apoptosis and both motor and behavioural deficits—of an aluminium adjuvant [55, 56]. What contribution cumulative doses of aluminium adjuvants might make to human chronic disorders, such as Alzheimer's disease [11, 57] or chronic bone disease [58], is simply unknown and warrants more thorough investigation. In particular, parenterally administered aluminium particles can behave very differently from soluble aluminium in the body, as these particles can be transported to unusual sites, such as the brain, after phagocytosis [54], whereas soluble aluminium ions on which current exposure limits are set are easily excreted by the kidneys [11]. The GACVS has characterized studies suggesting adverse effects of aluminium adjuvants in humans as 'seriously flawed' but unfortunately has failed to comment on the validity or otherwise of the animal toxicology data and their potential relevance to human immunization [59]. Any adverse finding against alum adjuvants would clearly have serious ramifications [60] in view of the current lack of adjuvant alternatives and the overwhelming public health benefit of current vaccines containing aluminium adjuvants, particularly in developing countries, where deaths from vaccine-preventable infectious diseases remain high. Hence, a very high standard of proof is required before any claim of aluminium adjuvant toxicity could be endorsed, and the risk–benefit of inclusion of alum adjuvants in vaccines, in the absence of a viable alternative, remains overwhelmingly positive.

It is important to note that not all forms of aluminium adjuvants are necessarily the same. For example, reports of MMF have been largely linked to use of aluminium hydroxide, which may reflect the fact that an interstitial fluid containing organic acids with an alpha-hydroxy carboxylic acid able to chelate aluminium reacted more readily with aluminium phosphate than with aluminium hydroxide, with the result that three times as much aluminium is excreted from rabbits vaccinated with aluminium phosphate, with aluminium hydroxide having a much longer tissue residence time [61] as was also suggested by a monkey study in which histopathological lesions similar to human MMF lesions were still present

12 months after aluminium hydroxide–adjuvanted vaccine administration, versus 3 months for aluminium phosphate [62]. In that study, none of the 24 immunized monkeys developed clinical symptoms despite the presence of MMF-like lesions [62], although this still does not exclude the possibility that clinical symptoms are associated with MMF lesions in some human subjects who are genetically or otherwise predisposed to developing this rare syndrome.

7 Oil Emulsion Adjuvants

This class of adjuvants includes a wide spectrum of oil-in-water or water-in-oil emulsions. Water-in-oil adjuvants, such as CFA, rank as the most reactogenic of known adjuvants and hence are unsuitable for human use. Oil-in-water emulsions have lower although still significant reactogenicity and include the squalene-based adjuvants (such as MF59, AS02 and AS03) [7], the various Montanide® oil adjuvants and the liposomal adjuvant CAF01, which is composed of a cationic liposome vehicle (dimethyldioctadecyl-ammonium [DDA]) stabilized with trehalose 6,6-dibehenate, a glycolipid synthetic variant of mycobacterial cord factor [63]. The mechanism of action of oil emulsions reflects their ability to induce a strong inflammatory reaction at the injection site, with local cell death leading to production of DAMPs and inflammasome activation [64]. The oil component also forms a potential long-term depot, which entraps the antigen and slows down its systemic release [65]. Local toxicities of oil emulsions include severe injection site pain due to local tissue damage followed by severe inflammatory reactions, which, in some cases, may progress to formation of a sterile granuloma or ulceration at the injection site [64]. Overall, emulsion adjuvants tend to be at the high end of the local reactogenicity scale and hence are not ideal for prophylactic vaccine use, particularly in paediatric populations [66].

Oil emulsions can also cause generalized systemic symptoms, including fever, headache, malaise, nausea, diarrhoea, arthralgia, myalgia and lethargy, reflecting induction of inflammation [6]. A major recurring concern is the potential association between oil emulsion adjuvants and autoimmune disease induction, as seen in animal models [67–69] and fish models [70]. A single intradermal injection of a range of oil emulsions, including squalene emulsions, induces adjuvant arthritis in susceptible murine and rat models [17]. Adjuvant arthritis is transferable using T cells, inhibited by anti-T-cell antibodies and associated with increased expression of pro-inflammatory cytokines, including IL-1 and IFN-γ, in the draining lymph nodes [71], indicating that oil emulsion adjuvants activate autoreactive arthritogenic T cells. Administration of CFA or IFA alone to C57Bl/6 mice can also induce experimental autoimmune hepatitis [72]. Susceptibility to oil emulsion–induced autoimmune disease is closely linked to genetic factors [73]. There is a theoretical risk that any humans who share genetic susceptibility features with these models could similarly be prone to developing adjuvant arthritis, lupus, autoimmune hepatitis, uveitis or some other form of autoimmune disease after exposure to oil emulsion adjuvants alone or combined with other potent innate immune activators, such as MPL [9, 74]. This might be relevant to the AS03 adjuvant containing squalene and tocopherol included in the narcolepsy-associated pandemic influenza vaccine [19, 20]. It is not known what causative factor(s) triggered the narcolepsy, but the AS03 adjuvant could have played a major role, as no increase in narcolepsy was seen in children who received alternative unadjuvanted vaccines [75]. Hence, it could be hypothesized that inflammation induced by the AS03 adjuvant could have contributed to the breaking of self-tolerance. IL-17 is thought to play a major role in autoimmune disorders, including multiple sclerosis, rheumatoid arthritis, psoriasis [76] and experimental allergic encephalitis (EAE) [77]. Oil emulsions are potent at inducing inflammatory cytokines, including IL-1 and IL-17 [78]. Given the importance of IL-17 for breaking self-tolerance and allowing T cells to cross the blood–brain barrier, this could explain why inflammatory oil emulsion adjuvants are so important to autoimmune disease induction in animal models [76], and it could also potentially explain the mechanism whereby the AS03-adjuvanted pandemic influenza vaccine caused narcolepsy in susceptible *HLA-DQB1**0602 (DR2-positive) children [19, 20].

8 Saponin Adjuvants

Saponins are tensoactive glycosides containing a hydrophobic nucleus of a triterpenoid structure with carbohydrate chains linked to the nucleus. Quil A is a saponin extract derived from the bark of *Quillaja saponaria* [79]. Fractions purified from this extract by reverse-phase chromatography, such as QS-21, induce strong humoral and T-cell responses [80]. Saponin adjuvants have been extensively utilized in experimental therapeutic cancer vaccines [81]. Through its detergent effects, saponin disrupts cell membranes, resulting in moderate to severe injection site pain and muscle cell damage and death, causing local redness, swelling and granuloma formation [82]. Saponin adjuvants also cause red blood cell haemolysis, reflecting the affinity of saponins for cholesterol present in erythrocyte membranes [83]. To make the saponin less toxic, QS21 can be mixed with cholesterol to form ISCOMs [84]. ISCOM particles induce less

haemolysis but still induce systemic side effects, including flu-like symptoms, fever and malaise [85–87]. The potential of saponin adjuvants to trigger autoimmunity in humans is not known. Some elderly human subjects in a clinical trial of a QS21-adjuvanted experimental Alzheimer's disease vaccine did develop meningoencephalitis [88], although the role, if any, of the QS21 adjuvant in these adverse reactions is not known [89].

9 TLR Agonist Adjuvants

The TLR adjuvant category covers an extremely broad spectrum of pathogen-derived compounds, including nucleic acids, proteins, lipopeptides and glycolipids, and synthetic analogues thereof [7]. These types of compounds are likely to have very different toxicities. All TLR agonists activate the inflammatory transcription factor nuclear factor (NF)-κB through the TLR adaptor proteins MYD88 and TRIF [90]. A consequence of NF-κB activation in monocytes is production of pyrogens and inflammatory cytokines, thereby resulting in potential for dose-limiting inflammation and pyrexia [91]. Attempts to detoxify TLR agonists inevitably lead to some loss of adjuvant activity. This is exemplified by conversion of the highly toxic TLR4 ligand lipopolysaccharide to the less toxic MPL [92]. Given its modest potency, MPL needs to be combined with aluminium or other adjuvants for best effect [92]. AS04 is an example of a combination adjuvant of MPL and aluminium, and it is included in an approved prophylactic hepatitis B virus (HBV) vaccine for low-responder renal dialysis patients [93] and a prophylactic human papilloma virus vaccine [94]. HBV-AS04 vaccine was more locally reactogenic than a standard aluminium-adjuvanted vaccine, with pain at the injection site occurring with 41 % of HBV-AS04 doses, versus 19 % of standard vaccine doses, consistent with increased vaccine reactogenicity due to the MPL component [93]. In animal models, TLR4 adjuvants have been shown to cause aberrant immune responses associated with toxicity [95]. For example, inclusion of a TLR4 agonist with an intranasal influenza vaccine in mice caused exacerbated illness and death when immunized animals were challenged with influenza, with the exacerbated lung pathology subsequently found to be due to the TLR4 agonist inducing an excessive IL-17 response [95]. TLR4 agonists have also been shown to be able to break tolerance and induce autoimmunity in susceptible animal models [96]. For example, TLR4 agonists—just like the inflammatory agents trehalose dimycolate, β-glucan, pristane and squalene oil—are potent inducers of inflammatory arthritis in susceptible strains [96]. However, the potential significance of these findings for human safety is not known, and relative doses used in human adjuvants are likely to be much lower than those used in animal models.

TLR9 agonists based on unmethylated CpG [97–99] are also under development as human vaccine adjuvants. Binding of CpG to TLR9 leads to activation of NF-κB and release of inflammatory cytokines [100], thereby stimulating T_h1 immune responses [101]. CpG can also bind directly to B-cell-expressed TLR9, leading to B-cell proliferation and antibody secretion [102]. Initially developed for anti-cancer use, CpG was shown to be well tolerated when injected intravenously in high doses in cancer subjects [103]. In general, the phosphodiester linkages in native CpG sequences are considered unsuitable for in vivo use because they are rapidly degraded by DNases [104]. Hence, the synthetic phosphorothioate backbone is almost exclusively used for current CpG adjuvants in human development. However, the phosphorothioate backbone has been shown to cause increased adverse effects in murine models, including splenomegaly, lymphoid follicle destruction and immunosuppression [103, 105]. In the last 10 years, phosphorothioate-backbone CpG adjuvants have been used in human clinical trials for a broad range of vaccine applications in infectious disease (hepatitis B, influenza, malaria, anthrax, human immunodeficiency virus [HIV]), cancer (melanoma, non-small cell lung cancer) and allergic rhinitis [106]. When CpG 7909 (0.5 or 1 mg) was added to an aluminium-adjuvanted hepatitis B vaccine, seroprotection after just a single dose was seen in ~50 % of subjects versus none that received the aluminium-adjuvanted vaccine alone [107]. Adverse events—including injection site reactions, flu-like symptoms and headache—were more frequent in the CpG 7909 groups but were predominantly of mild to moderate intensity [107]. 1018 ISS is a synthetic TLR9 agonist oligonucleotide used as an adjuvant in Heplisav®, a vaccine in development for hepatitis B prophylaxis. In one study, vaccine containing 1018 ISS (3 mg) promoted faster seroprotection than the comparator Engerix-B® vaccine [108]. Symptoms of local or systemic reactogenicity in the first 7 days post-immunization were not significantly different from those observed with an aluminium-adjuvanted control vaccine, although other studies have reported a higher rate of injection site reactions in subjects given HBsAg-1018 [109, 110]. Because a case of autoimmune Wegener's granulomatosis occurred in a subject receiving HBsAg-1018 in one trial [109], potential autoimmune events were monitored for in subsequent trials, where three new-onset autoimmune events, two cases of hypothyroidism and one case of vitiligo all occurred in the HBsAg-1018 group, whereas none occurred in the comparator group, although because of the small numbers and the 4:1 randomization ratio, this difference was not significant [111]. Nevertheless, in 2013, the US Food and Drug Administration (FDA) Vaccines and

Related Biological Products Advisory Committee reviewing the Biologic License Application for Heplisav® deemed that there were still insufficient data to adequately support the safety of Heplisav® [112].

A further TLR-based adjuvant approach that has been tested in preliminary human trials is the TLR5 ligand flagellin [113]. Since flagellin is a protein, it can be conveniently expressed as a fusion protein with the antigen itself, and this has been successfully applied to its use in an influenza hemagglutinin-based vaccine [114]. The globular head of the HA1 domain of A/Solomon Islands/3/2006 (H1N1) influenza virus fused to flagellin induced a functional antibody response, with the most common local adverse event being pain of mild or moderate intensity at the injection site. Systemic symptoms included fatigue and headache, and two subjects who received higher antigen doses had moderately severe systemic symptoms accompanied by substantial increases in serum C-reactive protein (CRP) levels consistent with a marked inflammatory response [114]. Clinical trials were also conducted with a fusion protein comprising four copies of the ectodomain of influenza matrix protein 2 fused to flagellin [115]. Following the first injection at higher doses (3 and 10 μg), self-limited but severe symptoms were noted in some subjects and were associated with elevated CRP levels believed to be mediated by TLR5-stimulated cytokine release [115]. Hence, the major challenge posed by flagellin-based adjuvant approaches, and also mirrored with TLR4 ligand adjuvants, is whether it is possible to titrate the dose to achieve sufficient vaccine immunogenicity on the one hand, while avoiding excess reactogenicity and inflammation on the other.

10 Enterotoxin Adjuvants

A major category of mucosal adjuvants includes cholera toxin (CT) and *Escherichia coli* heat-labile toxin (LT), and mutated variants thereof [116]. These mucosal adjuvants are thought to work via their ability to bind distinct ganglioside cell surface receptors and stimulate adenosine diphosphate (ADP)–ribosylating activity, thereby activating adenylate cyclase and increasing intracellular cyclic adenosine monophosphate (cAMP) levels [117, 118]. CT has a complex range of adjuvant activities, promoting CD40, CD80 and CD86 costimulatory molecule expression and IL-4 expression, thereby enhancing T_h2 responses and a B-cell isotype switch to IgA and IgG production, while suppressing IFN regulatory factor-8, IL-12 production and T-cell CD40 ligand expression, thereby suppressing T_h1 responses [119]. In gut epithelial cells, cAMP elevation leads to secretion of electrolytes and water into the gut lumen, with severe diarrhoea being the major dose-limiting toxicity of an unmodified CT adjuvant. While detoxified versions of CT and LT have been developed [116], human development of enterotoxin-based mucosal adjuvants was severely set back following a clinical influenza vaccine trial in which the use of a detoxified LT-based adjuvant with an intranasal inactivated vaccine caused facial nerve palsy in a small number of subjects [120].

11 Polysaccharide Adjuvants

The polysaccharides—including the polyglucans, polyfructans and mannans—share the benefit of biocompatibility and biodegradability while having potentially useful immunological activities [121]. Polysaccharide adjuvants can be separated into two classes based on whether they activate NF-κB and hence are pro-inflammatory (dextran, zymosan, β-glucan, mannan) or do not activate NF-κB and are non-inflammatory (delta inulin) [121]. The polysaccharide adjuvants that activate NF-κB and inflammation behave like emulsion adjuvants and are able to induce adjuvant arthritis in susceptible animal models [96]. The polysaccharide adjuvant known as delta inulin, or Advax™ [122], enhances humoral and cellular immune responses to a wide variety of viral and bacterial antigens but without evidence of inflammatory side effects [42, 123–127]. A delta inulin adjuvant has been safely administered to pregnant dams [128] and 7-day-old mouse pups [129], where it was able to induce protection with a single influenza vaccine dose. By contrast, MF59, a squalene emulsion adjuvant, failed to protect pups even after two vaccine doses [130]. A delta inulin adjuvant enhanced vaccine immunogenicity and was well tolerated in human clinical trials of hepatitis B [131], pandemic influenza [132] and bee sting allergy [133] vaccines. If inflammation is the key mechanism behind adjuvant-associated toxicity, including autoimmune disease induction, then a non-inflammatory adjuvant, such as delta inulin, may help avoid such toxicity and safety issues. This possibility warrants further exploration, as it could provide a route to the development of safer and better-tolerated adjuvants. With respect to safety, polysaccharides—particularly when in particulate form—activate complement, causing anaphylatoxin (C5a and C3a) release and basophil and mast cell activation, and potentially symptoms of anaphylactoid shock. In general, however complement activation sufficient to induce anaphylactoid shock is seen only after intravenous, but not after intramuscular or subcutaneous, injection. Furthermore, many polysaccharides, including dextran and delta inulin, bind plasma lipoproteins and may thereby provide negative feedback to downstream complement activation [134].

12 Glycolipid Adjuvants

A new class of adjuvants is based on glycolipids that bind the immune receptor CD1d and thereby activate natural killer T (NKT) cells, leading to cytokine production and enhanced vaccine responses. While the most characterized NKT cell agonist galactosyl ceramide has been extensively tested in humans as a potential anti-cancer therapy, no human data on its use as a vaccine adjuvant are yet available, despite promising data on its adjuvant potency in animal studies. However, ABX196, a synthetic analogue of galactosyl ceramide, was tested in a phase I/II human trial at doses of 0.2, 0.4 and 2.0 μg for its ability to enhance antibody responses to a hepatitis B vaccine [135]. There is known toxicity that can arise from activating NKT cells in the liver [136]. At high doses of ABX196 elevation of hepatic enzymes consistent with liver toxicity was seen in mice, and similarly some monkeys treated with ABX196 developed elevated transaminase levels [135]. A clinical trial was then undertaken in healthy adult subjects. Peripheral blood NKT cell activation and increased circulatory IFN-γ were seen 24 h post-immunization, and increased antibody titres were seen on day 43 in comparison with the antigen alone, consistent with an adjuvant effect. However, three of 29 subjects who received ABX196 had serious treatment-emergent adverse events, with major increases in hepatic transaminases (aspartate transaminase [AST] and alanine transaminase [ALT]) lasting for several weeks post-immunization, and had to be withdrawn from the study. It was concluded that the ABX196 as formulated was not safe for human use, because of NKT cell activation resulting in hepatotoxicity [135].

13 Animal Models for Adjuvant Safety Assessment

Both aluminium and squalene oil emulsion adjuvants already in broad human use can be shown to induce major adverse effects in animal models, although the relevance of such findings to humans remains unknown. Hence, data from such models are largely ignored when safety determinations are made on new vaccines containing these 'grandfathered' adjuvants. Regulators instead focus on vaccine safety data collected in rabbits or guinea pigs, together with data from human clinical trials to assess vaccine safety [137]. Notably, there remains a need for a better scientific explanation as to why specific animal model data showing adjuvant toxicity are not relevant to human use. For example, it has been known for many years that squalene oil emulsions, either alone or when formulated with relevant antigens, can induce autoimmune conditions (e.g. adjuvant arthritis [138]) in genetically susceptible animals. Hence, a consumer might reasonably ask why these animal toxicity data do not predict the possibility of the adjuvant causing autoimmune disease in human subjects who are also genetically susceptible. There is not currently any good answer to this question. Given the narcolepsy cases associated with use of a pandemic influenza vaccine containing an AS03 squalene oil emulsion adjuvant [19, 20], is it reasonable to ask whether the AS03 adjuvant was tested for its propensity to induce autoimmune disease in genetically susceptible animal models? Even if the influenza antigen in this vaccine turned out to be responsible for inducing narcolepsy—for example, through a process of antigen mimicry—it is still plausible that the AS03 adjuvant played a role in breaking self-tolerance, just as inflammatory adjuvants are critical to disease induction in models such as experimental allergic encephalomyelitis [139]. One possible mechanism worth investigating is whether the AS03 adjuvant induced an excessive T_h17 response, leading to opening of the blood–brain gate to autoreactive T cells, induced by influenza antigen mimicry [140].

Hence, any toxicity may depend on the adjuvant and antigen and other ingredients with which they are combined, together with the genetic background and the age of the population being immunized. This highlights the problem of trying to assess adjuvant safety by using traditional testing methods designed for assessment of small-molecule drugs for organ toxicity rather than for potential immunological toxicity. In the absence of agreement on appropriate assays to screen for potential immunological toxicity, existing adjuvants—most notably, aluminium and squalene oil emulsions—continue to be approved on a grandfathering basis, leaving extremely high barriers of entry to any new adjuvants. To remove obstacles to introduction of new adjuvants, there is a need for more adjuvant research, including research into mechanisms of adjuvant toxicity, thereby (hopefully) allowing development of better in vivo and in vitro models for adjuvant safety assessment. While the preceding sections have discussed adjuvant safety assessment generally, the following sections focus on safety aspects of specific adjuvants.

14 Approaches to Adjuvant Safety Testing

It is currently not clear what types of preclinical testing might be undertaken to prove that an adjuvant is immunologically safe or not. In this respect, it is important to distinguish 'immunological safety' (i.e. the risk of inducing, triggering or exacerbating immune disease in a susceptible individual) from 'toxicological safety' as assessed by current good laboratory practice (GLP) safety

tests using healthy animals [141]. GLP safety tests are designed to measure systemic safety in the context of potential direct organ damage by a substance—a method of testing that is most relevant to small-molecule drugs. With adjuvanted vaccines, the components themselves are likely to be non-toxic, but the immune responses they generate may have short- or long-term adverse effects, either spontaneously or upon exposure to a relevant pathogen. New vaccines, including those containing new adjuvants, need to pass standard toxicology tests with the issue of potential immunological toxicity in the 'too-hard basket' [141]. Hence, there is no agreement on what might be an appropriate predictive test of 'immunological toxicity' for an adjuvanted vaccine [142]. The situation is made more complex because most tests of immunological toxicity would need to be undertaken in susceptible animals, which may require substitution of the antigen and/or the adjuvant for the purposes of assessing the safety of each component separately. Current regulatory guidelines indicate that a vaccine adjuvant cannot be assessed or approved in its own right, independently of the vaccine antigen [142]. Notably, narcolepsy after influenza immunization affected only HLA DR2-positive children [8]; similarly, most other autoimmune diseases affect only very specific human subpopulations—for example, ankylosing spondylitis in HLA B27-positive individuals, multiple sclerosis in HLA DR2-positive individuals and type 1 diabetes in HLA DR3/4-positive individuals [143]. Hence, immunological safety cannot be easily assessed in animal strains that are not genetically susceptible to a particular autoimmune disease. With rare exceptions (e.g. adjuvant arthritis), testing for immunological toxicity also requires adjuvants to be tested in combination with one or more self-antigens. Thus, for example, EAE can be induced only by administering a neuronal self-antigen (e.g. myelin basic protein [MBP]) together with a pro-inflammatory adjuvant (e.g. CFA) to genetically prone animals [139]. Hence, the EAE model could be used to assess the immunological safety of a particular adjuvant if it were combined with MBP and administered to a susceptible animal. If EAE is not induced by a particular adjuvant, then this might provide reassurance that the adjuvant is unlikely to break self-tolerance and induce autoimmune disease, even if inadvertently formulated with a self-antigen mimic. In our hands, for example, neither aluminum and delta inulin adjuvant when formulated with MBP induced EAE in susceptible animals (unpublished data). The bigger problem is if the candidate adjuvant does induce EAE in this model. What is the risk if such an adjuvant is inadvertently formulated with a vaccine antigen that turns out later to be a self-antigen mimic, such as might have happened with the narcolepsy-associated pandemic influenza vaccine [144]? It would seem preferable not to include in prophylactic vaccines adjuvants that can be demonstrated to easily break self-tolerance. Nevertheless, such adjuvants may be ideal for use in cancer vaccines, where the ability to break self-tolerance might be a virtue. The EAE and adjuvant arthritis models teach us that induction of autoimmune disease is dependent on exposure of a genetically susceptible individual to the relevant self-antigen together with an inflammatory adjuvant able to break self-tolerance. By simply avoiding inclusion in prophylactic vaccines of an inflammatory adjuvant able to break self-tolerance, the risk of autoimmune disease should thereby be reduced, even if the vaccine includes a self-antigen mimic. In addition to EAE, there are many other well-established animal models of vaccine-inducible autoimmune diseases—including thyroiditis, arthritis and uveitis—that could be used to screen candidate adjuvant formulations for potential immunological toxicity due to ability to break self-tolerance. Predictably, highly pro-inflammatory adjuvants, such as oil emulsions, would fail these tests as, just like CFA, they can be shown to induce autoimmune disease in relevant models. Similarly, although regulatory bodies do not currently require testing of new adjuvants for potential for IgE induction or allergy exacerbation, it would seem sensible to require testing of all new adjuvants in a relevant allergy induction model, where they would be assessed against aluminium for their propensity to induce IgE-mediated anaphylaxis [40].

Another issue for adjuvant safety testing for adjuvants, such as TLR ligands, is that there may be species differences in the relevant receptor, downstream pathways and/or tissue distribution [145]. This may make it difficult to fully assess their safety in the absence of humanized animal models. In this situation, it would be useful to identify in vitro surrogates of adjuvant toxicity, using human cell lines or primary cells, with readouts such as potency of cytokine induction [146]. Unfortunately, such in vitro approaches may have limited value, as they cannot recapitulate the complexity of adjuvant action in vivo. For example, many adjuvants, including aluminium, have little effect on cytokine production in vitro and yet have potent adjuvant effects in vivo. Furthermore, toxicity may occur in distant and unexpected tissue compartments, such as the hepatotoxicity seen with injection of NKT cell agonists [135]. Hence, assessment of adjuvant potency, tolerability and safety will continue to require in vivo testing. Given that vaccine adverse effects may affect only rare individuals in a stochastic manner or because of underlying genetic susceptibilities, predictive animal models need to be able to recapitulate such factors. This necessitates research into the nature of human susceptibilities to adjuvant toxicity, with tools including whole-genome sequencing, gene expression arrays and deep sequencing approaches now readily available to start addressing such questions.

15 Consumer Perceptions of Adjuvant Safety

No medical intervention is completely without risk; hence, all human medicines, including vaccines, are approved by regulators on the basis of risk–benefit principles [147]. The interests of the public are protected by regulators such as the US FDA, whose role is to approve vaccines only if the proven benefits outweigh any measurable risks [147]. Assessment of risk–benefit is more complex for vaccines than for therapeutic interventions, as the benefits of vaccination can accrue to the population through herd immunity, while the risks of any adverse reactions are suffered by individuals, potentially raising complex ethical issues [148]. Hence, perceptions of risk–benefit at the individual level—i.e. "I do not want immunization, because any benefits do not justify the risk of a vaccine reaction" [149]—can sometimes be difficult to reconcile with risk–benefit assessments at the public health level—i.e. "if we allow too many individuals not to be immunized, herd immunity will be lost and serious infectious disease outbreaks may eventuate" [150]. Hence, policy makers and vaccine recipients might have very different perceptions of immunization risk–benefits [151]. This is also likely to shape the public's view of adjuvant risk–benefits, particularly in situations where both adjuvanted and unadjuvanted vaccines are available for the same indication. For example, an approved seasonal influenza vaccine in Europe contains MF59 squalene emulsion adjuvant, but the vast majority of influenza vaccines used in Europe are not adjuvanted [152]. Factors influencing consumer and practitioner utilization of adjuvanted versus unadjuvanted influenza vaccines could thereby be a useful area of study. No adjuvanted seasonal influenza vaccines are currently available in the USA, which could potentially reflect differences in regulatory and consumer views across continents [141]. During the 2009 influenza pandemic, both adjuvanted and unadjuvanted pandemic vaccines were utilized in Europe, with consumers not always given a choice regarding which vaccine was used [153]. By contrast, only unadjuvanted pandemic vaccines were used in the USA [154]. This imposed lack of consumer choice in some European countries may have acted to reinforce negative perceptions of adjuvanted vaccines, particularly when it was subsequently revealed that a squalene-adjuvanted vaccine used during the pandemic was associated with an increased risk of childhood narcolepsy [8]. Given potential public apprehension around the term 'adjuvant', there is a need for more research to identify the source of such fears and to develop strategies to alleviate them [155]. The origins of consumer apprehension surrounding adjuvants are likely to be multifactorial, with potential contributors being general mistrust towards governments and public health policy; perceptions of lack of choice; media coverage of rare adverse reactions; confusion between issues of aluminium, thiomersal and other vaccine excipients; and citing of papers on the role of adjuvants, such as oil emulsions, in autoimmune disease in animal models. With respect to the latter, scientists know that animal model findings may not always translate to humans, as reflected in the saying that 'mice lie'. However, in the absence of adequate education, many consumers are unlikely to appreciate this point and may place undue emphasis on such data when making risk–benefit assessments with respect to adjuvanted vaccines. It is also important that more research is undertaken to provide better understanding of such adverse effects in animal models and how they relate to the human context.

16 Public Health Views on Adjuvant Safety

Even if adjuvant causality is confirmed for a rare vaccine adverse event, this can create a disclosure dilemma—namely, should risks of rare vaccine-associated adverse events be publicized with the risks that consumers may overreact to such information. Alternatively, should such risks be downplayed to avoid damaging public confidence in immunization [60]. These are not easy questions to answer. To assist successful introduction of new adjuvants without risk of consumer backlash, it would be beneficial to have better understanding of public perceptions regarding adjuvants. This could then allow consumer education campaigns to be designed to address any misunderstandings or concerns [156]. Hence, alongside research into the mechanisms underlying potential adjuvant-associated adverse reactions, research is needed into consumer perceptions of adjuvants [157], as policies to improve immunization rates could easily backfire if not carefully researched [158]. While it can be argued that "society has the right and responsibility to establish laws, regulations, and choice frameworks that discourage vaccine refusal" [150], any mandatory action that reduces consumer choice needs to be considered very carefully. What is the role of bodies such as the WHO GACVS in adjudicating on vaccine or adjuvant safety? [159]. Arguably, the primary role of such bodies is to defend vaccine use in developing countries, where the risk–benefits of immunization are vastly different from those in developed countries, where infectious diseases are far less prevalent and old age and chronic diseases are far more prevalent. Notably, in most cases considered by the committee, reference is made to a lack of data and inadequate well-conducted controlled studies to confirm possible vaccine risks. This highlights the remarkable lack of research into vaccine and adjuvant

safety issues despite the fact that such research should fit within the framework for a 'global regulatory science agenda for vaccines' [160].

17 Conclusions

This paper highlights the inherent difficulties of assessing adjuvant safety and the poor state of knowledge of the mechanisms underlying potential adjuvant toxicity. Even aluminium—an adjuvant in widespread human use for almost a century and that has been given to billions of subjects—still has unanswered questions regarding its potential connection to conditions such as MMF or Alzheimer's disease. While there can be no such thing as a 100 % risk-free vaccine, any risks of immediate severe adverse reactions are extremely low for modern vaccines, and consumers should have high confidence in the safety of available vaccines. To facilitate the introduction of new adjuvants, it will be important that consumers are better educated regarding vaccine risk–benefit assessment. Given the vital importance of adjuvants to modern vaccines, additional resources are needed to support research to provide better understanding of adjuvant action and how this might relate to adjuvant toxicity. New adjuvants are needed that improve vaccine potency without compromising tolerability or safety. A hypothesis warranting further exploration is whether it is possible to design a non-inflammatory adjuvant able to enhance vaccine immunogenicity without causing reactogenicity or compromising vaccine safety.

Compliance with Ethical Standards

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