Published in final edited form as:

Pediatr Infect Dis J. 2015 December ; 34(12): 1395–1398. doi:10.1097/INF.0000000000000893.

Pediatric vaccine adjuvants: Components of the modern vaccinologist's toolbox

David J. Dowling, PhD^{a,b,*} and Ofer Levy, MD, PhD^{a,b,*}

^a Department of Medicine, Divisions of Infectious Diseases, Boston Children's Hospital, Boston, MA, 02115, USA.

^b Harvard Medical School, Boston, MA, 02115, USA.

Introduction

The goal of immunization is the induction of an immune response to protect from infection or disease (1). Until the end of the 20th century, adjuvantation science was limited to the use aluminum salts (alum) (2). Over the past 20 years there has been explosive growth of information regarding pattern recognition receptors (PRRs) that can activate leukocytes and thereby enhance immune responses (3). In parallel, a growing menu of adjuvants, agents that boost responses to vaccinal antigens following immunization, is now becoming available to immunologists and vaccinologists. However, immunity over life is not static but rather changes with age (4). Here, we will summarize the adjuvants currently in clinical use, new adjuvants in development and potential uses and benefits of a new “adjuvant toolbox” for developing novel early life vaccines (5).

Essential role of adjuvants in vaccinology

The word adjuvant is derived from the Latin *adjuvare*, meaning ‘to help’ (6). Adjuvants are used to enhance vaccine responses, increasing mean Ab titers and/or the fraction of the population that become protectively immunized. Early in the history of vaccinology, most vaccines contained live attenuated organisms, which express immune-stimulating components and were therefore “self”-adjuvanted. However, as vaccinology incorporated the tools of modern biotechnology, the field moved towards the development of defined antigen/molecule vaccine formulations, such as inactivated, subunit, and purified recombinant proteins and peptides. This approach has not only increased safety and lowered reactogenicity, but also lost many of the undefined immunological stimuli needed to trigger an effective immune response. Accordingly, the use of empirically tested adjuvants became an important, if incompletely understood, tool to stimulate innate immune factors (7).

Development of more fully characterized vaccine formulations has become a major goal for many vaccinologists and pharmaceutical companies (8). Considerations include 1)

*Corresponding can be directed to: David J. Dowling, PhD, Division of Infectious Diseases, Boston Children's Hospital, 300 Longwood Avenue, Boston, MA 02115, USA, Phone: 617-919-2906, Fax: 617-730-0255, david.dowling@childrens.harvard.edu, Ofer Levy MD, PhD, Division of Infectious Diseases, Boston Children's Hospital, 300 Longwood Avenue, Boston, MA 02115, USA, Phone: 617-919-2904, Fax: 617-730-0255, ofer.levy@childrens.harvard.edu.

formulation physicochemical characteristics, 2) adjuvant chemical structure, 3) route(s) of administration and 4) short and long term stability (9). A key aspect in common for both current and novel adjuvants is the ability to instruct adaptive immunity through the manipulation of antigen-presenting cells (APCs). As part of their function as professional APCs, dendritic cells (DCs) can integrate information from foreign stimuli (eg, components of pathogens or vaccines) and orchestrate these signals into appropriately regulated adaptive immune responses (10). As such, adjuvant discovery and vaccine delivery, along with improvement in antigen discovery and increased knowledge about human immune responses, are key technological advances fueling the current revolution in vaccine discovery and development (11).

The 9 adjuvants currently employed as components of approved vaccines fall into 5 classes: alum (of which there are 3), emulsions (a total of 4), naturally derived *Salmonella minnesota* monophosphoryl lipid A (MPLA) and virosomes (Fig. 1). Adjuvants can be combined. Indeed, 2 adjuvantation systems - adjuvant System (AS)-03 and AS04 - are components of vaccines approved for human use by the US Food and Drug Administration.

Adjuvants incorporated in licensed vaccine formulations

Alum

Aluminum salts (alum) have been used as components of human vaccine formulations since the early 20th century. Alum adjuvants, such as aluminum hydroxide, aluminum phosphate, and aluminum hydroxyphosphate are particulate in nature, to which the vaccine antigens are adsorbed, thereby increasing antigen stability. The molecular mechanisms by which alum interacts with the human immune system continue to be studied and involve multiple pathways, both direct and indirect. Alum enhances delivery of antigen to APCs as particulate vaccine formulations more readily interact with DCs and macrophages than soluble formulations of antigens alone (12). Crystalline alum binds lipids on the surface of DCs and triggers a cellular activation cascade leading to initiation of an immune response, but without itself being internalized by the cells (13). Second, alum may directly or indirectly trigger innate immunity through activation of inflammasome complexes, required for the processing of interleukin-1 family pro-inflammatory cytokines. This process is most likely nucleotide-binding oligomerization (NOD)-like receptor (NLR) mediated because the adjuvant effects of alum are not impaired in the absence of key Toll-like receptor (TLR)-dependent signal transduction adaptor molecules MyD88 and TRIF in knockout mice. Third, alum-induced cell death seems to modulate the local milieu in favor of enhancing adaptive immune stimulation. The release of damage-associated molecular patterns, such as uric acid and dsDNA, act as autologously derived autoadjuvants (14). Alum has some limitations. For instance, alum-adjuvanted vaccines often require multiple doses for induced protection (4), and drive Th2-polarized over Th1-polarized immunity. At present, there are multiple licensed pediatric vaccines, such as diphtheria, tetanus and hepatitis vaccines, listing alum as essential to produce effective Ab titers. However, there are also vaccinal antigens for which addition of alum may not be necessary for effective immunogenicity. For example, alum was excluded from GlaxoSmithKline's recent *Neisseria Meningitis* serogroup A, C, Y and W-135

(MenACWY) vaccine (trade name Menveo), because of the inability of alum adjuvantation to enhance serum bactericidal Ab titers in infant clinical trials (15).

Emulsions

Diverse water-in-oil emulsions, of which incomplete Freund's adjuvant is the best known, were originally evaluated in human trials during the mid 20th century (16). These consisted of emulsified water droplets in a continuous mineral oil phase but were soon withdrawn from commercial development because of unacceptable reactogenicity (increased risk of cysts at the injection site) and lack of formulation reproducibility. This led to the development of oil-in-water (O/W) emulsions, in which oil droplets are present in a continuous aqueous phase. Usually produced using microfluidic technologies, O/W formulations contain droplet like particles of 100 – 200 nm in size of naturally occurring oil metabolizable squalene and the sometimes non-ionic surfactants such as Tween 80 and Span 85. For the last 20 years, O/W emulsions have been popular adjuvants to enhance Ab responses in many seasonal and pandemic influenza vaccines licensed with European Medicines Agency (EMA) (17), using MF59 (Fluid, Focetria), AS03 (Pandemrix), and AF03 (Humenza) as adjuvants (16).

Unlike Alum, O/W emulsions do not seem to increase antigen stability. After intramuscular injection, the mechanism of action of O/W emulsions is TLR-independent and dependent on the creation an “immune-competent environment” within the muscle (16). For example, MF59 does not directly mature APCs but rather induces the production of chemokines and immune modulatory proteins from monocytes, macrophages, granulocytes and muscle cells that indirectly leads to increases migration of APCs to/from the site of injection and onto draining lymph nodes. This cell recruitment is greater than that induced by alum. MF59 may also instruct peripheral site monocyte differentiation into DCs and possibly induce the release of endogenous TLR agonists. Therefore, one major advantage of O/W adjuvants is the antigen dose-sparing potential (17). Of note, AS03 also contains α -tocopherol, a vitamin E compound with immune stimulant activity that is still under investigation (16).

TLR4 agonists and combination systems

The development of new adjuvants has expanded in part due to on-going discovery and characterization of cellular PRRs. At the forefront are detoxified congeners of endotoxin that stimulate TLR4 (18). A cell surface PRR, TLR4 recognizes several pathogen-associated molecular patterns, including lipopolysaccharides (LPS) from the outer membrane of Gram-negative bacteria and is the target for the adjuvant MPLA. Vaccine-grade natural MPLA is most commonly manufactured by passaging *S. minnesota* LPS through sequential acidic and basic hydrolysis steps. These processes result in dephosphorylated forms with significantly lower pyrogenicity/toxicity (approximately 1000-fold lower), but which retains robust adjuvant activity. To date, TLR4 adjuvants have been formulated with alum in licensed vaccine formulations. Most prominently, the adjuvant system AS04 includes 3-deacyl-MPLA (rendered even less pyrogenic by mild alkali hydrolysis) combined with aluminum phosphate is used in 2 approved vaccines: Cervarix (human papillomavirus) and Fendrix [hepatitis B vaccine (HBV)].

As MPLA extraction can present manufacturing hurdles, synthetic MPLA analogs have been developed by chemical synthesis. Glucopyranosyl lipid adjuvant (GLA) is a synthetic hexa-acyl form of MPLA, based on *Escherichia coli* - rather than *S. minnesota* LPS. GLA is in phases I and II clinical trials for multiple indications, including visceral leishmaniasis vaccine. A similar hexa-acyl MPLA, the adjuvant RC529, is a component of the alum-adjuvanted HBV (SuperVax) approved in Argentina (16). Combinations of the above-mentioned adjuvant classes are also in development. Both AS01 and AS02, consisting of MPLA and the purified plant bark extract/saponin QS21, are components of the Mosquirix (RTS,S) malaria vaccine (16, 19).

Virosomes

Virosomes are liposomal virus-like particles that act as both a vaccine carrier system and an adjuvant. They are typically produced from reconstituted empty envelopes of influenza viruses and function by adsorbing (or encapsulating) protein antigens into their liposomal membranes. Although the mode of action is still under investigation, it is hypothesized that virosomes may stimulate humoral and cellular immunity by binding to macrophages and APCs (16). Virosome-based influenza vaccines are licensed in Europe (as Inflexal) and as adjuvants for hepatitis A vaccine (as Epaxal).

New classes of adjuvants in advanced clinical development

There are several other adjuvants in advanced clinical development that do not fall into any of the aforementioned categories. A nonexhaustive list includes 1) various defined agonists of PRRs, 2) saponins, 3), polyelectrolytes, 4) outer membrane vesicles (OMVs) and 5) nanoparticles (Fig. 1).

PRR agonist adjuvants

In addition to TLR4 agonist adjuvants, various other PRR agonist adjuvants have been evaluated in human clinical trials. These target the innate immune system, in particular TLRs, NOD-like receptors, C-type lectin receptors and RIG-like receptors (RLRs). Agonists for the following TLRs have demonstrated adjuvant activity: TLR3 (dsRNA), TLR5 (bacterial protein called flagellin), TLR7 and 8 (ssRNAs rich in uridine residues, as is found in viral RNA) and TLR9 (bacterial DNA and synthetic single-stranded oligodeoxynucleotides with motifs containing unmethylated cytosine-phosphate-guanine (CpG) residues). Adjuvants directed toward the endosomal TLRs 7, 8, and 9 have demonstrated robust adjuvant activity. When applied as a topical skin adjuvant, the TLR7-activating, small synthetic imidazoquinoline molecule imiquimod enhances intradermal influenza vaccine responses (16). TLR7/8 agonists, such as imidazoquinolines, induce robust activation of human newborn leukocytes, suggesting utility as neonatal vaccine adjuvants (4). In addition, Dynavax's HBV vaccine (HEPLISAV), which is adjuvanted with the TLR9 activating CpG-rich DNA immunostimulatory sequence 1018-ISS, elicited seroprotective Abs with fewer immunizations in a recent phase III clinical trial.

Saponin-based adjuvants

Saponins are triterpenoid molecules with a complex sugar backbone extracted from a variety of plants (16). The most widely studied of these extracts is saponin (referred to as Quil-A), extracted from the bark of the South American tree *Quillaja saponaria* Molina. It has been used as an adjuvant in veterinary vaccines since the 1970s. However, because of unacceptable toxicity in humans, researchers took advantage of the high affinity of Quil-A saponins for cholesterol to produce cholesterol-saponin complexes that are less reactogenic than the parent saponin, yet maintain a strong adjuvant effect (16). Additionally, for large-scale manufacture, simple antigen coadministration was adopted over conjugation. For example, the Iscomatrix adjuvant is made from partially purified saponins from Quil-A, combined with cholesterol and phospholipids, forming small porous particles 50-60 nm in diameter. Iscomatrix is admixed with the antigen of interest.

The furthest advanced cholesterol-saponin complex is QS21, an isolated pure component of Quil-A. When combined with liposomes that contain cholesterol, QS21 has very high stability and low reactogenicity. As mentioned above, the combination adjuvant systems AS01 and AS02, components of the candidate RTS,S malaria vaccine, both contain MPLA and QS21. Although the exact mechanism of action of QS21 is not fully elucidated, their nanoparticulate size may lead to their preferential interaction and pore formation within cholesterol-rich DC membranes (16).

Polyelectrolytes

Polyelectrolytes are a class of water-soluble macromolecule polymers comprised of a phosphorus-nitrogen backbone and organic side groups. Such polymers, when formulated with various vaccine antigens, have demonstrated substantial immunopotential, antigen dose-sparing potential and acceptable safety thresholds during both animal and human *in vivo* studies. These include the synthetic polyphosphazene polyelectrolyte poly[di(carboxylatophenoxy)phosphazene] (PCPP) (20). Like Alum, PCPP can induce *in vitro* DC death. Importantly, PCPP, by engaging in covalent or noncovalent interactions, can form water-soluble multimeric complexes with antigens, thereby enhancing thermal stability, a critical requirement for vaccine formulation. Of note, the polyelectrolyte polymer, polyoxidonium, is the adjuvant component of the influenza vaccine Grippol licensed in Russia and routinely administered to both children and adults.

Nanoparticle adjuvants and delivery systems

Historically, the particulate nature of materials used in many vaccine formulations was empiric, often because of the need to stabilize antigens (i.e. adsorption onto alum). The biodegradable synthetic polymer, poly(D,L-lactic- *co*-glycolic acid), is a widely investigated nanoparticle adjuvant for controlled and effective delivery of vaccine antigens, including synthetic peptides. These are usually produced as solid block particles ranging from 50 to 500 nm in size, with antigens entrapped or adsorbed on the surface of the particles (21). More recently, advances in the field of immunoengineering, which are developing alongside vaccinology, have begun to guide vaccine formulation design (9, 22). Specifically, vaccine delivery systems have been engineered to mimic the size, shape and surface chemistry of pathogens (12), often referred to as “pathogen-like particles”. These can be engineered to

target subsets of immune cells such as DCs and specific subcellular compartments. For example, block copolymers of poly(ethylene glycol)-*bl*-poly(propylene sulfide) are novel macromolecular amphiphiles capable of forming a wide range of self-assembled morphologies when dispersed in water, including spherical or cylindrical micelles as well as liposome-like vesicles referred to as polymersomes (9).

Outer membrane vesicles

TLR4 agonist-containing OMVs are used in some serogroup B meningococci capsule vaccines (MenB). OMVs are produced by the blebbing of membranes of clinical derived live Gram-negative bacteria during in vitro growth and are useful vaccine components, as immunostimulatory membrane components (lipids, proteins, LPS etc.) from meningococci are represented (23). OMV-based MenB vaccines also typically combine recombinant proteins produced in *E. coli*. Novartis 4-component Alum adjuvanted MenB vaccine (Bexero) has been approved by the EMA and is composed of detergent-extracted OMV and 3 recombinantly produced *Neisseria meningitidis* proteins. Detergent extraction of the OMVs and genetic modification are approaches to ameliorate LPS toxicity and prevent excess reactogenicity.

Accelerating the discovery of adjuvants for the very young infant

The majority of global immunization schedules are focused on the pediatric age group. However, the majority of adjuvant discovery programs do not rationally select adjuvants for use in early life. As many of the above-mentioned adjuvant classes produce distinct and potentially suboptimal responses in the very young infants, it is critical to understand how adjuvants may best optimize vaccine efficacy by taking into account early life immune ontogeny (4, 11).

Identifying adjuvants active in the young infants

An increased appreciation of immune ontogeny may inform the design of rationally designed pediatric vaccine formulations. Such age-specific knowledge could enabled vaccine developers to exploit traditional drug discovery tools, including (1) high throughput screening of small molecules with desired activity towards infant leukocytes, (2) medicinal chemistry to design and synthesize compounds tailored to reflect distinct early life immunity (3) and computational approaches to elucidate structure-activity relationships and the optimization of adjuvant candidates (24). This approach may also have long term economic and licensure advantages. For example, use of animal models for vaccine formulation evaluation active in the young can be expensive and unpredictable (eg, variable responses between species and distinct immune ontogeny), with expensive non-human primate models becoming favored for late phase development. To the extent that they reflect activity in vivo, in vitro platforms to more accurately predict immune function in vitro, such as microphysiologic systems using primary human tissues and leukocytes (4), are also under investigation.

Reducing reactogenicity while optimizing safety

A key concern regarding adjuvanted vaccine development is reactogenicity, the propensity of a formulation to cause acute inflammatory events either locally- for example, erythema and tenderness - or systemically as fever. Of note, vaccine adjuvants are not licensed separately; rather, the adjuvant is a constituent of the licensed vaccine formulation. Therefore, adjuvants must be evaluated both alone and as a component of a vaccine formulation. Adjuvant optimization may entail (1) manipulating pharmacokinetic properties that affect compound biodistribution (eg, limit systemic exposure by covalent attachment of a hydrophobic group) (2) and facilitating interaction with formulations designed to ensure localized codelivery of the antigen and immunostimulatory compound (eg, nanoparticle encapsulation). Using in vitro biomarkers as surrogate markers of in vivo adjuvanticity and reactogenicity would be highly desirable. At this juncture, most studies have examined Th-polarizing cytokine induction as well as adjuvant-induced production of PGE₂ (25), a biomarker generated in vitro in human monomacs cells that correlates with pyrogenicity in rabbits but whose potential utility in predicting reactogenicity in the pediatric arena has yet to be defined.

Conclusion

Translational studies of adjuvants targeted towards newborns and young infants are challenging because of the transient nature of this phase of life, inherent logistical obstacles posed by the smaller size of infants and their distinct societal standing. However, the continuing high global burden of infections in the very young infants and consequent need for safe and effective early life vaccines provide a compelling rationale for on-going basic and translational studies of adjuvant discovery and development (19). Continued study of human early life immunity and translational studies of current and novel adjuvants will be integral to this effort.

Acknowledgments

Conflicts of interest: O.L. is supported by U.S. National Institutes of Health (NIH) grant 1R01AI100135-01, the National Institutes of Allergy and Infectious Diseases (NIAID), NIH, Department of Health and Human Services, Adjuvant Discovery Program Contract No. HHSN272201400052C as well as Global Health (OPPGH5284) and Grand Challenges Explorations (OPP1035192) awards from the Bill & Melinda Gates Foundation. The Levy Laboratory has received sponsored research support from VentiRx Pharmaceuticals, 3M Drug Delivery Systems, MedImmune and Crucell (Johnson & Johnson).

References

1. Levy O, Goriely S, Kollmann TR. Immune response to vaccine adjuvants during the first year of life. *Vaccine*. 2012
2. Rappuoli R, Mandl CW, Black S, De Gregorio E. Vaccines for the twenty-first century society. *Nat Rev Immunol*. 2011; 11:865–872. [PubMed: 22051890]
3. O'Neill LA, Golenbock D, Bowie AG. The history of Toll-like receptors - redefining innate immunity. *Nat Rev Immunol*. 2013; 13:453–460. [PubMed: 23681101]
4. Dowling DJ, Levy O. Ontogeny of early life immunity. *Trends Immunol*. 2014; 35:299–310. [PubMed: 24880460]
5. PrabhuDas M, Adkins B, Gans H, et al. Challenges in infant immunity: implications for responses to infection and vaccines. *Nat Immunol*. 2011; 12:189–194. [PubMed: 21321588]
6. Levitz SM, Golenbock DT. Beyond empiricism: informing vaccine development through innate immunity research. *Cell*. 2012; 148:1284–1292. [PubMed: 22424235]

7. Plotkin SA, Plotkin SL. The development of vaccines: how the past led to the future. *Nature reviews Microbiology*. 2011; 9:889–893.
8. Brito LA, Malyala P, O'Hagan DT. Vaccine adjuvant formulations: A pharmaceutical perspective. *Semin Immunol*. 2013; 25:130–145. [PubMed: 23850011]
9. Swartz MA, Hirose S, Hubbell JA. Engineering approaches to immunotherapy. *Sci Transl Med*. 2012; 4:148rv149.
10. Pulendran B, Ahmed R. Immunological mechanisms of vaccination. *Nat Immunol*. 2011; 12:509–517. [PubMed: 21739679]
11. Koff WC, Burton DR, Johnson PR, et al. Accelerating next-generation vaccine development for global disease prevention. *Science*. 2013; 340:1232910. [PubMed: 23723240]
12. Bachmann MF, Jennings GT. Vaccine delivery: a matter of size, geometry, kinetics and molecular patterns. *Nat Rev Immunol*. 2010; 10:787–796. [PubMed: 20948547]
13. Flach TL, Ng G, Hari A, et al. Alum interaction with dendritic cell membrane lipids is essential for its adjuvanticity. *Nat Med*. 2011; 17:479–487. [PubMed: 21399646]
14. Marichal T, Ohata K, Bedoret D, et al. DNA released from dying host cells mediates aluminum adjuvant activity. *Nat Med*. 2011; 17:996–1002. [PubMed: 21765404]
15. Snape MD, Perrett KP, Ford KJ, et al. Immunogenicity of a tetravalent meningococcal glycoconjugate vaccine in infants: a randomized controlled trial. *Jama*. 2008; 299:173–184. [PubMed: 18182599]
16. Plotkin, SA.; Orenstein, WA.; Offit, PA. *Vaccines*. 6th Ed.. Saunders; Philadelphia, PA: 2012.
17. Reed SG, Orr MT, Fox CB. Key roles of adjuvants in modern vaccines. *Nat Med*. 2013; 19:1597–1608. [PubMed: 24309663]
18. Duthie MS, Windish HP, Fox CB, Reed SG. Use of defined TLR ligands as adjuvants within human vaccines. *Immunol Rev*. 2011; 239:178–196. [PubMed: 21198672]
19. Rappuoli R, Aderem A. A 2020 vision for vaccines against HIV, tuberculosis and malaria. *Nature*. 2011; 473:463–469. [PubMed: 21614073]
20. Palmer CD, Ninkovic J, Prokopowicz ZM, et al. The effect of stable macromolecular complexes of ionic polyphosphazene on HIV Gag antigen and on activation of human dendritic cells and presentation to T-cells. *Biomaterials*. 2014; 35:8876–8886. [PubMed: 25023392]
21. Azmi F, Ahmad Fuaad AA, Skwarczynski M, Toth I. Recent progress in adjuvant discovery for peptide-based subunit vaccines. *Hum Vaccin Immunother*. 2014; 10:778–796. [PubMed: 24300669]
22. Hubbell JA, Thomas SN, Swartz MA. Materials engineering for immunomodulation. *Nature*. 2009; 462:449–460. [PubMed: 19940915]
23. Sanders H, Kaaijk P, van den Dobbelsteen GP. Preclinical evaluation of MenB vaccines: prerequisites for clinical development. *Expert Rev Vaccines*. 2013; 12:31–42. [PubMed: 23256737]
24. Flower DR. Systematic identification of small molecule adjuvants. *Expert opinion on drug discovery*. 2012; 7:807–817. [PubMed: 22724523]
25. Mastelic B, Garcon N, Del Giudice G, et al. Predictive markers of safety and immunogenicity of adjuvanted vaccines. *Biologicals : journal of the International Association of Biological Standardization*. 2013; 41:458–468. [PubMed: 24071553]

Figure 1.
Classes of clinically used and tested adjuvants.