0031-3998/09/6505-0098R
PEDIATRIC RESEARCH

Vol. 65, No. 5, Pt 2, 2009
Printed in U.S.A.

Developmental Biology of the Innate Immune Response: Implications for Neonatal and Infant Vaccine Development

VICTORIA JANE PHILBIN AND OFER LEVY

Department of Medicine, Children's Hospital Boston and Harvard Medical School, Boston, Massachusetts 02115

ABSTRACT: Molecular characterization of mechanisms by which human pattern recognition receptors (PRRs) detect danger signals has greatly expanded our understanding of the innate immune system. PRRs include Toll-like receptors, nucleotide oligomerization domain-like receptors, retinoic acid inducible gene-like receptors, and C-type lectin receptors. Characterization of the developmental expression of these systems in the fetus, newborn, and infant is incomplete but has yielded important insights into neonatal susceptibility to infection. Activation of PRRs on antigen-presenting cells enhances costimulatory function, and thus PRR agonists are potential vaccine adjuvants, some of which are already in clinical use. Thus, study of PRRs has also revealed how previously mysterious immunomodulators are able to mediate their actions, including the vaccine adjuvant aluminum hydroxide that activates a cytosolic protein complex known as the Nacht domain leucine-rich repeat and pyrin domain-containing protein 3 inflammasome leading to interleukin-1β production. Progress in characterizing PRRs is thus informing and expanding the design of improved adjuvants. This review summarizes recent developments in the field of innate immunity emphasizing developmental expression in the fetus, newborn, and infant and its implications for the design of more effective neonatal and infant vaccines. **(*Pediatr Res* 65: 98R–105R, 2009)**

Need for Effective Neonatal and Infant Vaccines

On a global basis, infections result in ~2 million deaths per year in those younger than 6 mo (World Health Organization) (1). Common pathogens in neonates and/or infants include Gram-positive bacteria (*e.g.*, group B Streptococcus, *Streptococcus pneumoniae*), Gram-negative bacteria (such as *Escherichia coli* and *Bacillus pertussis*), herpes simplex virus, respiratory syncitial virus, and rotavirus. This susceptibility highlights the unmet need for effective vaccines for newborns and infants and the functional immunodeficiencies that must be overcome in designing vaccines that adequately protect the very young. As birth is the most reliable point of healthcare contact worldwide, vaccines that are active at birth are a major global health imperative (2). Design and development of such vaccines will require understanding of the developmental expression of innate immune pathways whose activation enhances the adaptive immune response.

Received November 25, 2008; accepted January 24, 2009.

Correspondence: Ofer Levy, M.D., Ph.D., Division of Infectious Diseases, Children's Hospital Boston, 300 Longwood Avenue, Boston, MA 02115; e-mail: ofer.levy@childrens.harvard.edu

Supported in part by AHA postdoctoral fellowship (V.J.P.) and NIH R01 grant AI067353, the Patterson Trust, reagent support from 3M Pharmaceuticals, and a sponsorship by Idera Pharmaceuticals.

Distinct Aspects of Neonatal Innate Immunity That Pose Challenges to Effective Vaccination Early in Life

The fetal immune system is heavily Th2-biased, presumably to avoid proinflammatory Th1-type alloimmune responses to maternal tissues that may trigger preterm birth or spontaneous abortion.

Birth triggers a dramatic shift in environment that places further demands on the neonatal immune system, mediating the transition from a normally sterile intrauterine compartment to a foreign antigen (Ag)-rich external environment. This transition includes the first colonization of the skin and gastrointestinal tract. In contrast to low levels of Th1-type cytokines [*e.g.*, tumor necrosis factor (TNF), interleukin (IL)-12p70, interferon (IFN)-γ], human neonatal plasma contains high levels of the Th2-type cytokine IL-6 *in vivo* at birth and throughout the first week of life (3). IL-6 induces an acute phase response at birth that may serve to shield against bacterial infections and clear microbial products and/or pattern recognition receptor (PRR) agonists (4).

The distinct polarization of fetal and early neonatal immune responses presents obstacles to effective neonatal immunization, including impaired antigen-presenting cell (APC) responses (*e.g.*, impaired IFNγ production) to many (but not all) stimuli, a Th2 bias to immune responses, impaired antibody (Ab) affinity maturation (5), and the potential inhibitory effect of maternal Abs (6).

Quantitative and qualitative differences exist between neonatal and adult APCs. Qualitative differences are evident in human monocytes *in utero* as assessed by flow cytometry indicating reduced expression levels of major histocompatability complex (MHC) class II molecules (7). Several mechanisms have been implicated in skewing neonatal APCs toward Th2-type responses, including a) the production by placental tissues of transforming growth factor-β, progesterone and prostaglandin E2 that enhance Th2 cytokine production (8) and b) the presence in neonatal blood plasma of relatively high concentrations (~300 nM) of adenosine, an immunosuppressive endogenous purine metabolite (4,9). The patterns of neonatal cytokine production appear to be relevant *in vivo*, in that after birth, during the first week of

Abbreviations: APC, antigen-presenting cell; **BCG,** Bacille Calmette-Guerin; **MyD88,** myeloid differentiation factor 88; **NALP3,** Nacht domain leucine-rich repeat and PYD-containing protein 3; **RLRs,** retinoic acid inducible gene-like receptors; **TLR,** Toll-like receptor; **Treg,** T-regulatory cell

life, human neonatal peripheral serum levels of TNF remain low (relative to human adult serum) whereas levels of IL-6, a Th2-polarizing cytokine with anti-inflammatory properties, increase.

Multiple studies document that human neonatal monocytes and APCs function suboptimally when tested *in vitro* with respect to costimulatory responses to most stimuli (10). Studies of murine neonatal dendritic cells (DCs) have demonstrated that Ag-presentation increases during ontogeny, correlating with increased costimulatory molecule expression and increased responses to protein-conjugated, T cell-dependent polysaccharide Ags (11). However, C57BL/6 neonatal mice (1–7 d) demonstrated stronger LPS-induced inflammatory cytokine production by splenocytes in the presence of T cells *in vitro* and after intraperitoneal injection *in vivo*, ascribed to neonatal quantitative deficiency of $CD4^+$ and $CD8^+$ T cells (12). Thus, inflammatory responses in neonates seem to be dependent on the species [noting that innate immune genes are hypervariable between species, including between humans and mice (13,14)], experimental model (*in vitro versus in vivo*), extracellular culture medium [autologous 100% (vol/vol) adenosine-rich neonatal plasma/serum (15) *versus* low concentrations of heat-inactivated fetal calf serum that are used in many studies], and particular stimulus studied.

In contrast to neonatal APCs, neonatal $CD4^+/CD25^{high}$ T regulatory cells (Treg) are fully functional and found at high abundance in human fetal lymphoid tissues (16) and newborn cord blood (17,18). Neonatal Treg cells suppress both T-responder cell proliferation and Th1-cytokine (*e.g.*, IFN-γ) production, induced by self-Ags, to limit adaptive immune responses. Treg mediate their effects by both cell contact-dependent and contact-independent mechanisms, including secretion of IL-10, CD39, and CD73 (ectonucleotidase)-mediated generation of extracellular adenosine, and adenosine A2A receptor-mediated enhancement of cyclic AMP concentrations in target T-responder cells (19,20). Neonatal inhibition of autoimmunity *via* Treg suppression has clear advantages as neonates first encounter the foreign-Ag rich world; however, these effects may be detrimental to neonatal immunity to infection (21) and to neonatal vaccine responses (22,23).

Innate Immune Activation Enhances Adaptive Immune Responses

Activation of PRRs on APCs such as macrophages and DCs enhances Ag-presenting activity and adaptive immune responses *via* direct and indirect mechanisms (24). PRR signaling is influenced by cross signals mediated *via* diverse PRR classes, tyrosine kinases, tyrosine phosphatases, ubiquitinating systems, and glucocorticoids, and therefore varies between different cell types (25). On activation, DCs efficiently process and present Ags in the context of MHC, increase production of Th1-polarizing cytokines such as IL-12p70, and up-regulate costimulatory molecules (*e.g.*, CD40, CD80, CD86) and chemokine receptors mediating cell migration from the tissues into the draining lymph nodes. Once inside the lymph nodes, DCs interact with naïve T and B cells inducing their differentiation into effector cells, thereby triggering an acquired immune response.

In addition to their direct effect on APCs, PRR agonists also enhance the transition from innate to adaptive immune responses *via* indirect mechanisms. For example, Toll-like receptor (TLR)-mediated DC activation induces IL-6 that renders T-responder cells refractory to inhibition by suppressive Treg (26). Tissue-derived signals may also influence APCs and control the type of effector class generated (27). TLR-mediated activation of DCs enhances lymph node function *via* triggering DC migration to lymph nodes, expression of vascular endothelial growth factor, increasing high endothelial venule proliferation, remodeling primary feed arterioles, and increasing nodal blood flow and recruitment of rare Ag-specific lymphocytes (28,29).

The impressive progress in defining the potential roles of PRRs has provided an opportunity to better understand the development of innate immune responses after birth, with potential implications for the optimization of vaccine development. Later, we review the current state of knowledge regarding the developmental expression of key PRRs, highlighting both recent progress and gaps in our knowledge.

Developmental Expression of PRRs

Responses to microbial infection are initiated through an innate immune system that features diverse PRRs poised for activation in extracellular and intracellular locations (Fig. 1).

Toll-like receptors. TLRs are type I transmembrane proteins with an extracellular amino terminus and an intracellular carboxy terminus. They are composed of various domains including extracellular leucine rich repeat (LRR) with one or two cysteine-rich regions and an intracellular toll/IL-1 receptor (TIR) domain, named after its homology with the IL-1

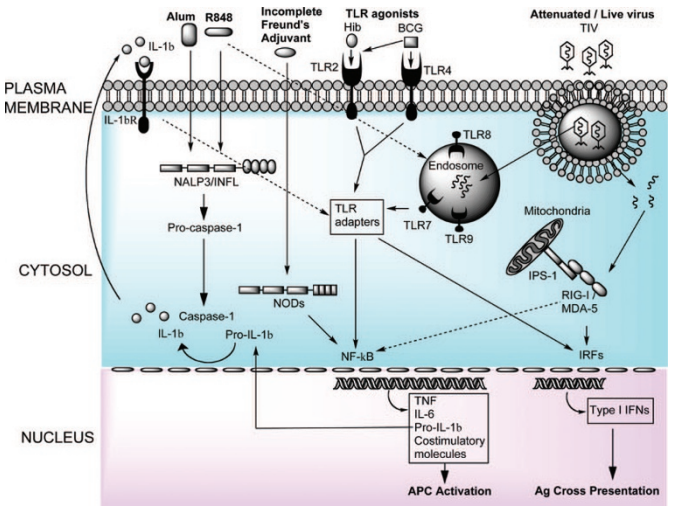

Figure 1. Mechanisms of innate immune activation induced by vaccine adjuvants. TLR agonists found in multiple neonatal vaccines (Table 1) activate either cell associated or intracellularly located TLRs or NODs. These PRRs then interact with specific adaptor molecules culminating in NF-κB or IRF activation. Viral-derived products (dsRNA or ssRNA) can also activate endosomal TLRs, along with RLRs (such as RIG-I), which induce type I IFN production. The vaccine adjuvant Alum activates the cytosolic NALP3/inflammasome leading to proIL-1β cleavage into bioactive IL-1β.

receptor. Humans express 10 TLRs: a) surface expressed TLRs include TLR2 (bacterial lipopeptides), TLR4 (lipopolysaccharide), and TLR5 (flagellin); b) endosomal TLRs include TLR7 and 8 [single stranded RNA (30,31)] and TLR9 (unmethylated CpG DNA). Engagement of TLRs activates intracellular signaling cascades *via* myeloid differentiation factor 88 (MyD88)-dependent and MyD88-independent pathways (32), including IL-1 receptor-associated kinase-4 (IRAK-4) recruitment, culminating in NF-κB activation and expression of proinflammatory cytokines, such as TNF, IL-6, and pro-IL1β [note that processing to mature IL-1β requires Nacht domain leucine-rich repeat and pyrin domain (PYD)-containing protein 3 (NALP3) inflammasome (INFL) action]. TLR stimulation can also lead to the activation of several other intracellular signaling pathways such as those involving Jun N-terminal kinase, mitogen activated protein kinase (33,34), interferon regulatory factor (IRFs), and the FAS-associated death domain-induced apoptosis pathway (32).

The importance of the TLR pathway for host defense in newborns and infants is apparent in the clinical consequences of TLR pathway defects. Defects of signaling molecules downstream of TLRs, including IRAK4 and MyD88 deficiency result in selective susceptibility to pyogenic infections (often streptococcal and staphylococcal) during childhood with improvement later in life (35–37).

Cord blood monocytes of full-term human newborns express normal amounts of TLRs, yet on TLR-mediated stimulation in whole cord blood (*i.e.*, 100% vol/vol autologous, adenosine-rich plasma), agonists of TLRs 1–7 demonstrate a 1–3 log impairment in TNF-α production relative to adult peripheral blood monocytes (15). One of the mechanisms accounting for this impairment is that during the hypoxia and stress accompanying the birth process, plasma concentrations of adenosine, an endogenous immunosuppressive purine metabolite, increase. Adenosine can act *via* A3 adenosine receptors on neonatal mononuclear cells to induce production of cAMP, a key second messenger that inhibits TLR-mediated production of Th1-polarizing cytokines (4,9). Other studies have demonstrated that LPS-induced responses of newborn mononuclear cells are diminished at birth because of reduced expression of the TLR adaptor molecule MyD88 (38) and by failure of nucleosome remodeling at the IL-12 promoter (39). Impaired TLR agonist-induced production of type I IFNs from human neonatal plasmacytoid DCs (pDCs) and neonatal monocyte-derived DCs (moDC) has also been described (40,41).

In contrast to agonists of TLRs 1–7, TLR8 agonists, including TLR7/8 agonists, are able to induce robust immune responses by human neonatal APC, comparable with those of healthy adult controls (42). Exposure of human neonatal cord blood-derived monocytes and APCs, including myeloid dendritic cells and moDCs, to TLR8 agonists induces robust (*i.e.*, adult level) phosphorylation of p38 MAP kinase, NF-κB activation, proinflammatory cytokine production (TNF, IL-12), and upregulation of costimulatory molecules (*e.g.*, CD40).

Retinoic acid inducible gene-like receptors. During the infection cycle of some viruses, double stranded RNA is produced that can be detected in the cell cytosol by retinoic acid inducible gene-like receptors (RLRs), such as retinoic acid inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA-5). These cytoplasmic proteins are composed of amino terminal caspase-recruitment domains (CARDs) and a carboxy-terminal helicase domain (43,44). RLR activation induces CARD domain interaction with the CARD domain-containing adaptor protein, IFN-β promoter-stimulator (IPS)-1 (also known as mitochondrial antiviral signaling protein, virus-induced signaling adaptor, and CARD adaptor inducing IFN-β) leading to IRF3 and NF-κB activation. Little is known regarding the developmental expression of RLRs at birth and in the neonate, an important area of future study.

Nucleotide oligomerization domain-like receptors. Nucleotide oligomerization domain-like receptors (NLRs) detect bacterial components and include members of the nucleotide-binding oligomerization domain (NOD) subfamily and NLRs associated with the INFL. The NOD proteins in humans are a family of $>$20 cytosolic proteins. Structurally they are composed of: a) a variable N-terminal effector-binding domain, usually consisting of a PYD or CARD, capable of regulating homotypic and heterotypic binding; b) a centrally located NOD domain; and c) a C-terminal ligand-recognition domain, which can be composed of LRRs. As with the RLRs, little is known regarding the developmental expression of NODs at birth and in the neonate.

The INFL is a cytosolic multicomponent protein complex, including caspase-1, which on activation cleaves pro-IL-1β to the potent proinflammatory cytokine IL-1β. IL-1β has a central role in the onset of parturition, particularly in the context of intrauterine infection/inflammation. A recent study, found that: a) caspase-1 concentration in the amniotic fluid increases as a function of gestational age; b) women with spontaneous term labor had a higher median caspase-1 amniotic fluid concentration than women at term without labor, suggesting that the INFL may be activated in spontaneous parturition at term; and c) higher caspase-1 levels were associated with infection/inflammation (45). Human neonatal monocytes, in particular those of preterm newborns, demonstrate impaired IL1-β production to LPS (45a) and to lipoteichoic acids (45b). A search of Pubmed did not yield any publications relating to INFL function at birth.

C-type lectin receptors. C-type lectin receptors (CLRs) can be produced as secreted soluble proteins, including mannose binding lectin (MBL), lung surfactant protein A, or as transmembrane proteins such as selectins, mannose receptor, and the DC-specific ICAM-3 grabbing nonintregrin (DC-SIGN). MBL is an acute phase plasma protein whose expression in the liver is upregulated during inflammation. MBL recognizes a variety of carbohydrate patterns found on infectious microorganisms including bacteria (*e.g.*, *Staphylococcus aureus*), fungi (*e.g.*, *Saccharomyces cerevisiae*), and viruses (*e.g.*, HIV) and on altered selfglycoproteins (*e.g.*, aberrant glycosylation of cancer cells). MBL binding to carbohydrate targets triggers activation of MBL-associated serine protease, cleaving complement proteins, and promoting opsonisation/membrane attack complex formation (46). MBL can also act directly as an opsonin by binding to receptors, which promote

phagocytosis (46) and can modulate cytokine production *in vitro* and *in vivo* (47) thereby playing important roles in neonatal infection. Plasma MBL concentrations in both premature and full-term neonates are lower than those of adults (48), but steadily increase during the first weeks of life, possibly as a consequence of the skew of neonatal cytokine responses toward IL-6, which induces the acute phase response (3,4). MBL deficiency is associated with an increased risk of bacterial sepsis (49).

From the standpoint of innate immune modulation of vaccine responses, MBL deficiency enhances mouse Ag-specific IgG production after immunization with tetanus toxoid-conjugated group B *Streptococcus* polysaccharide vaccine or tetanus toxoid alone (50). These data suggest that under certain circumstances MBL can inhibit Ab production. Further characterization of the MBL pathway may inform design of vaccines that minimize such inhibitory MBL effects.

Developmental expression of antimicrobial proteins and peptides. APPs expressed by leukocytes and epithelial cells are ancient components of innate immune defense that are best known for their ability to kill microorganisms and neutralize their surface components. However, some APPs also demonstrate activity in modulating adaptive immune responses. For example, bactericidal/permeability-increasing protein (BPI), a neutrophil-derived primary granule protein with endotoxin binding activity, was recently shown to enhance APC function (50a). Neonates are deficient in BPI expression, increasing the possibility that may also impair delivery of naturally shed Gram-negative bacterial LPS outer membrane blebs to APCs. Defensin peptides can enhance production of antiviral IFNs, which are important to Th1 polarization and cross-presentation (51). Cathelicidin peptides, found in neutrophil secondary granules, enhance adaptive immune responses *in vivo*. Indeed there is an age-dependent maturation in the expression in human neonatal blood plasma of a broad range of APPs, and thus to the extent that these molecules contribute to modulating adaptive immune responses, deficiencies in their expression could contribute to impaired adaptive responses.

The recent progress in defining PRRs and APPs involved in triggering innate immune responses and thereby modulating adaptive immunity provides new opportunities to understand that function of adjuvants in currently administered vaccines, as outlined below.

Engagement of Innate Immunity by Currently Approved Neonatal and Infant Vaccines

It is increasingly appreciated that an important determinant of vaccine efficacy is the ability of a given vaccine to activate the innate immune system to enhance APC function and Th1-polarizing adaptive responses (52). Currently, the two vaccines that are regularly given at birth in humans are the Bacillus Calmette-Guerin (BCG) vaccine and the hepatitis B (HepB) vaccine (Table 1). BCG is capable of inducing a strong Th1-type immune responses in human neonatal cells *in vitro* (53) and *in vivo* (54), which is in part due its ability to activate multiple TLRs expressed by APCs (55). The BCG vaccine demonstrates that under certain conditions, the human neonatal immune system is capable of mounting a protective Th1-type response (56), but the underlying mechanisms of its efficacy at birth is not fully understood. Recent work has indicated that it is very likely that activation of multiple PRRs by BCG plays important roles in its efficacy. The BCG vaccine is a live bacterium and able to activate TLR2 (57,58) and TLR4 (57,58) by virtue of its cell wall that consists of

Table 1. *Activation of PRRs by approved pediatric vaccines (0–2 y)*

Vaccine	First administration	Adjuvant	PRR	References
Hepatitis B (Hep B) vaccine	Birth	Alum	NALP3/INFL	67, 68
Bacille Calmette-Guerin (BCG)	Birth (not in US)	CWS*	TLR2	57–60
		CWS*	TLR4	57–59
		ssRNA	TLR8†	61
		CpG DNA	TLR9†	60
			NODs, CTLs†	97
Diphtheria, tetanus, acellular pertussis (DTaP) vaccine	2 mo	Alum	NALP3/INFL	67, 68
Haemophilus influenzae type b conjugate vaccine (Hib)	2 mo	Alum	NALP3/INFL	67, 68
		OMPC	TLR2	64
			TLR4†	63
Pneumococcal conjugate vaccine (PCV7)	2 mo	Alum	NALP3/INFL	67, 68
Trivalent inactivated Influenza vaccine (TIV)	6 mo	dsRNA	TLR3†	98
		ssRNA	TLR7	30, 99
		ssRNA	TLR8†	31
		dsRNA	RIG-1†	100
Measles, mumps, Rubella (MMR) vaccine	12 mo	hemagglutinin protein (Measles)	TLR2†	65
		dsRNA (Measles)	MDA-5†	66
Varicella vaccine	12 mo	?	TLR2†	101
Hepatitis A (HepA) vaccine	12 mo	Alum	NALP3/INFL	67, 68

* CWS consists of a purified noninfectious material consisting of peptidoglycan, arabinogalactan, and mycolic acids.

† PRR activation inferred based on published engagement of PRRs by wild-type strains. Note: the minimum age is given for vaccination (102).

OMPC, outer membrane protein complex; INFL, inflammasome; Alum, aluminium hydroxide; MDA-5, melanoma differentiation-associated gene 5; CTLs, C-type lectin receptor; CWS, cell wall skeleton; ss, single stranded.

peptidoglycan, arabinogalactan, and mycolic acids (59), and TLR9 through its CpG-rich DNA (60). More recently, the possibility that BCG also engages TLR8 has also been raised based on associations of TLR8 polymorphisms with susceptibility to pulmonary tuberculosis, increased susceptibility in males (TLR8 is encoded on the X chromosome), and the ability of BCG to induce macrophage TLR8 expression (61).

Hepatitis B virus (HBV) is a global public health threat that has chronically infected >350 million people worldwide (62). The HepB vaccine is composed of a virus-like particle containing the viral envelope protein hepatitis B surface Ag (HBsAg), prepared using recombinant DNA technology. HepB vaccine is composed of an aluminum-containing ajuvant, but is still incompletely immunogenic as ~10% of vaccinated populations fail to mount immune responses to HBsAg after immunization (Table 1).

Haemophilus influenzae type b (Hib) conjugate vaccines are initially administered at 6–8 wk of age. Hib activates transfected HEK293 cells in a TLR2-dependent and TLR4-dependent manner, likely reflecting expression of bacterial lipopeptides (TLR2) and lipopolysaccharide (TLR4) (63,64) (Table 1). Indeed, the outer membrane protein complex (OMPC) found within the Hib-OMPC glycoconjugate vaccine is TLR2 and MyD88-dependant, and in the absence of TLR2, the immunogenicity of the Hib-OMPC vaccine is significantly reduced (64).

In addition to TLR-induced vaccine responses (65), activation of RLRs [RIG-I and MDA-5 (66)] has been reported for the wild-type measles virus, suggesting that the attenuated strains used for vaccination may also engage this innate immune pathway (Table 1).

To date, the US Food and Drug Administration (FDA) have approved a limited number of human vaccine adjuvants, chief among which is aluminum hydroxide, aluminum phosphate (typically referred to as "Alum"). Alum is a commonly used adjuvant present in human and animal vaccines worldwide (HepB, DTaP, Hib, PCV7, and HepA; Table 1) and is known for its ability to induce protective Th2-type responses. It was recently demonstrated that the key to Alum's adjuvant activity is its ability to activate the NALP3/INFL (67,68) (Fig. 1). These conclusions were based on the observations that IL-1β and IL-18 production by macrophages in response to Alum *in vitro* requires intact INFL signaling. Moreover, mice deficient in NALP3, apoptosis-associated speck-like protein containing a CARD or caspase-1 failed to mount a significant Ab response to an Ag administered with Alum, whereas the response to complete Freund's adjuvant remained intact. These data highlight the crucial role of the NALP3/INFL in Alum's adjuvant activity.

MF59 is an oil-in-water squalene emulsion and the precise mechanism of its adjuvant effects is still largely unknown (69). Recent studies have revealed that MF59 is a very efficient adjuvant due to its ability to activate and sustain tissue-resident DCs (69a). Studies using fluorescently labeled MF59 injected intramuscularly then observed 2 d later indicated partial localization in T cell areas of the draining lymph node (70). MF59 has also been documented to induce a significant influx of macrophages to the site of infection in mice, which is chemokine receptor 2 dependant (71). Of interest, the oil emulsion incomplete Freund's adjuvant (not licensed in humans due to its toxicity) induces optimal IgG1 and IgG2c in a NOD2-dependant manner (72).

Translational Research Toward Improved Adjuvants for Newborns and Infants

Given that newborns and young infants are susceptible to multiple bacterial and viral pathogens, neonatal immunization is of particular importance because: a) birth represents a likely point of contact of the newborn with healthcare providers, b) early life immunization is associated with a substantially higher rate of vaccination coverage than immunization given at later time points (2,73), c) vaccines that require multiple dosing regimens throughout infancy increase cost and reduce compliance, and (d) strategies involving immunization of the mother pose substantial logistical and medico-legal challenges. There is, therefore, an unmet medical need to develop vaccines that would be more effective very early in life or that would require fewer doses to generate durable and protective immune responses.

A novel approach to neonatal vaccination has recently been described, which uses an attenuated strain of the intracellular pathogenic bacterium *Listeria monocytogenes* to deliver Ag to the cytoplasm of APC (74). Vaccinated neonatal mice induced strong $CD8^+$ and $CD4^+$ T cell responses and were protected after wild-type challenge. Of note, *L. monocytogenes* is able to activate several PRRs including multiple NODs (IPAF, NALP3) (75) and can induce the expression of RIG-I (76), possibly accounting for its immunostimulatory properties. Therefore, this approach could potentially overcome preexisting hurdles of maternal immune response interfering with neonatal vaccine responses (77). This live attenuated vaccine seemed safe in this murine model in that there was no associated mortality and no bacteria were recovered from the spleens or liver of immunized newborn mice 7 d postvaccination.

Among the TLR agonists, TLR7/8 agonists hold particular promise as potential adjuvants for use in neonatal and infant vaccines as they induce robust production of the Th1-polarizing cytokines TNFα and IL-12 from neonatal (and adult) APCs that substantially exceeds responses induced by agonists of TLR-2, TLR-4, or TLR-7 (alone) (42,78). Therefore, TLR7/8 agonists have potential as novel neonatal vaccine adjuvants, due to their ability to activate both TLR-dependant and independent pathways (NALP3/INFL) mediating Th1-type responses from APC (78). In addition, human Treg express TLR8 and mediate reversal of Treg function on exposure to TLR8 agonists (79) (Fig. 2). Indeed TLR7/8 agonists, such as the synthetic low-molecular weight (<400 Da) antiviral imidazoquinoline compounds imiquimod and resiquimod (R848), have already been used as immunomodulators for many years against specific viral infections. The US FDA approved imiquimod for the treatment of external genital and perianal warts caused by certain strains of human papilloma virus (80), and it may also have activity against molluscum contagiosum (mulluscipox virus) (81–

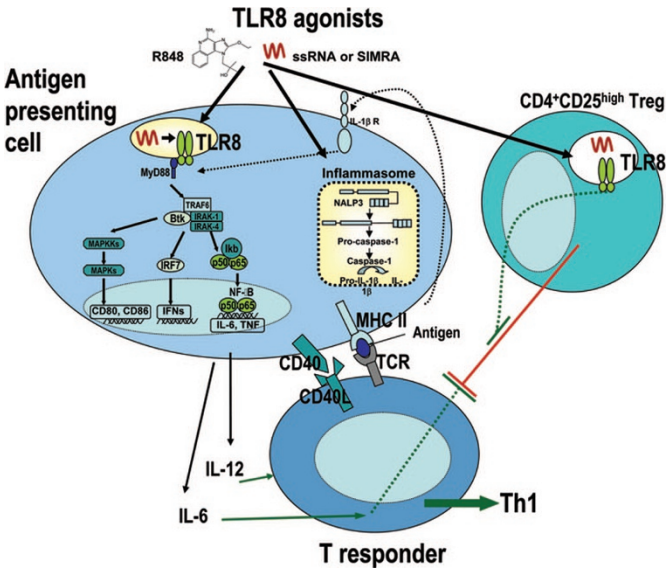

Figure 2. TLR8 agonists activate human APCs and reverse human Treg function. TLR8 agonists, such as R848, ssRNA and stabilized immune modulatory RNA (SIMRA) strongly activate human APCs *via* TLR8-dependent and TLR8-independent mechanisms including activation of the NALP3 inflammasome inducing IL-1β production. Exposure of human neonatal APCs to TLR8 agonists induces robust phosphorylation of p38 MAP kinase and profound/prolonged disappearance of IkB-κ, resulting in robust induction of protective Th1-type immune responses, including production of IL-12 and up-regulation of the costimulatory molecule CD40. TLR8 agonists also reverse suppression mediated by human Treg cells, *via* both direct action on Treg as well as by induction of APC production of IL-6, a cytokine that renders Tresponder cells refractory to Treg-mediated inhibition.

83). One of the main cellular targets of imiquimod is pDCs (IFN-α producing cells), which express high amounts of TLR7, whose engagement induced IFN-α in a MyD88-dependent manner (80,84).

R848 is an effective adjuvant for HBsAg vaccination in mice, increasing humoral and cellular immune responses. R848 used in combination with the TLR9 agonists CpG ODN further strengthened the immune response and long-lasting HBsAg-specific T cells displaying effector memory phenotype were detected in mice (85). R848 or topical application of imiquimod administered with Leishmania Ag induced protective Th1 immune responses in mice, compared with Leishmania Ag alone. In addition, s.c. vaccination also induced protective immunity whereas intramuscular vaccination did not (86). Indeed, conjugation of the TLR7/8 agonist to the HIV Gag protein improved the magnitude of Th1 and CD8 responses in adult *Rhesus macaques* (87). A combined TLR7/8 agonist compared with a pure TLR8 agonist can also induce greater Th1 responses and IFNα production from pDCs, which express TLR7 but not TLR8. IFNα is a key cytokine within a vaccine adjuvant setting, inducing Th1 differentiation (80,88), induction of cytotoxic T lymphocytes, enhancement of cross presentation and of primary Ab responses and DC activation (89). Novel TLR7 and TLR8 agonists referred to as stabilized immune modulatory RNA (SIMRA) compounds have recently developed and have distinct pharmacodynamic characteristics (90). SIMRA compounds demonstrate greater stability in human sera compared with linear RNA, which is rapidly degraded by ubiquitous RNases. In addition, SIMRA compounds and are able to activate TLR7 or TLR8 in HEK293 cells without the need for lipid carriers.

TLR9 agonists are also undergoing biopharmaceutical development for multiple indications, including as vaccine adjuvants for HBV by linking an immunostimulatory DNA sequence to the recombinant HBsAg. This vaccine formulation, which has currently completed phase III trials, may help drive Th1-type responses and reduce Th2 responses (91).

A murine study demonstrated that although the IPS-1 signaling pathway seems to be important for initial type I IFN responses, the TLR7/MyD88 pathway is needed for induction of protective immune responses to influenza A infection. Inactivated influenza virus vaccine failed to confer protection against lethal challenge with live influenza virus in TLR7-deficient and MyD88-deficient mice. Thus, protective adaptive immune responses to live attenuated influenza A virus strains are likely dependent on the TLR7-MyD88 pathway (92).

Priorities for Future Studies

In summary, infectious diseases account for >2 million deaths per year in newborns and infants younger than 6 mo. Early life vaccination offers several advantages, particularly for developing countries in which birth maybe the only point of contact with the healthcare system. There is, thus, an unmet medical need for improved neonatal vaccine adjuvants, which are able to improve the level, speed, and longevity of protection, particularly by inducing Th1-polarizing cell-mediated immunity. In this context, neonatal vaccination is a feasible approach that is likely to be expanded in future years, an effort that will require the development of new adjuvants capable activating specific PRRs. The recent expansion in knowledge of PRRs in the neonate as described earlier provides new opportunities for developing novel delivery systems (*e.g.*, intracellular/cytosolic) and/or adjuvants. As always, safety considerations will be paramount, but the possibility of vaccinating this vulnerable population provides a strong motivation to pursue effective vaccines for neonates and infants. Although neonatal vaccination offers many advantages safety concerns will be paramount and have recently been reviewed (93). There are concerns that neonatal vaccination could trigger autoimmunity through molecular mimicry or Ag immune system polarization (94,95); however, self-reactive neonatal T and B cells are eliminated by peripheral tolerance and there is substantial evidence that multiple pediatric vaccines, including BCG, are not linked to allergy or autoimmunity (96). Nevertheless, as with any new drugs, all novel adjuvant/Ag combinations, must undergo rigorous safety analysis. The rapidly expanding menu of Ag-adjuvant combinations will require federal authorities to update and enhance the pathways to establishing vaccine safety and efficacy.

Acknowledgments. We thank Drs. Michael Wessels, Eva Guinan, Jerrold Weiss, Peter Elsbach, Raif Geha, and Richard Malley for their mentorship.

REFERENCES

1. WHO 2005 The World Health Report. World Health Organization, Geneva
2. Siegrist CA 2003 Immunologic requirements for vaccines to be used in early life. In: Bloom B, Lambert PH (eds) The Vaccine Book. Academic Press, Boston, pp 73–83
3. Angelone DF, Wessels MR, Coughlin M, Suter EE, Valentini P, Kalish LA, Levy O 2006 Innate immunity of the human newborn is polarized toward a high ratio of IL-6/TNF-alpha production in vitro and in vivo. Pediatr Res 60:205–209
4. Levy O 2007 Innate immunity of the newborn: basic mechansims and clinical correlates. Nat Rev Immunol 7:379–390
5. Kallewaard NL, McKinney BA, Gu Y, Chen A, Prasad BV, Crowe JE Jr 2008 Functional maturation of the human antibody response to rotavirus. J Immunol 180:3980–3989
6. Morein B, Blomqvist G, Hu K 2007 Immune responsiveness in the neonatal period. J Comp Pathol 137:S27–S31
7. Jones CA, Holloway JA, Warner JO 2002 Phenotype of fetal monocytes and B lymphocytes during the third trimester of pregnancy. J Reprod Immunol 56:45–60
8. Saito S 2000 Cytokine network at the feto-maternal interface. J Reprod Immunol 47:87–103
9. Levy O, Coughlin M, Cronstein B, Roy RM, Desai A, Wessels MR 2006 The adenosine system selectively inhibits TLR-mediated TNF-alpha production in the human newborn. J Immunol 177:1956–1966
10. Langrish CL, Buddle JC, Thrasher AJ, Goldblatt D 2002 Neonatal dendritic cells are intrinsically biased against Th-1 immune responses. Clin Exp Immunol 128: 118–123
11. Muthukkumar S, Goldstein J, Stein KE 2000 The ability of B cells and dendritic cells to present antigen increases during ontogeny. J Immunol 165:4803–4813
12. Zhao J, Kim KD, Yang X, Auh S, Fu YX, Tang H 2008 Hyper innate responses in neonates lead to increased morbidity and mortality after infection. Proc Natl Acad Sci USA 105:7528–7533
13. Roach JC, Glusman G, Rowen L, Kaur A, Purcell MK, Smith KD, Hood LE, Aderem A 2005 The evolution of vertebrate Toll-like receptors. Proc Natl Acad Sci USA 102:9577–9582
14. Philbin VJ, Iqbal M, Boyd Y, Goodchild MJ, Beal RK, Bumstead N, Young J, Smith AL 2005 Identification and characterization of a functional, alternatively spliced Toll-like receptor 7 (TLR7) and genomic disruption of TLR8 in chickens. Immunology 114:507–521
15. Levy O, Zarember KA, Roy RM, Cywes C, Godowski PJ, Wessels MR 2004 Selective impairment of TLR-mediated innate immunity in human newborns: neonatal blood plasma reduces monocyte TNF-alpha induction by bacterial lipopeptides, lipopolysaccharide, and imiquimod, but preserves the response to R-848. J Immunol 173:4627–4634
16. Michaelsson J, Mold JE, McCune JM, Nixon DF 2006 Regulation of T cell responses in the developing human fetus. J Immunol 176:5741–5748
17. Godfrey WR, Spoden DJ, Ge YG, Baker SR, Liu B, Levine BL, June CH, Blazar BR, Porter SB 2005 Cord blood CD4(+)CD25(+)-derived T regulatory cell lines express FoxP3 protein and manifest potent suppressor function. Blood 105:750–758
18. Wing K, Larsson P, Sandstrom K, Lundin SB, Suri-Payer E, Rudin A 2005 CD4+ CD25+FOXP3+ regulatory T cells from human thymus and cord blood suppress antigen-specific T cell responses. Immunology 115:516–525
19. Deaglio S, Dwyer KM, Gao W, Friedman D, Usheva A, Erat A, Chen JF, Enjyoji K, Linden J, Oukka M, Kuchroo VK, Strom TB, Robson SC 2007 Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. J Exp Med 204:1257–1265
20. Bodor J, Fehervari Z, Diamond B, Sakaguchi S 2007 Regulatory T cell-mediated suppression: potential role of ICER. J Leukoc Biol 81:161–167
21. Fernandez MA, Puttur FK, Wang YM, Howden W, Alexander SI, Jones CA 2008 T regulatory cells contribute to the attenuated primary CD8+ and CD4+ T cell responses to herpes simplex virus type 2 in neonatal mice. J Immunol 180:1556–1564
22. Toka FN, Suvas S, Rouse BT 2004 CD4+CD25+ T cells regulate vaccine-generated primary and memory CD8+ T-cell responses against herpes simplex virus type 1. J Virol 78:13082–13089
23. Stober CB, Lange UG, Roberts MT, Alcami A, Blackwell JM 2005 IL-10 from regulatory T cells determines vaccine efficacy in murine Leishmania major infection. J Immunol 175:2517–2524
24. Iwasaki A, Medzhitov R 2004 Toll-like receptor control of the adaptive immune responses. Nat Immunol 5:987–995
25. O'Neill LA 2008 When signaling pathways collide: positive and negative regulation of toll-like receptor signal transduction. Immunity 29:12–20
26. Pasare C, Medzhitov R 2003 Toll pathway-dependent blockade of CD4+CD25+ T cell-mediated suppression by dendritic cells. Science 299:1033–1036
27. Matzinger P 2007 Friendly and dangerous signals: is the tissue in control? Nat Immunol 8:11–13
28. Soderberg KA, Payne GW, Sato A, Medzhitov R, Segal SS, Iwasaki A 2005 Innate control of adaptive immunity via remodeling of lymph node feed arteriole. Proc Natl Acad Sci USA 102:16315–16320
29. Webster B, Ekland EH, Agle LM, Chyou S, Ruggieri R, Lu TT 2006 Regulation of lymph node vascular growth by dendritic cells. J Exp Med 203:1903–1913
30. Diebold SS, Kaisho T, Hemmi H, Akira S, Reis e Sousa C 2004 Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science 303:1529–1531
31. Heil F, Hemmi H, Hochrein H, Ampenberger F, Kirschning C, Akira S, Lipford G, Wagner H, Bauer S 2004 Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. Science 303:1526–1529
32. O'Neill LA, Bowie AG 2007 The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. Nat Rev Immunol 7:353–364
33. Dumitru CD, Ceci JD, Tsatsanis C, Kontoyiannis D, Stamatakis K, Lin JH, Patriotis C, Jenkins NA, Copeland NG, Kollias G, Tsichlis PN 2000 TNF-alpha induction by LPS is regulated posttranscriptionally via a Tpl2/ERK-dependent pathway. Cell 103:1071–1083
34. Huang Q, Yang J, Lin Y, Walker C, Cheng J, Liu ZG, Su B 2004 Differential regulation of interleukin 1 receptor and Toll-like receptor signaling by MEKK3. Nat Immunol 5:98–103
35. von Bernuth H, Picard C, Jin Z, Pankla R, Xiao H, Ku CL, Chrabieh M, Mustapha IB, Ghandil P, Camcioglu Y, Vasconcelos J, Sirvent N, Guedes M, Vitor AB, Herrero-Mata MJ, Arostegui JI, Rodrigo C, Alsina L, Ruiz-Ortiz E, Juan M, Fortuny C, Yague J, Anton J, Pascal M, Chang HH, Janniere L, Rose Y, Garty BZ, Chapel H, Issekutz A, Marodi L, Rodriguez-Gallego C, Banchereau J, Abel L, Li X, Chaussabel D, Puel A, Casanova JL 2008 Pyogenic bacterial infections in humans with MyD88 deficiency. Science 321:691–696
36. Ku CL, von Bernuth H, Picard C, Zhang SY, Chang HH, Yang K, Chrabieh M, Issekutz AC, Cunningham CK, Gallin J, Holland SM, Roifman C, Ehl S, Smart J, Tang M, Barrat FJ, Levy O, McDonald D, Day-Good NK, Miller R, Takada H, Hara T, Al-Hajjar S, Al-Ghonaium A, Speert D, Sanlaville D, Li X, Geissmann F, Vivier E, Marodi L, Garty BZ, Chapel H, Rodriguez-Gallego C, Bossuyt X, Abel L, Puel A, Casanova JL 2007 Selective predisposition to bacterial infections in IRAK-4-deficient children: IRAK-4-dependent TLRs are otherwise redundant in protective immunity. J Exp Med 204:2407–2422
37. Medvedev AE, Lentschat A, Kuhns DB, Blanco JC, Salkowski C, Zhang S, Arditi M, Gallin JI, Vogel SN 2003 Distinct mutations in IRAK-4 confer hyporesponsiveness to lipopolysaccharide and interleukin-1 in a patient with recurrent bacterial infections. J Exp Med 198:521–531
38. Yan SR, Qing G, Byers DM, Stadnyk AW, Al-Hertani W, Bortolussi R 2004 Role of MyD88 in diminished tumor necrosis factor alpha production by newborn mononuclear cells in response to lipopolysaccharide. Infect Immun 72:1223–1229
39. Goriely S, Van Lint C, Dadkhah R, Libin M, De Wit D, Demonte D, Willems F, Goldman M 2004 A defect in nucleosome remodeling prevents IL-12(p35) gene transcription in neonatal dendritic cells. J Exp Med 199:1011–1016
40. De Wit D, Olislagers V, Goriely S, Vermeulen F, Wagner H, Goldman M, Willems F 2004 Blood plasmacytoid dendritic cell responses to CpG oligodeoxynucleotides are impaired in human newborns. Blood 103:1030–1032
41. Aksoy E, Albarani V, Nguyen M, Laes JF, Ruelle JL, De Wit D, Willems F, Goldman M, Goriely S 2007 Interferon regulatory factor 3-dependent responses to lipopolysaccharide are selectively blunted in cord blood cells. Blood 109:2887–2893
42. Levy O, Suter EE, Miller RL, Wessels MR 2006 Unique efficacy of Toll-like receptor 8 agonists in activating human neonatal antigen-presenting cells. Blood 108:1284–1290
43. Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, Taira K, Akira S, Fujita T 2004 The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. Nat Immunol 5: 730–737
44. Andrejeva J, Childs KS, Young DF, Carlos TS, Stock N, Goodbourn S, Randall RE 2004 The V proteins of paramyxoviruses bind the IFN-inducible RNA helicase, mda-5, and inhibit its activation of the IFN-beta promoter. Proc Natl Acad Sci USA 101:17264–17269
45. Gotsch F, Romero R, Chaiworapongsa T, Erez O, Vaisbuch E, Espinoza J, Kusanovic JP, Mittal P, Mazaki-Tovi S, Kim CJ, Kim JS, Edwin S, Nhan-Chang CL, Hamill N, Friel L, Than NG, Mazor M, Yoon BH, Hassan SS 2008 Evidence of the involvement of caspase-1 under physiologic and pathologic cellular stress during human pregnancy: a link between the inflammasome and parturition. J Matern Fetal Neonatal Med 21:605–616

45a. Forster-Waldl E, Sadeghi K, Tamandl D, Gerhold B, Hallwirth U, Rohrmeister K, Hayde M, Prusa AR, Herkner K, Boltz-Nitulescu G, Pollak A, Spittler A 2005 Monocyte toll-like receptor 4 expression and LPS-induced cytokine production increase during gestational aging. Pediatr Res 58:121–124

45b. Sadeghi K, Berger A, Langgartner M, Prusa AR, Hayde M, Herkner K, Pollak A, Spittler A, Forster-Waldl E 2007 Immaturity of infection control in preterm and term newborns is associated with impaired toll-like receptor signaling. J infect Dis 195:296–302

46. Gadjeva M, Takahashi K, Thiel S 2004 Mannan-binding lectin—a soluble pattern recognition molecule. Mol Immunol 41:113–121
47. Jack DL, Read RC, Tenner AJ, Frosch M, Turner MW, Klein NJ 2001 Mannose-binding lectin regulates the inflammatory response of human professional phagocytes to Neisseria meningitidis serogroup B. J Infect Dis 184:1152–1162
48. Oudshoorn AM, van den Dungen FA, Bach KP, Koomen I, Fetter WP, Catsburg A, Savelkoul PH, van Elburg RM 2008 Mannose-binding lectin in term newborns and their mothers: genotypic and phenotypic relationship. Hum Immunol 69:344–348
49. Dzwonek AB, Neth OW, Thiebaut R, Gulczynska E, Chilton M, Hellwig T, Bajaj-Elliott M, Hawdon J, Klein NJ 2008 The role of mannose-binding lectin in susceptibility to infection in preterm neonates. Pediatr Res 63:680–685
50. Guttormsen HK, Stuart LM, Shi L, Carroll MC, Chen J, Kasper DL, Ezekowitz RA, Takahashi K 2008 Deficiency of mannose-binding lectin greatly increases antibody response in a mouse model of vaccination. Clin Immunol 130:264–271

50a. Schultz H, Hume J, Zhang de S, Gioannini TL, Weiss JP 2007 A novel role for the bactericidal/permeability increasing protein in interactions of gram-negative bacterial outer membrane blebs with dendritic cells. J Immunol 179:2477–2484

51. Oppenheim JJ, Biragyn A, Kwak LW, Yang D 2003 Roles of antimicrobial peptides such as defensins in innate and adaptive immunity. Ann Rheum Dis 62:ii17–ii21
52. Kornbluth RS, Stone GW 2006 Immunostimulatory combinations: designing the next generation of vaccine adjuvants. J Leukoc Biol 80:1084–1102
53. Vekemans J, Amedei A, Ota MO, D'Elios MM, Goetghebuer T, Ismaili J, Newport MJ, Del Prete G, Goldman M, McAdam KP, Marchant A 2001 Neonatal bacillus Calmette-Guerin vaccination induces adult-like IFN-gamma production by CD4+ T lymphocytes. Eur J Immunol 31:1531–1535
54. Marchant A, Goetghebuer T, Ota MO, Wolfe I, Ceesay SJ, De Groote D, Corrah T, Bennett S, Wheeler J, Huygen K, Aaby P, McAdam KP, Newport MJ 1999 Newborns develop a Th1-type immune response to Mycobacterium bovis bacillus Calmette-Guerin vaccination. J Immunol 163:2249–2255
55. Pasare C, Medzhitov R 2004 Toll-like receptors: linking innate and adaptive immunity. Microbes Infect 6:1382–1387
56. Ridge JP, Fuchs EJ, Matzinger P 1996 Neonatal tolerance revisited: turning on newborn T cells with dendritic cells. Science 271:1723–1726
57. Means TK, Wang S, Lien E, Yoshimura A, Golenbock DT, Fenton MJ 1999 Human toll-like receptors mediate cellular activation by Mycobacterium tuberculosis. J Immunol 163:3920–3927
58. Uehori J, Matsumoto M, Tsuji S, Akazawa T, Takeuchi O, Akira S, Kawata T, Azuma I, Toyoshima K, Seya T 2003 Simultaneous blocking of human Toll-like receptors 2 and 4 suppresses myeloid dendritic cell activation induced by Mycobacterium bovis bacillus Calmette-Guerin peptidoglycan. Infect Immun 71:4238–4249
59. Tsuji S, Matsumoto M, Takeuchi O, Akira S, Azuma I, Hayashi A, Toyoshima K, Seya T 2000 Maturation of human dendritic cells by cell wall skeleton of Mycobacterium bovis bacillus Calmette-Guerin: involvement of toll-like receptors. Infect Immun 68:6883–6890
60. Bafica A, Scanga CA, Feng CG, Leifer C, Cheever A, Sher A 2005 TLR9 regulates Th1 responses and cooperates with TLR2 in mediating optimal resistance to Mycobacterium tuberculosis. J Exp Med 202:1715–1724
61. Davila S, Hibberd ML, Hari Dass R, Wong HE, Sahiratmadja E, Bonnard C, Alisjahbana B, Szeszko JS, Balabanova Y, Drobniewski F, van Crevel R, van de Vosse E, Nejentsev S, Ottenhoff TH, Seielstad M 2008 Genetic association and expression studies indicate a role of toll-like receptor 8 in pulmonary tuberculosis. PLoS Genet 4:e1000218
62. Williams R 2006 Global challenges in liver disease. Hepatology 44:521–526
63. Mogensen TH, Paludan SR, Kilian M, Ostergaard L 2006 Live Streptococcus pneumoniae, Haemophilus influenzae, and Neisseria meningitidis activate the inflammatory response through Toll-like receptors 2, 4, and 9 in species-specific patterns. J Leukoc Biol 80:267–277
64. Latz E, Franko J, Golenbock DT, Schreiber JR 2004 Haemophilus influenzae type b-outer membrane protein complex glycoconjugate vaccine induces cytokine production by engaging human toll-like receptor 2 (TLR2) and requires the presence of TLR2 for optimal immunogenicity. J Immunol 172:2431–2438
65. Bieback K, Lien E, Klagge IM, Avota E, Schneider-Schaulies J, Duprex WP, Wagner H, Kirschning CJ, Ter Meulen V, Schneider-Schaulies S 2002 Hemagglutinin protein of wild-type measles virus activates toll-like receptor 2 signaling. J Virol 76:8729–8736
66. Berghall H, Siren J, Sarkar D, Julkunen I, Fisher PB, Vainionpaa R, Matikainen S 2006 The interferon-inducible RNA helicase, mda-5, is involved in measles virus-induced expression of antiviral cytokines. Microbes Infect 8:2138–2144
67. Eisenbarth SC, Colegio OR, O'Connor W, Sutterwala FS, Flavell RA 2008 Crucial role for the Nalp3 inflammasome in the immunostimulatory properties of aluminium adjuvants. Nature 453:1122–1127
68. Kool M, Petrilli V, De Smedt T, Rolaz A, Hammad H, van Nimwegen M, Bergen IM, Castillo R, Lambrecht BN, Tschopp J 2008 Cutting edge: alum adjuvant stimulates inflammatory dendritic cells through activation of the NALP3 inflammasome. J Immunol 181:3755–3759
69. Ott G, Barchfeld GL, Chernoff D, Radhakrishnan R, van Hoogevest P, Van Nest G 1995 MF59. Design and evaluation of a safe and potent adjuvant for human vaccines. Pharm Biotechnol 6:277–296
69a. Mosca F, Tritto E, Muzzi A, Monaci E, Bagnoli F, Iavarone C, O'Hagan D, Rappuoli R, De Gregorio E 2008 Molecular and cellular signatures of human vaccine adjuvants. Proc Natl Acad Sci USA 105:10501–10506
70. Schultze V, D'Agosto V, Wack A, Novicki D, Zorn J, Hennig R 2008 Safety of MF59 adjuvant. Vaccine 26:3209–3222
71. Dupuis M, Denis-Mize K, LaBarbara A, Peters W, Charo IF, McDonald DM, Ott G 2001 Immunization with the adjuvant MF59 induces macrophage trafficking and apoptosis. Eur J Immunol 31:2910–2918
72. Moreira LO, Smith AM, Defreitas AA, Qualls JE, El Kasmi KC, Murray PJ 2008 Modulation of adaptive immunity by different adjuvant-antigen combinations in mice lacking Nod2. Vaccine 26:5808–5813
73. Lambert PH 1997 Vaccines for the world: major challenges for the future. Southeast Asian J Trop Med Public Health 28:122–126
74. Kollmann TR, Reikie B, Blimkie D, Way SS, Hajjar AM, Arispe K, Shaulov A, Wilson CB 2007 Induction of protective immunity to Listeria monocytogenes in neonates. J Immunol 178:3695–3701
75. Warren SE, Mao DP, Rodriguez AE, Miao EA, Aderem A 2008 Multiple nod-like receptors activate caspase 1 during Listeria monocytogenes infection. J Immunol 180:7558–7564
76. Imaizumi T, Sashinami H, Mori F, Matsumiya T, Yoshida H, Nakane A, Wakabayashi K, Oyama C, Satoh K 2006 Listeria monocytogenes induces the expression of retinoic acid-inducible gene-I. Microbiol Immunol 50:811–815
77. Starks H, Bruhn KW, Shen H, Barry RA, Dubensky TW, Brockstedt D, Hinrichs DJ, Higgins DE, Miller JF, Giedlin M, Bouwer HG 2004 Listeria monocytogenes as a vaccine vector: virulence attenuation or existing antivector immunity does not diminish therapeutic efficacy. J Immunol 173:420–427
78. Philbin VJ, Levy O 2007 Immunostimulatory activity of Toll-like receptor 8 agonists towards human leucocytes: basic mechanisms and translational opportunities. Biochem Soc Trans 35:1485–1491
79. Peng G, Guo Z, Kiniwa Y, Voo KS, Peng W, Fu T, Wang DY, Li Y, Wang HY, Wang RF 2005 Toll-like receptor 8-mediated reversal of CD4+ regulatory T cell function. Science 309:1380–1384
80. Hemmi H, Kaisho T, Takeuchi O, Sato S, Sanjo H, Hoshino K, Horiuchi T, Tomizawa H, Takeda K, Akira S 2002 Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway [comment]. Nat Immunol 3:196–200
81. Bayerl C, Feller G, Goerdt S 2003 Experience in treating molluscum contagiosum in children with imiquimod 5% cream. Br J Dermatol 149:25–29
82. Hengge UR, Cusini M 2003 Topical immunomodulators for the treatment of external genital warts, cutaneous warts and molluscum contagiosum. Br J Dermatol 149:15–19
83. Miller RL, Meng TC, Tomai MA 2008 The antiviral activity of Toll-like receptor 7 and 7/8 agonists. Drug News Perspect 21:69–87
84. Jurk M, Heil F, Vollmer J, Schetter C, Krieg AM, Wagner H, Lipford G, Bauer S 2002 Human TLR7 or TLR8 independently confer responsiveness to the antiviral compound R-848 [comment]. Nat Immunol 3:499
85. Ma R, Du JL, Huang J, Wu CY 2007 Additive effects of CpG ODN and R-848 as adjuvants on augmenting immune responses to HBsAg vaccination. Biochem Biophys Res Commun 361:537–542
86. Zhang WW, Matlashewski G 2008 Immunization with a Toll-like receptor 7 and/or 8 agonist vaccine adjuvant increases protective immunity against Leishmania major in BALB/c mice. Infect Immun 76:3777–3783
87. Wille-Reece U, Flynn BJ, Lore K, Koup RA, Kedl RM, Mattapallil JJ, Weiss WR, Roederer M, Seder RA 2005 HIV Gag protein conjugated to a toll-like receptor 7/8 agonist improves the magnitude and quality of Th1 and CD8+ T cell responses in nonhuman primates. Proc Natl Acad Sci USA 102:15190–15194
88. Gibson SJ, Lindh JM, Riter TR, Gleason RM, Rogers LM, Fuller AE, Oesterich JL, Gorden KB, Qiu X, McKane SW, Noelle RJ, Miller RL, Kedl RM, Fitzgerald-Bocarsly P, Tomai MA, Vasilakos JP 2002 Plasmacytoid dendritic cells produce cytokines and mature in response to the TLR7 agonists, imiquimod and resiquimod. Cell Immunol 218:74–86
89. Bracci L, La Sorsa V, Belardelli F, Proietti E 2008 Type I interferons as vaccine adjuvants against infectious diseases and cancer. Expert Rev Vaccines 7:373–381
90. Lan T, Kandimalla ER, Yu D, Bhagat L, Li Y, Wang D, Zhu F, Tang JX, Putta MR, Cong Y, Trombino AF, Sullivan T, Agrawal S 2007 Stabilized immune modulatory RNA compounds as agonists of Toll-like receptors 7 and 8. Proc Natl Acad Sci USA 104:13750–13755
91. Sung JJ, Lik-Yuen H 2006 HBV-ISS (Dynavax). Curr Opin Mol Ther 8:150–155
92. Koyama S, Ishii KJ, Kumar H, Tanimoto T, Coban C, Uematsu S, Kawai T, Akira S 2007 Differential role of TLR- and RLR-signaling in the immune responses to influenza A virus infection and vaccination. J Immunol 179:4711–4720
93. Demirjian A, Levy O 2008 Safety and efficacy of neonatal vaccination. Eur J Immunol 39:36–46
94. Offit PA 2004 Multiple vaccines and the immune system. In: Plotkin SA, Orenstein WA (eds) Vaccines. Saunders, Philadelphia, pp1583–1589
95. Goriely S, Goldman M 2007 From tolerance to autoimmunity: is there a risk in early life vaccination? J Comp Pathol 137:S57–S61
96. Gruber C, Nilsson L, Bjorksten B 2001 Do early childhood immunizations influence the development of atopy and do they cause allergic reactions? Pediatr Allergy Immunol 12:296–311
97. Jo EK 2008 Mycobacterial interaction with innate receptors: TLRs, C-type lectins, and NLRs. Curr Opin Infect Dis 21:279–286
98. Le Goffic R, Pothlichet J, Vitour D, Fujita T, Meurs E, Chignard M, Si-Tahar M 2007 Cutting edge: influenza A virus activates TLR3-dependent inflammatory and RIG-I-dependent antiviral responses in human lung epithelial cells. J Immunol 178:3368–3372
99. Geeraedts F, Goutagny N, Hornung V, Severa M, de Haan A, Pool J, Wilschut J, Fitzgerald KA, Huckriede A 2008 Superior immunogenicity of inactivated whole virus H5N1 influenza vaccine is primarily controlled by toll-like receptor signalling. PLoS Pathog 4:e1000138
100. Matikainen S, Siren J, Tissari J, Veckman V, Pirhonen J, Severa M, Sun Q, Lin R, Meri S, Uze G, Hiscott J, Julkunen I 2006 Tumor necrosis factor alpha enhances influenza A virus-induced expression of antiviral cytokines by activating RIG-I gene expression. J Virol 80:3515–3522
101. Wang JP, Kurt-Jones EA, Shin OS, Manchak MD, Levin MJ, Finberg RW 2005 Varicella-zoster virus activates inflammatory cytokines in human monocytes and macrophages via toll-like receptor 2. J Virol 79:12658–12666
102. American Academy of Pediatrics Committee on Infectious Diseases 2009 Recommended childhood and adolescent immunization schedules–United States, 2009. Pediatrics 123:189–190