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Immune Response to Vaccine Adjuvants during the First Year of Life

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Abstract

Subunit vaccine formulations often include adjuvants that primarily stimulate innate immune cells. While young infants represent the major target population for vaccination, effective immunization in this age group remains a challenge. Many parameters of innate immune responses differ quantitatively and qualitatively from newborns to infants and adults, revealing a highly regulated developmental program. Herein, we discuss the potential implications of innate immune ontogeny for the activity of adjuvants contained in licensed infant vaccines, as well as future directions for rational design of adjuvanted vaccines for this age group.

Keywords

adjuvants; innate immunity; vaccines; newborn; infant; immunization

1. Introduction

The goal of immunization is the induction of an immune response to protect from infection or disease [1–3]. While immunogens contained in a vaccine provide the antigen-specific stimulus, vaccine adjuvants direct the quantity and quality of the ensuing immune response [2, 4, 5]. For most live vaccines, such as Bacillus Calmette Guérin (BCG), measles mumps and rubella (MMR), and varicella zoster virus (VZV), and for some inactivated vaccines, antigen and adjuvant activity reside in the same vaccine constituent [2, 4, 6]. However, vaccines consisting of purified microbial subunits often lack the necessary adjuvant activity to induce and optimally shape an immune response. Most of the vaccines currently given early in life are examples of such subunit vaccines [7–10]. Inclusion of adjuvants has been key to the efficacy of these subunit vaccine formulations [11].

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It is surprising that for most adjuvants we still lack insight into if and how their impact changes in an age-dependent manner [7, 12, 13]. Only in recent years has the development of the innate immune system, and in particular the response to danger- or pathogen associated molecular patterns (DAMPs or PAMPs) from birth onward received attention. We summarize here what is known about the ontogeny of the response to the most common adjuvants. Much of the literature we reviewed is based on *in vitro* studies of human cord or infant peripheral blood (or blood-derived leukocytes), and *in vivo* studies of newborn mice or adult humans. Clearly, a good deal of translational research in this crucial area remains to be undertaken. Accordingly, we also identify the pivotal areas of research that urgently need to be addressed in order to advance adjuvant, and with that vaccine, design from the empirical to the rational [14–16].

2. Developmental program of adjuvant responses early in life

2.1. Ontogeny of non-TLR adjuvant activity

Aluminium salts—Upon phagocytosis by antigen presenting cells (APCs), alum particles trigger lysosomal membrane damage and cathepsin B-dependent activation of the NALP3 (NLR family, pyrin domain containing 3) inflammasome [17]. This pathway is required for caspase 1-mediated processing and subsequent release of IL-1β, IL-18 and IL-33. While initial studies also indicated a role for the inflammasome for Alum adjuvanticity *in vivo*, this suggestion remains controversial, as subsequent reports did not confirm the initial findings [18, 19]. *In vitro*, Alum crystals also activate DCs through direct interactions with membrane lipids [20]. And *in vivo*, the effect of alum may be further mediated and/or amplified by mediators released by dying cells, such as uric acid [21] and DNA [22]. Alum-induced production of TNFα and IL-1β from human newborn and adult monocytes tested *in vitro* appears to be similar [Philbin 2012]. However, whole blood innate immune responses to Alum decreased with age in infancy in Papua New Guinean children [23]. This may imply that the adjuvant effect of alum in pediatric vaccines could be age-related. With respect to in vivo studies, Alum-adjuvated vaccines enhance Ab responses in neonatal mice (Ref Schallert et al Eur J Immunol 2002). Administration of Alum-adjuvanted pneumococcal conjugate vaccine at birth to human newborns induced antibody responses, but resulted in Th2 skewing of subsequent whole blood TLR-mediated cytokine responses in vitro, raising the possibility that Alum-adjuvanted vaccines may impact long term innate immune polarization (Ref Van den Bigelaar et al Vaccine).

Emulsion & Saponins—Oil-in-water emulsions such as MF59 and AS03 are licensed as adjuvants for seasonal and pandemic influenza vaccination. They are mainly composed of squalene, a cholesterol precursor and polysorbate [24]. Such emulsions trigger local recruitment of innate cells at the injection site and draining lymph node and enhance subsequent induction of antibody responses [25]. The adjuvant effect of MF59 on antibody production appears to be independent of the NALP3 inflammasome, while both MyD88-dependent signaling and apoptosis-associated speck-like protein containing CARD (ASC) may be required; however, their exact contributions currently are uncertain [26, 27]. MF59 adjuvanted vs. non-adjuvanted trivalent influenza vaccine resulted in higher antibody titres that were maintained for prolonged periods of time [24]. While the initially reported data

regarding the adjuvanted vaccine appeared promising, concerns raised upon further analysis preclude current assessment of efficacy or adverse events of MF59 adjuvanted vaccines administered in children < 1 year of age [28–30].

Saponins such as QuilA or QS21 have potent immunostimulatory capacities, potentiate antibody production and favor both CD4 and CD8 T cell responses [31]. The little that is known about their mechanism of action suggests that similar to Alum, saponins activate the NALP3 inflammasome pathway [32]. The new RTS,S malaria vaccine uses a combination of the TLR4 agonist monophosphoryl lipid A (MPL; see next section), liposomes and QS21, a saponin [33]. Based on an interim data analysis, the RTS,S vaccine administered to children as young as 6 weeks of age was well tolerated [Vekemans 2011], immunogenic [Horowitz 2012] and provided protection against severe malaria in African children [33]; however, pending the full results emerging towards the end of 2014, there currently hremain some uncertainties regarding overall efficacy [34].

Virosomes, delivery systems comprised of phospholipid membrane vesicles containing viral proteins that can fuse with target cells, have been effectively used as an adjuvantation system in human infants, as has been demonstrated with virosomal hepatitis A vaccine REF

2.2. Ontogeny of TLR-based adjuvant activity

TLR2 & Outer Membrane Protein (OMP)—The Neisserial outer membrane protein (OMP) complex has been used as a vaccine adjuvant in some *Haemophilus influenzae* type b (“Hib”) vaccines. Only years after widespread us of OMP-adjuvanted Hib vaccine in infants was it discovered that OMP is in fact a TLR2 agonist [35]. OMP induces up-regulation of co-stimulatory molecules such as CD86 on antigen-presenting cells [36], as well as homing receptor expression and IgA production [37]. The use of OMP as a carrier protein for Hib polyribosylribitol phosphate (PRP) displays a stronger response after the first dose given at 2 months of age as compared to PRP conjugated to tetanus toxoid (TT); however, upon subsequent boosting, OMP-PRP in contrast to TT-PRP did not result in further increases of antigen-specific titres [Granoff 1992; Bulkow 1993]. Furthermore, when Hib-OMPC was given to neonates, not only was antibody not induced, but little or no antibody was induced by booster immunizations in the first 6 months of life [38, 39]. The basis for the induction of persistent unresponsiveness/tolerance by Hib-OMPC is unknown, but could be related to the apparent propensity for TLR2 ligands to induce the immuno- suppressive cytokine IL-10 [40]. These studies suggest markedly different effects of the TLR2 ligand OMPC if given at 2 months vs. at birth.

TLR4 & Monophosphoryl Lipid A (MPL)—MPL is a TLR4 agonist adjuvant component of several licensed vaccines. MPL is a low-toxicity derivative of LPS that activates TLR4 preferentially via the TRIF-dependent pathway [41]. This mechanism of action appears to decrease inflammasome priming and IL-1β production, and thus reactogenicity, while maintaining overall adjuvanticity [42]. Adsorbed to alum, MPL it is part of human papilloma virus (HPV) and hepatitis B virus (HBV) vaccines (which has been given to HBV vaccine non-responders [Jacques 2002] or after liver transplantation [Di Paolo 2010]) enhancing antibody responses in comparison to alum alone [43]. MPL has also been

compounded with additional components, such as saponins providing a more potent immunostimulatory effect [44]. To our knowledge, there have been no published studies examining the early life ontogeny of the innate response to MPL. Only for LPS, MPL's parent compound, have *in vitro* studies examined the developmental change from birth onward. TLR4 and it's downstream signaling molecules are expressed and demonstrate sensor function at or near adult levels around birth and throughout the first year of life [45–48]. However, the functional consequences of TLR4 activation are distinct at birth vs. later in life, in part reflecting the impact of soluble plasma factors that contribute to neonatal cytokine polarization [49]. The production of cytokines following stimulation of whole blood with TLR agonists, that in turn influence the ensuing adaptive immune response [50–52], has been examined in several cohorts of newborns and infants [40, 53–63]:

- Overall pro-inflammatory responses of the innate immune system to LPS such as TNF-α and IL-1β are quantitatively below adult levels at birth, but reach adult level production by around 1 year of age.
- LPS-induced production of the anti-inflammatory cytokine IL-10 is similar to adults, but drops to below adult levels by 1 year of age.
- Production of IL-6 and IL-23 are at or even above those of adults at birth. However, the IL-6 and IL-23 responses to LPS then decline to below adult levels by 1 year of age.
- LPS is an overall relatively weak inducer of IFN-α, IFN-γ, or IL-12p70 (as compared to other TLR agonists); in general, the newborn and infant produces any of these three cytokines at much lower levels than the adult [64, 65].

This paradigm has found general experimental support [66, 67]. To the extent that *in vitro* assays of peripheral blood or blood-derived mononuclear cells predict *in vivo* responses to vaccine adjuvants, the developmental trajectory for the LPS response would predict that immune responses to an MPL-containing vaccine would likely differ if given at birth vs. later in life. Thus, MPL-containing vaccines given neard birth may more likely support a Th17- or Th2-polarized response, instead of a Th1 type response. Of note, MPLA-adjuvanted RTS, S malaria vaccine was safe and effective in children 6 to 12 weeks of age (Ref Agnandji, ST NEJM 2011 365:1863). Further studies on the ontogeny of MPLA activity in vitro and in vivo are needed.

Other TLR agonists under biopharmaceutical development: TLR3, TLR7/8, and TLR9—A subset of TLRs, including TLRs -3,-7,-8 and -9, are expressed in endosomal compartments and serve to recognize microbial nucleic acids. TLR7 and 9 are expressed primarily on plasmacytoid DCs (pDCs) that produce key immunomodulatory cytokines, including type 1 IFN important for antigen cross-presentation and other aspects of cell-mediated immunity [68]. A TLR3 synthetic adjuvant enhances innate and adaptive immunity similar to live viral infection in adult volunteers [69]. *In vitro* experiments employing whole blood or PBMCs indicate that a TLR3 agonist (pI:C) induces very high IFNα, IFNγ and IL-12p70 production in adults, with very few other cytokines being produced. Studies on the ontogeny of the TLR3-mediated responses indicate that newborns and infants up to 1 year of age produce these three cytokines at much lower levels as

compared to adults [40, 56]. Presuming that responses of whole blood or mononuclear cells *in vitro* predict *in vivo* responses to adjuvant-containing formulations, these observations would suggest that TLR3 targeting adjuvants would be unlikely to significantly enhance vaccine-induced immune responses if given in the first year of life. This however, has not been tested *in vivo*, and thus is speculative extrapolation from the limited data that currently are available.

The TLR7 agonist imiquimod, a low molecular weight synthetic imidazoquinoline compound, is FDA-approved as a topical antiviral agent to treat human papilloma virus (HPV) infection (warts). In a human adult clinical trial, topical administration of imiquimod as adjuvant followed by injection of vaccinal antigen through the treated skin enhanced recruitment of mononuclear cells, activation of dendritic cells and enhanced both humoral and cellular adaptive immunity *in vivo* [70]

TLR8 agonists are in development for a variety of indications [71]. Yellow Fever vaccine, which activates multiple TLRs, including TLR8 and appears to be safe in infants over 9 months of age [72]. BCG, which also engages multiple TLRs including TLR8 [73], is generally safe and effective at birth [74]. Although selective molecular agonists of TLR-7 and -8 have promise, as their *in vitro* activity exceeds that of Alum and several other TLR agonists [75], they have yet to be tested in newborn or infant animals that express TLR8 that is similar in function to that of humans [76]. *In vitro* stimulation of cord or peripheral blood with TLR7/8 induces the broadest cytokine response of all tested TLR agonists, and importantly does so in the newborn, infant and adult [40, 61, 77]. However, there are important qualitative differences in the response of the various age groups to TLR7/8 stimulation. So far as is known, in response to *in vitro* TLR7/8 stimulation

- Production of IL-6 at birth is similar to adults, but subsequently drops to below that of adults by 1 year of age.
- IL-23 production at birth if is above that of adults; and while it declines from the neonatal high, it remains above that of adults even at 1 year of age.
- TNF-α and caspase-1-mediated IL-1β production is strong even at birth, far exceeding that induced by Alum [75], but depending on the study at or below adult levels; however, it reaches adult level production by ~ 1 year of age.
- IL-10 is produced by the neonate at levels far above those of the adult; and while it declines from this neonatal high, IL-10 production remains above that of adults even at 1 year of age.
- Even IFN-α, IFN-γ, and IL-12p70 are induced, however at levels below those of the adult; as for TLR9, only IFN-α production reaches adult levels within the first few weeks of life, while those of IFN-γ and IL-12p70, remain below adult levels even at 1 year of age.

Overall, these findings regarding the development of TLR7/8 indicate that this pathway is one of the most active around birth and in the first year of life, and thus promises to provide potential adjuvant activity for neonatal and early life vaccination. However, to date, no clinical data or even appropriate animal data are available to determine if the theoretical

benefits of selective TLR7/8 molecular agonists as a neonatal and early life adjuvant can materialize.

While newborn cord blood pDCs produce significantly less IFN-α following CpG (TLR9) stimulation, this response rapidly reaches adult levels within the first few weeks of life [40, 53, 60, 80]. In addition, TLR9 stimulation also induces a strong IL-10 response in the newborn, which does not occur in the adult; the cellular source of the IL-10 in response to CpG at birth has not been identified. These findings argue that TLR9 based adjuvants could possibly substantially aid vaccine responses if given after the first few months of life. In murine models, TLR9 agonists such as CpGs have adjuvant activity *in vitro* and *in vivo* [78]. Indeed, in studies of newborn mice, CpG can circumvent Th2 Polarization of Neonatal Responses to Vaccines, but apparently does not fully redirect Th2 Responses after neonatal priming (REF Kovarik et al). In humans, immune-enhancing effects of CpG-based adjuvants have been demonstrated *in vivo* in HIV-infected individuals [79]. Thus, TLR8- and TLR9-based adjuvants hold some promise for early life immunization.

3. Safety considerations

Safety is paramount in vaccine development, especially for newborns/infants. Concerns have been raised that powerful adjuvantation systems may increase risks of reactogenicity, systemic inflammation, and possibly even autoimmune diseases. The potential risks of exposing newborns and infants to pro-inflammatory stimuli, especially if they result in systemic inflammation, are illustrated by a murine study indicating that systemic, repetitive, high dose exposure to TLR2 agonists can result in perinatal brain injury [81]. However, in order to support efficient cellular responses, for example in the context of malaria, TB or HIV, triggering multiple signaling pathways through combinations of TLR agonists or use of live vectors may be required. The safety and efficacy of neonatal BCG, a live vaccine containing multiple TLR agonists, is encouraging and suggests that adjuvants based on TLRs or other PAMPs and DAMPs could be tested in clinical trials. However, the potential relationship between vaccination and autoimmunity, although they have yet to be causally linked, continues to require careful risk assessment [82]. As reviewed in this article, the quality of the innate immune response triggered in infants and adults differs from one another, and changes significantly over at least the first few years of life. Hence, there is an unmet need for age-specific considerations for each new formulation. For example, there is potential risk for Th17-biased hyperinflammatory responses as a high IL-23 to IL-12 ratio is generally observed until the age of one year. In contrast, the risk associated with type I IFN may be limited, as both IRF3-dependent production by myeloid cells and IRF7-dependent production of IFN by plasmacytoid cells tend to be reduced very early in life. A key focus in developing safe and effective adjuvants is localizing the adjuvant effect in both space (i.e., local site of administration) and time. An example is the recent development of 3M-052, an imidazoquinloine TLR7/8 agonist that is chemically substituted with a lipid tail in order to in crease hydrophobicity and local tissue retention, thereby retaining efficacy but avoiding systemic cytokine induction (REF Smirnov et al Vaccine 2011).

4. Implications & future directions regarding use of adjuvants in early life

Adjuvants have been used for nearly a century and by now have an established track record of having helped save billions of lives [83, 84]. Thus, there clearly is no question that adjuvants contained in our current vaccines work even if given early in life [7–9, 12, 13]. However, we do not know if the adjuvants we currently give, nor the age when they are given are optimal. While we now have the tools at hand to identify age-dependent changes in adjuvant activity [15], rational vaccine design for early life [14, 16] is complicated by the fact that from birth throughout infancy the immune response to innate stimuli and thus adjuvants appears to rapidly change, i.e. represents a moving target [23, 40]. We currently possess insufficient insight into the mechanistic underpinnings that drive these changes in early life immune ontogeny and therefore cannot predict their specific function or time of change, nor the consequence of changing specific developmental trajectories. In the absence of such insight, vaccine design would remain confined to the empiric. We must unravel the molecular basis of early life immune ontogeny and establish *in vitro* platforms that accurately model this process if we are to efficiently develop safe and effective vaccine adjuvants for use in newborns and young infants.

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