



# BioPharm

INTERNATIONAL.COM

August 2, 2007

## New-Age Vaccine Adjuvants: Friend or Foe?

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*A major unsolved challenge in adjuvant development is how to achieve a potent adjuvant effect while avoiding reactogenicity or toxicity*

### ABSTRACT

Older vaccines made from live or killed whole organisms were effective, but suffered from high reactogenicity. As vaccine manufacturers developed safer, less reactogenic subunit vaccines, they found that with lower reactogenicity came reduced vaccine effectiveness. Somewhat ironically, the solution proposed to boost immunogenicity in modern vaccines is to add back immune-activating substances such as toll-like receptor agonists—the very same contaminants removed from old-style vaccines. This raises the question of whether the vaccine field is moving forward or backward. We propose that by avoiding adjuvants that work through toll-like receptor (TLR) pathways, and instead focusing on adjuvants stimulating B- and T-cell immunity directly, one can minimize inflammatory cytokine production and consequent reactogenicity. We present data on a polysaccharide-based adjuvant candidate, Advax, that enhances immunogenicity without reactogenicity, suggesting that potent and well-tolerated vaccines for both adult and pediatric use are indeed possible.



A major bottleneck in vaccine development is the lack of suitable adjuvants for adult and pediatric prophylactic vaccine use. Aluminum salts were introduced for human use in the 1930s when the regulatory environment was less stringent. The desire for new and improved adjuvants stems not only from the need to make existing inactivated vaccines more potent, but also to gain features such as antigen-sparing ability, more rapid seroprotection, stimulation of T-cell immunity, and longer-lasting protective immunity. Significant regulatory and other hurdles exist for developing new adjuvants, as evidenced by the complete absence of new FDA-approved adjuvants.

Safety and tolerability are critical regulatory issues confronting new adjuvants, and pose the greatest barrier to new adjuvant approvals. In addition to preclinical studies on the adjuvant itself, the combined antigen–adjuvant formulation must pass animal toxicology screens in at least two species at a dose and frequency similar to, or higher than, the proposed human dose, and using the same route of administration, to assess safety and tolerability before clinical tests can begin. Therefore, the benefits of incorporating any adjuvant into vaccines must be balanced against any increased reactogenicity or risk of adverse reactions. Unfortunately, in most cases, increased adjuvant potency is associated with increased reactogenicity and toxicity. The best example of this is complete Freund's adjuvant (CFA). While it remains the gold standard in terms of adjuvant potency, its extreme reactogenicity and toxicity precludes its use in human vaccines, and there have been discussions of banning CFA even in veterinary vaccines.

Vaccine-caused adverse effects can be separated into two types: local and systemic reactions. Local reactions range from injection site pain, inflammation, and swelling, to granulomas, sterile abscess formation, lymphadenopathy, and ulceration. Systemic vaccine reactions may include nausea, fever, adjuvant arthritis, uveitis, eosinophilia, allergic reactions, organ-specific toxicity, anaphylaxis, or immunotoxicity mediated by liberation of cytokines, immunosuppression, and induction of autoimmune diseases.<sup>1,2</sup> While some systemic reactions such as allergy and anaphylaxis are clearly due to the antigen, others, such as adjuvant arthritis, may be caused directly by or exacerbated by the adjuvant. It can be difficult to identify which adverse reactions are mediated by the antigen, which by the adjuvant, and which by both.

A major unsolved challenge in adjuvant development is how to achieve a potent adjuvant effect while avoiding reactogenicity or toxicity.<sup>3</sup> Most newer human adjuvants including MF59,<sup>4</sup> ISCOMS,<sup>5</sup> QS21,<sup>6</sup> AS02,<sup>7</sup> and AS04<sup>8</sup> have substantially higher local reactogenicity and systemic toxicity than alum. Even alum, despite being FDA-approved, has significant adverse effects including injection site pain, inflammation, and lymphadenopathy, and less commonly injection-site necrosis, granulomas, or sterile abscess.<sup>9</sup> Although many adjuvant-caused vaccine reactions are not life-threatening and do resolve over time, they remain one of the most important barriers to better community acceptance of routine prophylactic vaccination. This particularly applies to pediatric vaccination where prolonged distress in the child due to increased reactogenicity may lead directly to parental and community resistance to vaccination.<sup>10</sup> Hence, particularly in the context of childhood prophylactic vaccines, it is critical that suitable adjuvants be developed with lower reactogenicity and greater safety. Ideally, in addition to being safe and well tolerated, adjuvants should promote an appropriate (humoral and/or cellular) immune response, have a long shelf-life, and should be stable, biodegradable, cheap to produce, and not induce immune responses against themselves.<sup>11</sup> A brief description and history of potential human adjuvants follows (Table 1).

Adjuvant	Formulation	Route	Indication	Year	Country
Alum	Aluminum hydroxide	IM	Various	1930s	USA
MF59	Squalene emulsion	IM	Various	1980s	USA
ISCOMS	Iscomatrix	IM	Various	1990s	USA
QS21	Quilichitin	IM	Various	1990s	USA
AS02	Alum + squalene	IM	Various	2000s	USA
AS04	Alum + squalene + cholesterol	IM	Various	2000s	USA
Advax	Polysaccharide-based	IM	Various	2000s	USA

Table 1. A range of human adjuvants under development with comparative features.

## Aluminum Salts (Alum)

**Mechanism of action.** While the exact mechanism of action of aluminum adjuvants remains uncertain, proposed mechanisms include formation of a local antigen depot, efficient uptake of aluminum-adsorbed antigen particles by antigen-presenting cells due their particulate nature and optimal size, stimulation of immune-competent cells of the body through activation of complement, induction of eosinophilia, and activation of macrophages.<sup>12</sup> Yet, none of these theories fail to adequately explain aluminum's adjuvant ability.

We propose an alternative unifying theory of aluminum action based on its toxicity. In our model, aluminum particles together with absorbed antigen are phagocytosed by tissue macrophages, which then become activated and mobilize into the lymph. Aluminum, once ingested, is toxic to cells<sup>13</sup> and by the time they reach the draining lymph node most of the macrophages that have ingested aluminum particles will be dead or dying. Once necrotic, the macrophages release their cytoplasmic contents, including alum-adsorbed antigen and inflammatory mediators such as IL-1 and TNF, into the lymph. This provides a source of macrophage cell debris, antigen, and co-stimulatory cytokines flowing into the draining lymph node, a potent mix to stimulate antigen-specific plasma cells and antibody production. Interestingly, a similar mechanism was proposed many years ago to explain the adjuvant action of beryllium, a compound which is even more toxic to macrophages than aluminum, and has potent adjuvant activity.<sup>14</sup>

**Limitations of alum.** Although aluminum salts remain the most commonly used adjuvants and the only ones currently approved for use in humans by the FDA, they suffer from a number of downsides, including inability to induce cytotoxic T-lymphocyte (CTL) responses critical in many cases for viral protection and clearance.<sup>15</sup> Well-recognized problems of aluminum adjuvants include local injection site reactions, stimulation of eosinophilia, augmentation of IgE antibody responses, ineffectiveness for some antigens, and failure to enhance CTL responses. Alum is reasonably well tolerated when injected intramuscularly, with only mild to moderate injection pain and occasional granulomas. Risk of granulomas becomes particularly high when alum-based vaccines are injected subcutaneously or intradermally. Consequently, alum-containing vaccines are generally given by intramuscular injection.<sup>16,17</sup>

The mechanism for alum's tendency to stimulate eosinophilia and enhance IgE production is unknown, but its consequence is an increased risk of vaccine allergy and anaphylaxis.<sup>9,16,18,19</sup> This potential has been demonstrated in animal models of ovalbumin-induced asthma or anaphylaxis, which are dependent on alum in the initial priming. In humans, there have been reports of a chronic inflammation syndrome called macrophagic myofasciitis (MMF) being induced by alum-based vaccines.<sup>20</sup> The original description of the syndrome was based on a group of patients with presumptive diagnoses of myopathy mimicking polymyositis. Symptoms included myalgias, arthralgias, marked asthenia, muscle weakness, and fever. Abnormal laboratory findings included elevated CK levels, increased ESR, and myopathic EMG, with muscle biopsies showing infiltration by sheets of macrophages with granular periodic-acid-Schiff positive content. The syndrome is due to the persistence of vaccine-derived aluminum at vaccine injection sites in the muscle, causing a chronic inflammatory reaction.<sup>21</sup> Since its original description in 1993,<sup>22</sup> more than 200 cases of MMF have been described in multiple countries.<sup>23</sup> Neurological manifestations resembling multiple sclerosis have been reported in some patients.<sup>24</sup> Children with MMF present with hypotonia and motor or psychomotor delay. A conclusive diagnosis is made by showing an aluminum peak in the lesion by energy-dispersive X-ray microanalysis. When Cynomolgus monkeys were immunized in the quadriceps muscle with diphtheria-tetanus vaccine, histopathological lesions similar to MMF in humans were observed up to three and 12 months after aluminum phosphate and aluminum-hydroxide-adjuvanted vaccine administration, respectively.<sup>25</sup> Aluminum is widely used as an adjuvant in human vaccines, and children can receive up to 3.75 mg of parenteral aluminum during the first six months of life. Intraperitoneal injection of aluminum-adsorbed vaccines in mice causes a transient rise in brain tissue aluminum levels peaking around day.<sup>2-3</sup> It is likely that aluminum is transported to the brain by the iron-binding protein transferrin and enters the brain via specific transferrin receptors.<sup>26</sup> Of major concern is the finding in cats of feline fibrosarcomas at the site of aluminum-adjuvanted vaccination. The tumors are sometimes surrounded by lymphocytes and macrophages that have taken up aluminum (with lesions identical to MMF), leading to the hypothesis that persistent inflammatory and immunological reactions associated with aluminum derange fibrous connective tissue repair responses, leading to neoplasia.<sup>27</sup>

## Oil-in-Water Emulsions

**General mechanism of action.** Oil-in-water emulsions include Montanide, Adjuvant 65, and Lipovant.<sup>28</sup> (MF59, also an oil-in-water emulsion, is discussed separately below.) Oil-in-water particles are irritants and cause local inflammation, inducing a chemotactic signal that elicits local macrophage invasion. The oil particles, along with associated antigen, are rapidly ingested by macrophages, which traffic to the draining lymph node. Because of frequent adverse reactions, the major human use of oil-in-water emulsions has been in therapeutic cancer and HIV vaccines<sup>29</sup> although Adjuvant 65 was previously used in a prophylactic influenza vaccine. Montanide adjuvants are variously formulated as water in oil, oil-in-water, or water in oil-in-water emulsions.<sup>30,31</sup> The water-in-mineral-oil adjuvant Drakeol/ISA-51 has been used in HIV-infected individuals.<sup>32</sup> Water-in-squalene emulsion (ISA-720) has been evaluated in a malaria vaccine trial.<sup>31</sup> Although potent, such adjuvants induced severe local reactions in some recipients.<sup>33,34</sup> Emulsions have also been used as delivery systems for immunostimulatory adjuvants, including MPL and QS21. An oil-in-water emulsion containing MPL and QS21 (SBAS-2) induced protection in a mouse model of malaria equivalent to that seen with CFA35 and was subsequently shown to confer short-lived protection in a malaria challenge in human volunteers, though with a high reactogenicity profile.<sup>36</sup> In trials with a HIV vaccine, SBAS-2 induced high antibody titers and proliferative but not CTL responses.<sup>37</sup>

**Limitations of oil-in-water emulsions.** Use of oil-in-water emulsions has been limited by their reactogenicity and potential for adverse reactions. Various types of emulsions have been used, with different natural oils, in order to try to find more stable, potent, less reactogenic formulations.<sup>38</sup> However, they still suffer from excessive reactogenicity and toxicity which restricts their suitability for prophylactic vaccines, particularly those intended for children.

## MF59

**Mechanism of action.** Originally, Syntex adjuvant (containing squalene oil, a non-ionic surfactant, poloxamer L121, and threonyl muramyl dipeptide) was developed as a replacement for CFA.<sup>39</sup> However, this adjuvant proved too toxic for human use<sup>40</sup> and Chiron subsequently

developed MF59 adjuvant as an alternative.<sup>41</sup> MF59 is a submicron oil-in-water emulsion which contains 4–5% w/v squalene, 0.5% w/v Tween 80, 0.5% Span 85, and optionally, varying amounts of muramyl tripeptide phosphatidyl-ethanolamine (MTP-PE), which activates non-TLR sensing receptors known as NOD-LRRs (reviewed in Akira<sup>42</sup>). Because of excessive reactogenicity and/or toxicity, the current version of MF59 used in an adjuvanted influenza vaccine (FLUAD) registered in Italy does not contain MTP but instead just squalene oil and surfactants.<sup>43,44</sup> Published data suggests addition of MF59 only induces a modest (about 25%) increase in antibody levels in the elderly and no difference in younger individuals when compared to unadjuvanted influenza vaccine.<sup>4,45</sup> Furthermore, there was little evidence that MF59 is antigen-sparing for influenza vaccines, since the same antigen dose is required for MF59 as for the unadjuvanted vaccine.<sup>4,45</sup> MF59 has been shown to be superior to alum in inducing antibody responses to hepatitis B vaccine in baboons<sup>46</sup> and humans.<sup>47</sup>

**Limitations of MF59.** On the negative side, MF59, like all other oil-in-water adjuvants, is associated with major increases in injection site pain and reactogenicity.<sup>4</sup> Another concern with squalene oil is its ability to induce chronic inflammatory arthritis in susceptible animal models.<sup>48</sup> Susceptibility to squalene arthritis is genetically determined, raising the risk that adjuvants based on squalene oil may also induce or exacerbate inflammatory arthritis in genetically susceptible humans.<sup>48</sup>

### Monophosphoryl Lipid A (MPL)

**Mechanism of action.** Bacteria-derived immunostimulants constitute a major potential source of adjuvants. Lipopolysaccharide (LPS),<sup>49</sup> containing the active Lipid A moiety, is very potent but too toxic for human use. MPL is a chemically detoxified derivative of native Lipid A from *Salmonella minnesota* R595, which is used in complex adjuvant formulations with alum, QS21, liposomes, and emulsions, and is a component of GlaxoSmithKline's proprietary AS02 and AS04 adjuvants.<sup>7,8,50</sup> Like LPS, MPL interacts with TLR4 on macrophages, resulting in the release of proinflammatory cytokines including TNF, IL-2 and IFN-gamma, which promote the generation of Th1 responses.<sup>51,52</sup> MPL has been extensively evaluated in human subjects for applications including vaccines for cancer and infectious disease (genital herpes, HBV, malaria, and HPV), and allergies. Approved vaccines containing MPL include a melanoma vaccine approved in Canada, a hepatitis B vaccine for hemodialysis patients approved in Europe, and an HPV vaccine approved in Australia.

**Limitations of MPL.** Although MPL lacks some of the more extreme toxicities of LPS, it is nevertheless able to strongly activate via TLR-4, inducing pro-inflammatory cytokines, and thereby significant reactogenicity. In terms of production, like any bacterially-derived material, there are issues of consistency of preparation, formulation, and cost.

### CpG

**Mechanism of action.** The immunostimulatory effect of bacterial DNA is due to the presence of unmethylated CpG dinucleotides which are both rare and methylated in vertebrate DNA.<sup>53–55</sup> CpG's effect is mediated by endocytic TLR9 receptors<sup>56</sup> expressed on B cells and plasmacytoid dendritic cells in humans, triggering the release of inflammatory cytokines<sup>57</sup> and biasing responses towards Th1 immunity and induction of CTL.<sup>58</sup> CPG 7909, developed by Coley Pharmaceuticals, has been tested in conjunction with an alum-adjuvanted Hepatitis B vaccine. This vaccine resulted in faster achievement of protective antibody levels and higher overall titer. There was an indication of enhanced CD8 CTL responses, but only in higher CpG dose groups.<sup>59</sup> In Phase 2 cancer trials using a CpG adjuvanted Melan-A vaccine in melanoma patients, there was evidence of induction of CD8 CTL's specific for Melan-A expressed by tumor cells, but little effect on outcome.<sup>60</sup>

**Limitations of CpG.** In human trials of CpG adjuvants, adverse events included injection site reactions, flu-like symptoms, and headache, and were all more frequent in CpG versus alum adjuvanted groups.<sup>59</sup> This is due to TLR9 activating NK-kB, a major inducer of inflammatory cytokines such as TNF-alpha, which are largely responsible for reactogenicity of adjuvants using TLR pathways.<sup>61–63</sup> Overall, reactogenicity, toxicity, and safety remain a barrier to acceptance of CpG adjuvants for human prophylactic vaccines. Additionally, TLR9 signalling has shown to play a critical role in experimental allergic encephalitis (EAE), a model of human multiple sclerosis,<sup>64</sup> and can even break tolerance and trigger EAE in otherwise healthy animals,<sup>65</sup> raising concern that CpG adjuvants could induce or exacerbate multiple sclerosis or other autoimmune diseases in susceptible individuals. Activation by CpG-DNA also has been shown to trigger and exacerbate systemic erythematous (SLE), with TLR9 activation in genetically prone mice triggering lupus nephritis.<sup>66</sup> CpG-DNA triggers lupus nephritis due to its potent immunostimulatory effects at multiple levels, including B-cell IL12p40 production, B-cell proliferation, double-stranded DNA autoantibody secretion, and dendritic cell IFN-alpha production.<sup>67</sup>

### QS21

**Mechanism of action.** QS21 is derived from Quil A, itself a collection of triterpenoid glycosides (saponins) derived from the bark of the South American soap bark tree, *Quillaja saponaria*. QS21 induces Th1 cytokines and antibodies of the IgG2a isotype in mice, consistent with a Th1 bias.<sup>68–70</sup> Saponins integrate into cell membranes through interaction with cholesterol, resulting in pores<sup>71</sup> through which antigens enter. Subsequently, peptides from these antigens may be processed and presented via MHC class I, stimulating a CD8 CTL response. Numerous clinical trials have been conducted using QS21 in cancer vaccines and infectious disease, including HIV-1, influenza, herpes, malaria, and hepatitis B.<sup>72</sup> The saponins have also been used in adjuvant formulations such as immunostimulatory complexes (ISCOMs) which will be discussed separately.

**Limitations of QS21.** Severe injection site pain is a major limiting factor in QS21 use. In addition to pain on injection and granulomas, toxicity of QS21 includes severe hemolysis,<sup>3,6,69,73,74</sup> making such adjuvants unsuitable for human prophylactic uses. This was highlighted in a recent trial of a QS21 adjuvanted influenza vaccine in healthy young adults where vaccination site pain and postvaccination myalgias were far greater in the QS21 group, and the QS21-containing vaccine had no advantage in terms of antibody response compared with the unadjuvanted vaccine.<sup>75</sup> In a trial of QS21 in a cancer vaccine, virtually all of the patients experienced inflammation and/or pruritis at the site of injection attributed to the QS21 adjuvant.<sup>76</sup> Other common side effects were fever (71%), fatigue (44%), flu-like symptoms (58%), chills (29%), myalgias (48%), and headache (66%). These toxicities were thought by the investigators to be all due to QS21, given there was no correlation between vaccine dose and

toxicity.<sup>77</sup>

In a trial of a malaria vaccine using QS21, two of 89 individuals developed severe vaccine allergy, a high complication rate for a prophylactic vaccine.<sup>78</sup> Further issues of QS21 safety have also surfaced with deaths of human subjects in an Alzheimer's disease vaccine trial using QS21, although the contribution of QS21 to these encephalitis deaths is not clear.<sup>79</sup>

## ISCOMs

**Mechanism of action.** ISCOMs are immunostimulating complexes containing a saponin, a sterol, and, optionally, a phospholipid. The preferred saponins are Quil A or QS21, the preferred sterol cholesterol, and the phospholipid is generally phosphatidylethanolamine. ISCOMs have been shown to help generate protective immunity in a variety of experimental models, and generate CTL responses to such antigens as HIV envelope glycoprotein and influenza hemagglutinin.<sup>80,81</sup> The principal advantage of ISCOMs is to reduce the dose of the highly toxic QS21 adjuvant component (the saponin component is bound to cholesterol and is less free to interact with cell membranes, thereby reducing QS21 hemolytic activity.)<sup>82,83</sup> ISCOMs, being particles, are also more likely to be phagocytosed directly by macrophages. The adjuvant activity of ISCOMs is related to their ability to induce cytokines, including IFN-g and IL-12,<sup>5,84</sup> consistent with an ability to skew immune responses in a Th1 direction.

**Limitations of ISCOMs.** ISCOMs have suffered from issues including cost, manufacturing difficulty, and stability, in addition to reactogenicity, toxicity, and safety concerns.<sup>6,85</sup> Side effects in a Phase 1 human cancer trial included flu-like symptoms, fever, and malaise.<sup>86</sup> A major part of ISCOM reactogenicity and toxicity reflects the inclusion of Quil A or QS21 as an active ingredient. Hence all safety concerns about QS21 apply to ISCOMs.

## Liposomes

**Mechanism of action.** Liposomes are synthetic phospholipid spheres consisting of lipid layers that can encapsulate antigens and act as both vaccine delivery vehicle and adjuvant.<sup>87</sup> The adjuvanticity of liposomes depends on the number of lipid layers,<sup>88</sup> electric charge,<sup>89</sup> composition,<sup>90</sup> and method of preparation.<sup>90-92</sup> Their use enhances both humoral and cell-mediated immunity to protein and polysaccharide antigens.<sup>89,91</sup>

Liposome-based vaccines based on virosomes are approved in Europe for hepatitis A and influenza.<sup>93</sup> They have been shown to better induce CTL to influenza in elderly humans compared to unadjuvanted vaccine.<sup>94</sup> INFLUSOME-VAC, which contains IL2-supplemented trivalent liposomal influenza vaccine, showed enhanced immunogenicity when compared with standard split-virion vaccine in elderly and young subjects, but at the expense of an overwhelming (83%) incidence of pain at the injection site.<sup>95</sup> The mechanism of liposomes is fusion with the cell membranes of macrophages, enabling delivery of proteins into the cytoplasm where they can enter the MHC class I pathway and activate CD8 CTLs.<sup>96,97</sup> Liposomes can be made with various charge properties and cationic lipid vesicles comprising cationic cholesterol derivatives, and optionally neutral phospholipids<sup>98</sup> are able to bind antigen on the surface and thereby enhance antigen presentation. Modified proteo-liposomal structures termed cochleates have also shown utility as systemic adjuvants.<sup>99</sup>

**Limitations of liposomes .** Liposomes have suffered from manufacturing difficulties, stability, and high cost, which have limited their use. Furthermore, they are more antigen vehicles than true adjuvants and hence require addition of immunostimulatory components such as MPL for potent adjuvant action. Injection site pain can be a major limitation in some liposome vaccines.

## Advax

**Mechanism of action.** Nanocrystalline particles of inulin, a natural plant-derived polysaccharide consisting of a linear chain of fructose molecules capped by a single glucose, are the active constituent of Advax. A relatively hydrophobic backbone structure gives inulin unique physicochemical properties: it can be crystallized into a number of different isoforms.<sup>100</sup> Specific isoforms of inulin have the unique ability to enhance antigen-specific humoral and cellular immune responses without reactogenicity.<sup>101-103</sup> Another advantage of Advax is that inulin can be prepared in exceptionally pure form, free of endotoxin or other contaminants, is heat stable with an extremely long shelf-life, and has had no safety issues over many decades of human use in intravenous injections for renal function testing (*British Pharmacopoeia*). Inulin is not metabolized in humans but is excreted unchanged in the urine as fructose and a small quantity of glucose. Advax's excellent safety and tolerability make it well suited to inclusion in childhood as well as adult vaccines.

**Limitations of Advax.** Currently, one of the main obstacles facing Advax is the presumption within the vaccine community that adjuvant potency is proportionate to inflammation and reactogenicity.<sup>104-110</sup> This dogma has arisen from uncritical acceptance of the "danger" hypothesis, which suggests that immunogenicity is linked to activation of the innate immune system. Advax gives good humoral and cell mediated immune responses in the absence of inflammation<sup>100-103,111-114</sup> or reactogenicity, thereby refuting the idea that "danger" signals are essential to eliciting potent adaptive immune responses.

## Summary

This paper highlights that the major differences between current adjuvants is not their efficacy, but their reactogenicity and safety. Increased reactogenicity reflects either an adjuvant's intrinsic tissue irritant effect, e.g., for MF59 and other oil emulsions or QS21, or its ability to induce inflammatory cytokines, e.g., LPS or MPL (through TLR activation). While alum has a modest tissue irritant effect, it does not directly induce inflammatory cytokine production, thereby explaining its lower reactogenicity.

Advax polysaccharide adjuvant has no local tissue irritant effects and does not induce cytokine production *in vitro*, explaining its almost complete lack of reactogenicity, which is unique among the known adjuvants. Adjuvant potency must be balanced against potential to do harm. Microbial cell components and TLR agonists including MDP, LPS, trehalosedimycolate, and beta glucan, and also oils such as pristane and squalene, are potent inducers of inflammatory arthritis in arthritis-prone animal strains. Since rheumatoid arthritis affects 1% of the population, there is significant risk of exacerbating or inducing such autoimmune syndromes in humans. Similarly, TLR agonists such as CpG have been shown to induce and exacerbate EAE and lupus. The ability of TLR agonists to break immune tolerance, potentially leading to autoimmunity in susceptible

individuals, may preclude their inclusion in prophylactic vaccines, particularly for children.

Similarly, the severe reactogenicity of compounds such as QS21 and oil emulsions preclude their inclusion in prophylactic vaccines. They may have restricted use in applications such as vaccine treatment of life-threatening conditions such as cancer and HIV. Although alum is the current gold standard and has a favorable reactogenicity profile compared to other adjuvants, major long-term safety issues continue to cloud its future, with concerns including MMF and vaccine allergy.

Liposome technology is highly promising and appears to offer significant advantages, providing reactogenicity is not excessive and sufficient immunogenicity is obtained. Currently, Advax is the only adjuvant that is non-reactogenic and without safety concerns in pre-clinical and clinical trials. This profile makes it ideal for inclusion in prophylactic vaccines, including those intended for use in children where maximum safety and tolerability are paramount.

This article demonstrates the relative under-development of the science of adjuvants, compared with the rapidly advancing knowledge of vaccine antigens. It is extraordinary that the exact mechanism of action remains unknown for many adjuvants, including alum, the oldest known vaccine adjuvant. Given the increasing importance of adjuvants to modern vaccines, national and international funding agencies urgently need to institute policies to address this imbalance and provide major new support for adjuvant basic science and clinical development.

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**Table 1.** A range of human adjuvants under development with comparative features.

Adjuvant	Mechanism of action	Induction auto-immunity/allergy	IgG1	IgG2	IgE	CD4 T Cells	CD8 T Cells	Induction inflammatory cytokines	Reactogeni
Alum	APC death	+	+++	+/-	++	++	-	++	++
MF59	APC apoptosis	++	++++	+/-	-	++	-	++	++++
CpG	TLR9	++	++	+++	-	++++	++++	++++	+++
LPS/MPL	TLR4	++	++++	+++	-	++++	++++	++++	++++
Oil emulsions	Irritant	+	++++	+++	-	++++	++	++	++++
Liposomes	Antigen delivery	-	++++	+++	-	+++	++	++	+++
QS21	Irritant	++	++	+++	-	+++	+++	++++	++++
Advax	Adaptive immune stimulant	-	++++	+++	-	+++	+++	-	-

(References to Table 1: (1, 3, 11, 18, 19, 21, 28, 73, 74, 101-103, 115-127)

Table 1. A range of human adjuvants under development with comparative features.



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