Adjuvants in Pediatric Vaccines

Nathalie Garçon

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5.1 Introduction

The exponential evolution of scientific knowledge during the first half of the twentieth century led to the emergence of new and improved ways of producing vaccines. Vaccines were produced from cultivating the pathogens, but this has not always been possible in sufficient quantities. The rise of molecular biology and a better understanding of the key components of immune protection have allowed the development and production of what is known as recombinant antigens. Most, if not all purified and recombinant antigens, require to be effective the addition of what is known today as adjuvants. They are an important part of the development of improved or new vaccines against infectious diseases, alongside DNA or vector-based vaccines, and may be required with oligonucleotide-based vaccines if not self adjuvanted.

5.2 Definition of Adjuvants

An adjuvant, from the Latin word adjuvare meaning to help or aid, is a substance used to improve a vaccine's immune response by accelerating, prolonging, or enhancing the immune responses specific to the vaccine antigen(s), in particular by increasing mean antibody (Ab) titers of the population being immunized.

It is clearly accepted today that all current whole, attenuated, subunit, purified recombinant protein and peptide vaccines are adjuvanted endogenously (part of the pathogen) or exogenously (added to the antigen formulation).

Indeed, during this evolution moving from whole killed or attenuated pathogens to particulate vaccines, combined with the tools of modern biotechnology, vaccines have not only seen an increased safety and lowered reactogenicity profile but also the loss of many of immunological stimuli needed to trigger an effective immune response. For these vaccines, adjuvants became an important tool to ensure efficient and lasting immune response.

Until the early 1980s, adjuvantation science was limited to the use of aluminum salts. Following the emergence of HIV and the following attempts to develop HIV vaccines, it appeared that aluminum salts were not enough to induce a protective immune response when combined with recombinant antigens. This revived the interest in adjuvants, and over the past 30 years, there has been an exponential growth of information regarding pattern recognition receptors (PRRs) that can activate leukocytes and thereby enhance immune responses.

When properly designed, selected, and combined with the relevant antigen(s), adjuvants can enable the appropriate and long-lasting immune response required to protect against the disease, with a safety profile acceptable in the targeted population. To date, no combination of recombinant antigen and adjuvant has demonstrated the ability to induce a CD8 immune response in naive human subjects, and adjuvants that enhance CD4 T cell responses are critical for durable vaccine immunity.

The understanding of the mode of action of adjuvants has greatly benefited from the discovery of pathogen-associated molecular patterns (PAMPs) and their associated receptors (Toll-like receptors [TLRs], nucleotide-binding oligomerization domain [NOD]-like receptors [NLR]) and inflammasome components and has been critical to the understanding of the link between innate and adaptive immunity and the associated pivotal role of dendritic cells. Despite these advances, a rational design approach would clearly benefit from a better understanding of the roles of innate and adaptive immunity and their impact on vaccine safety and immunogenicity.

5.3 Adjuvants in Vaccines

New vaccines based on recombinant antigens and adjuvants have put vaccine formulation at the center of vaccine development. Chemical structure, physicochemical characteristics, stability, the nature of the induced immune response, the impact on innate immune response, and the mode of action are key for their evaluation and use.

Table 5.1 Adjuvants in vaccines licensed for pediatric populations

Aluminum salts	Phosphate or hydroxide	D, T, Pa, Hib, HBV, HAV, IPV, pneumococcus, HPV
Emulsion	MF59	Seasonal influenza
	AS03	Pandemic influenza H1N1 and H5N1
	AF03	Pandemic influenza H1N1
Liposomes	Virosomes	Seasonal influenza
Combination	Aluminum + MPL	HPV

To date, there are nine different adjuvants present in licensed adjuvanted vaccines. Amongst those, seven are licensed for use in pediatric populations (Table 5.1).

5.4 Aluminum Salts

The evaluation and use of aluminum salts in vaccines emerged in 1921 when a diphtheria vaccine based on inactivated diphtheria toxin (toxoid) was shown to be protective against diphtheria toxin. In 1926, aluminum precipitation was shown to enhance antibody response to diphtheria toxoid in guinea pigs, and in 1932 it was shown that alum enhances response to diphtheria toxoid immunization in humans. In 1939, Al-hydrogel became commercially available, and since then, several billions of aluminum-containing vaccine doses have been used around the world. Several types of aluminum salts have been developed. They are particulate in nature and are different with regard to their surface charge, allowing effective adsorption of the antigen depending on its point of zero charge (pH at which the antigen has a neutral charge). The antigen adsorption increases the specific immune response and the antigen stability. Aluminum adjuvants are present in most of the currently licensed vaccines (Table 5.2). Although aluminum-containing vaccines are licensed across the world, the amount of Al present in a vaccine can vary depending on the country considered (Table 5.3).

Table 5.2 Aluminum-containing vaccines licensed for pediatric vaccines

Adjuvant	Vaccine
Alum: aluminum potassium sulfate Alhydrogel: aluminum hydroxide Adju Phos: aluminum phosphate Proprietary aluminum hydroxide and phosphate	DTaP (pediatric diphtheria, tetanus and acellular pertussis)
	DTaP, polio and *Haemophilus influenzae* type b
	DTaP, polio, *Haemophilus influenzae* type b and hepatitis B
	Hepatitis A
	Hepatitis B
	Hepatitis A/B
	Human papillomavirus-6/11/16/18
	Influenza (H5N1)
	Pneumococcus (conjugated)
This is not an exhaustive list; it focuses on the USA and Europe	

The mode and mechanism of action by which aluminum salts have an impact on the human immune system are not fully deciphered and appear to be both direct and indirect. Through the transformation of antigens into a particulate through their adsorption on aluminum salts, antigen interaction with antigen-presenting cells (APCs) and macrophages is optimized compared to a soluble antigen formulation. To date, various possible mechanisms of action have been described (Table 5.4).

Aluminum salts have the longest and largest safety track record of all adjuvanted vaccines, with more than three billion vaccine doses used during the past 80 years and a positive risk–benefit ratio. Focal histological lesions were observed in vaccinees with diffuse muscular symptoms that included persistent myalgias, arthralgias, and persistent fatigue. In

Table 5.3 Limits of elemental aluminum (Al^{3+}), reported per human dose

Region	Reference/product	Limit (Al^{3+}) mg/dose
USA	21CFR Part 610 "General Biological Products Standards"	0.85
EU	European pharmacopoeia "Vaccines for Human Use"	1.25
WHO	WHO technical report series	1.25
China	DTPa	0.17–0.26
	Diphtheria vaccine adsorbed	0.52
	Tetanus vaccine adsorbed	0.52
	Diphtheria and tetanus combined vaccine, adsorbed	0.43
	HAV	0.60
	HBV	0.18–0.31
Japan	Adsorbed purified pertussis	0.15
	Adsorbed diphtheria-purified pertussis-tetanus	0.15
	HPV	0.42–0.58
	Recombinant adsorbed hepatitis B vaccine	0.325
India	HBV	1.25
	DTP	1.25

Table 5.4 Mode of action of aluminum

Crystalline alum binds lipids on the surface of DCs	Cellular activation cascade triggering an immune response
Directly or indirectly triggers innate immunity through activation of inflammasome complexes	Likely nucleotide-binding oligomerization (NOD)-like receptor (NLR)-mediated effect is still present in MyD88 and TRIF in knockout mice
Induces cell death, which modulates the environment towards an enhanced adaptive immune response	Damage-associated molecular pattern release, such as uric acid and dsDNA, act as autologously derived autoadjuvants

the approximately 130 cases observed, these lesions were identified as macrophagic myofasciitis (MMF). Intracytoplasmic inclusions in the infiltrating macrophages have been identified as containing aluminum by electron microscopy, microanalysis, and atomic adsorption spectroscopy. There is no established relationship between the presence of aluminum and MMF and the clinical symptoms, however. The Vaccine Safety Advisory Committee of the World Health Organization (WHO) reviewed MMF during a meeting in 1999 and found no basis for recommending a change in vaccination practices (vaccine selection, schedule, delivery practices, or information on aluminum-containing vaccines). Studies have been undertaken since then, to evaluate the clinical, epidemiological, and basic science aspects of MMF. Although it is recognized that aluminum salts may be found months or years later at the intramuscular injection site after vaccination, to date, no link has been clearly established with the MMF syndrome.

5.5 Emulsions

Since the development of Freund's adjuvant, numerous emulsions have been evaluated in human. Water-in-oil emulsions (emulsified water droplets in a continuous oil phase) have been removed from testing following unacceptable reactogenicity (cysts at the injection site) and a lack of formulation reproducibility. The development of alternative emulsions (oil-in-water where oil droplets are in a continuous aqueous phase) was then undertaken. They represent the class of emulsion currently licensed in pediatric vaccines. They are made of particles of less than 200 μm (allowing for sterile filtration), are made of metabolizable naturally occurring oils such as squalene, and are stabilized by nonionic surfactants such as Tween 80 and Span 85. They have been shown

to enhance antibody responses and allow for antigen dose sparing particularly seasonal and pandemic influenza vaccines, using MF59 (Fluad, Focetria), AS03 (Pandemrix), and AF03 (Humenza) as adjuvants. Oil-in-water emulsion can have a deleterious effect on antigen stability depending on the nature of the antigen and has not yet been shown to improve antigen stability. Their mechanism of action may vary depending on the emulsion considered. Post H1N1pdm09 vaccination, reports of narcolepsy caused great concern. Narcolepsy was observed following the use of ASO3 adjuvanted H1N1sw vaccine in several European countries, including Sweden, Finland, and the UK. The current hypothesis points towards a role of a CD4 T cell mimicry sequence in the nucleoprotein and neuraminidase proteins of A/H1N1pdm09. The role of ASO3 adjuvant cannot be excluded. The H1N1pdm09 vaccine is recommended for individuals above 20 years of age by EMA, but is not in use anymore (see ► Chap. 14).

5.6 Virosomes

Virosomes are liposome-based formulations that can incorporate hydrophobic components within their membrane and hydrophilic ones as a cargo within the particle internal volume. They can act both as antigen carrier and adjuvant through the incorporation of immunomodulatory molecules.

In the case of Inflexal (seasonal influenza vaccine), the virosomes are made up of empty influenza virus envelopes that present the HA antigen within their membranes.

The mode of action of virosomes is not yet understood. It is, however, hypothesized that it relies on binding to macrophages and APC membranes, leading to the engagement of the innate and adaptive immune mechanisms.

5.7 TLR4 Agonists and Adjuvant Systems

At the forefront of PRRs are detoxified congeners of endotoxin that stimulate TLR4. Present in Cervarix, one of the human papilloma virus vaccines, it is derived from lipopolysaccharide, the *Salmonella minnesota* lipopolysaccharide, through a specific process that allows for a very significant reduction of its pyrogenicity (2–3 log) while retaining its adjuvant effect. In this vaccine, monophosphoryl lipid A (MPL) is combined with aluminum hydroxide and is known as AS04 adjuvant. Its mode and mechanism of action have been thoroughly evaluated. The efficacy and safety report in the target population has allowed for vaccine registration worldwide, making AS04 the first adjuvant, other than aluminum salts, to be present in a licensed vaccine in the USA.

5.8 Additional Adjuvants in Development

Building on the successful results obtained with MPL, and a better understanding of the mechanisms of action of the current immunomodulators, a number of additional adjuvants are being evaluated in the context of various vaccines.

5.8.1 Defined Agonists of PRRs

Numerous PRR agonists targeting TLRs, NOD-like receptors, or retinoic acid-inducible gene (RIG)-like receptors have been evaluated in adult human clinical trials. Several TLR agonists such as double-stranded ribonucleic acid (dsRNA), flagellin, single-stranded RNA, or CpG have demonstrated different levels of activity. Several have also been shown to be capable of inducing an effective immune response in animal models, including mucosal adjuvants. Those capable of targeting the endosomal compartment have demonstrated the most robust impact on cellular immunity so far.

5.8.2 Saponins

As most of the adjuvants used or developed for human vaccines have shown strong local

reactogenicity, efforts have been undertaken to purify out from the mixture a specific molecule (QS21) that presents the optimum ratio between adjuvant effect and low local reactogenicity. This, however, was not sufficient to fully abrogate the lytic activity observed, and improvement through formulation was developed. The ability of Quil-A saponins to interact strongly with cholesterol was the cornerstone of the two formulations that were developed: one, known as ISCOMs/ISCOMATRIX, uses specific fractions of Quil-A; the other uses specific cholesterol-containing liposomes that are able to completely quench the lytic activity while retaining the adjuvant activity. This later adjuvant combined with MPL is known as AS01 and is present in the malaria candidate vaccine RTS,S, as well as the recombinant zoster vaccine. AS01 acts through the TLR4 activation capability of MPL and increases APC recruitment and activation, leading to a stronger and more persistent immune response.

5.8.3 Particulates

The use of particulates in vaccines goes back to the early 1920s when G. Ramon, then at the Pasteur institute, developed a method of increasing the production of hyperimmune sera while avoiding the frequent abscesses observed in horses after toxoid administration. It is the adsorption of antigens on those particles that increases the immune response (the principle used for aluminum salts) and decreases or prevents abscesses by the concomitant adsorption of endotoxins. Biodegradable polymers (such as polylactic, polyglycolic) have been extensively explored with the hope of designing nano- or microparticles, where the antigens could be entrapped within or adsorbed on their surface. This should allow for a slow release of the antigens, leading to a single-shoot vaccine approach. Those polymers, however, due to their sensitivity to hydrolysis, need to be lyophilized and kept in a humidity-controlled environment until use.

Recent advances in polymer synthesis and particle engineering have allowed for the development of delivery systems with defined size, shape, and components, allowing for an approach tailored to the antigen to be delivered and cell or cell compartment to be targeted. This has the potential for a rational design approach to the field of vaccine delivery systems.

5.9 Specific Needs for the Pediatric Population

Today, pediatric populations are the primary beneficiary of vaccination, whereas most adjuvant research and development is done for vaccines to be used in older populations. As many of the adjuvants described above can have a varying impact on immunogenicity and reactogenicity when applied to younger populations, a better understanding of the immune status and its evolution across ages, in addition to the impact of adjuvants in those settings, is critical to understand how adjuvants may be best used in children.

5.9.1 Immunogenicity

The emergence and development of new tools first applied to drug discovery such as medicinal chemistry for the design and synthesis of molecules tailored to the need for early life immunity, their evaluation in high-throughput models based on infants' leukocytes, and their optimization through modern computational algorithms can reasonably be seen as the next step toward the rational design of adjuvants for all target populations, including pediatrics.

The evaluation of a vaccine's immunogenicity and efficacy in animal models predictive of infant human populations can be expensive and unpredictable. In vitro approaches, which have the potential to accurately reflect the in vivo activity of those adjuvants in the target population, would allow for a rational design and selection of the adjuvant to be used and a focused preclinical evaluation. Given the leaps that are being made today, both in fundamental science and in technology development such as organ on a chip, these approaches may be a reality in the near future.

5.9.2 Reactogenicity

A key concern regarding adjuvanted vaccine development is reactogenicity, i.e., the ability of a formulation to cause acute inflammatory events locally or systemically (such as fever). Their optimization may require adaptations such as modifying their pharmacokinetic properties to affect their biodistribution or tailoring the formulation to ensure co-delivery of the antigen(s) and adjuvant to the same APC. The discovery of biomarkers as surrogate markers of in vivo reactogenicity would allow for the rational screening of potential candidates and accelerate the selection of the optimal candidate for a specific vaccine.

Vaccines and Adjuvants

The emergence of SARS-CoV-2, its speed of spread, and case fatality in specific populations have prompted a fast track development of candidate vaccines. To date tens of vaccines are in clinical trials, based on classical technology platforms (recombinant antigens and adjuvant, attenuated or killed pathogens vaccines) or more pioneering ones such as mRNA and live vectors. Three vaccines are being developed based on the spike protein S adjuvanted with AS03, CpG or matrix adjuvant. Matrix adjuvant is a saponin lipid particle-based adjuvant which is combined to a subunit S protein in Novavax NVX-CoV2373 vaccine. It has demonstrated an 89.3% efficacy against the primary and England strains and a protection of 60% against the South African variant. Two other recombinant antigen vaccines based on S protein (Sanofi Pasteur and Medicago) are using AS03 (or matrix for Medicago). Both vaccines are in PIII clinical trials. It will be of value to see if when using AS03 adjuvant the immune response induced will allow for a broader protection against the emerging variants.

To date, mRNA vaccines have demonstrated their ability to be produced and delivered faster than any current platforms. Long-lasting protection as well as reactogenicity profile is still to be established, and one cannot exclude that addition of adjuvant will be needed.

5.10 Conclusion

The emergence of new diseases that can affect populations of all ages worldwide, in addition to the re-emergence of childhood diseases, needs to be tackled using new or improved technologies. Adjuvants have been, for the past decades, one of the most promising advances in the development of new or improved vaccines. They have been developed and tested to a large extent for and in the adult population. Little has been done to specifically design adjuvants that are best suited to pediatric populations, in part because of the less advanced understanding of the pediatric immune system and the challenges posed by the small size of infants.

Given the evolution of knowledge and technologies observed during the last few decades, it is possible today to envision the identification of biomarkers predictive of better safety and immunogenicity that allow for their targeted use in pediatric populations when and where needed.

Further Reading

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