

Neonatal innate immunity in allergy development

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Purpose of review

The neonate is born with a distinct immune system that is biased against the production of T-helper cell 1 (Th1) cytokines. Birth imposes a great challenge on the neonatal immune system, which is confronted with an outside world rich in foreign antigens. Exposure to these antigens shapes the developing neonatal immune system. Inducing Th-1 or Th-2 polarized responses that may extend beyond the neonatal age and counteract or promote allergic sensitization. This review describes how engagement of the innate immune system might contribute to the development of allergy in children.

Recent findings

The exact role of innate immune stimulation in the development of allergies is a controversial area. Epidemiological literature suggests that microbial exposure in early childhood protects against the development of allergies, whereas a large amount of experimental data demonstrates that innate immune stimulation enhances Th2 responses upon primary and secondary antigen exposure.

Summary

Dose, site and timing of allergen exposure are likely to modulate the innate immune response, polarizing the maturing neonatal immune system towards Th1 or Th2-type responses, thereby protecting from or predisposing to asthma and allergies. Modulation of neonatal innate immune responses may be a novel approach to prevent asthma and allergies.

Keywords

allergy, asthma, neonate, review, toll-like receptor

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Introduction

Neonatal immunity is immature. Adaptive immune responses are impaired at birth by lack of preexisting memory and low frequency and impaired function of effector B cells and T cells [1]. Therefore, the neonate is largely dependent on passively acquired antibodies and innate immune responses in the defense against micro-organisms. It is also well documented that neonatal innate immunity is distinct from that of adults in its response to bacterial and viral pathogens [1,2]. Perinatal life is characterized by massive and constant exposure to new antigens, predominantly in the mucosa of the digestive and respiratory tract. The neonatal impairment in production of proinflammatory/T-helper cell 1 (Th1)-polarizing cytokines is believed to prevent potentially harmful hyperinflammation caused by these antigens. In addition, during this period, tolerance to many new allergens is induced, which is the key mechanism to prevent the development of allergy. This process is orchestrated by cells from the innate immune system. The distinct characteristics of the neonatal innate immune system may be essential in the induction of

tolerance. Here, we review the role of the distinct neonatal innate immune system in the development of allergy.

Neonatal innate immunity

Quantitative and qualitative differences between the neonatal and adult innate immune system may contribute to neonatal Th2 polarization. Although cord blood contains a higher number of monocytes than adult venous blood, many phenotypic differences have been described, including lower baseline human leukocyte antigen (HLA)-DR expression and decreased upregulation of CD40 on ex-vivo stimulation with lipopolysaccharide (LPS) [3]. Cord blood also contains a higher ratio of plasmacytoid dendritic cells (pDCs) over myeloid dendritic cells (mDCs) compared with adult blood. Activation of pDC generally leads to production of Th2-polarizing cytokines, whereas mDCs are associated with Th1-type responses [4,5]. In addition, neonatal cord blood contains lower numbers of differentiated natural killer T (NKT) cells than adults [6]. Because of their central role in the induction and polarization of the

Table 1 Evidence for distinct neonatal toll-like receptor-mediated cytokine responses

TLR	Culture system					Ref.
	Whole blood	PBMC	Monocytes	mDC	moDC	
1	TNF α ($\downarrow$)					[7,8]
2	TNF α ($\downarrow$)			IL-23 (=)		[7–9]
3	IL-6 ($\uparrow$) IL-12p70 ($\downarrow$) IL-10 (=)		IL-12p35 ($\downarrow$)	TNF α (=) IL-1 β (=) IL-6 (=) IL-8 (=)	IL-12p40 ($\downarrow$)	[4 $\bullet$,9–11]
4	IL-12p70 ($\downarrow$) IL-10 $\uparrow$ TNF α ($\downarrow$ /=) IL-8 (=) IFN γ (=)	IL-6 ($\uparrow$) IL-10 (=) TNF α (=/ $\downarrow$) IL-23 ($\uparrow$)	IL-12p35 ($\downarrow$) TNF α ($\downarrow$) IL-1 α (=) IL-12p40 ($\downarrow$)	IL-12p35 ($\downarrow$) IL-12p40 (=) IL-23 ($\uparrow$) IL-1 α (=) TNF α ($\downarrow$)	IFN β ($\downarrow$) TNF α ($\downarrow$)	[3,4 $\bullet$,7–9,11,12,13 $\bullet$,14,15]
6	TNF α ($\downarrow$)					[7]
7	IL-10 (=) TNF α ($\downarrow$) IFN α ($\downarrow$)				IL-12p70 ($\downarrow$) TNF α ($\downarrow$)	[4 $\bullet$,7]
8	TNF α (=)				IL-23 ($\uparrow$)	[7,9,16]
9	TNF α ($\downarrow$) IFN α (=/ $\downarrow$)					[4 $\bullet$,17]

Cytokine concentrations after in-vitro stimulation with TLR agonists are depicted. ($\downarrow$) decreased compared with adults, (=) similar to adults, ($\uparrow$) increased compared with adults. IFN, interferon; IL, interleukin; mDC, myeloid dendritic cells; moDC, monocyte-derived dendritic cells; PBMC, peripheral blood mononuclear cells; TLR, toll-like receptor; TNF α , tumor necrosis factor alpha.

immune response, most studies [17,18] on neonatal innate immunity have focused on antigen-presenting cells (APCs) and toll-like receptors (TLRs). Upon stimulation with various TLR agonists, human neonatal cord blood, as well as cord blood-derived monocytes and APCs, generally produces decreased amounts of Th1-polarizing cytokines compared with adults (Table 1) [3,4 $\bullet$,7–9,11,12,13 $\bullet$,14–16,19].

The inability to produce Th1-polarizing cytokines persists throughout the neonatal period [4 $\bullet$]. In contrast, cord blood demonstrates similar or increased production of the Th2-polarizing cytokine interleukin (IL)-6 and elevated production of IL-10 upon stimulation with agonists for TLR2, TLR4 or TLR7 [4 $\bullet$,8]. An interesting exception is TLR8 agonists, such as single-stranded RNAs and imidazoquinolines, which induce a similar magnitude of Th1-polarizing responses in newborn and adult blood, monocytes and APCs [16].

Multiple mechanisms have been reported to contribute to decreased cord blood TLR agonist-induced cytokine production. First, studies investigating neonatal TLR expression report that TLR4 expression in cord blood monocytes from preterm neonates is lower than adult expression but increases during gestational aging [20]. At birth, newborn and adult blood monocytes express similar basal levels for various TLRs [7]. However, a study [21] investigating in-vitro stimulus-induced TLR4 expression reported an elevated LPS-induced expression of TLR4 in cord blood monocytes compared with adult monocytes. The differential regulation of stimulus-induced TLR

expression in neonates and adults remains to be determined. Second, neonates demonstrate defects in signaling pathways downstream of TLRs. LPS-induced phosphorylation of p38 mitogen-activated protein kinase (MAPK) is decreased in whole cord blood, suggesting that the mechanism for impaired neonatal TLR responses is localized at the level of, or upstream of, kinase phosphorylation [7,22]. Expression of the MyD88 adapter, which is used by all TLRs except for TLR3, is decreased in cord blood monocytes compared with adults [12]. Cord blood MyD88-independent signaling is also impaired, as illustrated by decreased whole blood cytokine responses to agonists for TLR3 and decreased LPS-induced interferon regulatory factor 3 (IRF-3)-mediated production of the IL-12p35 subunit in cord blood monocytes [14,15,23].

Soluble mediators may induce or modulate innate immune responses. Human breast milk contains several TLR-modulating factors, including soluble CD14 (sCD14), soluble TLR2 and a thus far unidentified approximately 80 kDa protein that inhibits signaling through membrane TLR2 but activates TLR4 in human intestinal epithelial and mononuclear cells [24]. Breast milk-mediated intestinal immune modulation may be an important factor guiding postnatal immune development, preventing harmful inflammation caused by intestinal colonization, but selectively allowing proinflammatory responses needed for immune maturation.

The complement system can be activated by innate immune responses, resulting in generation of the

anaphylatoxins C3a and C5a, recruitment of innate immune cells and formation of the membrane attack complex [25]. In the airways, C3a production favors Th2-type responses, whereas C5a can induce both inflammation and tolerance. Concentrations of complement components in neonatal plasma are diminished compared with those in adults [26]. Levels of complement in other organs, including the neonatal lung, and the role of neonatal complement deficiency in allergy development remain to be defined.

Neonatal plasma contains high concentrations of adenosine, an endogenous purine metabolite that induces intracellular cyclic AMP (cAMP), thereby inhibiting TLR2-mediated production of tumor necrosis factor alpha (TNF α) [8]. Interestingly, cAMP is also known to inhibit the production of several other cytokines that are impaired in newborns [IL-12 and interferon alpha (IFN α)], while preserving IL-10 and IL-6 [27]. Elevated concentrations of adenosine in neonatal blood may thus be a general mechanism underlying distinct polarization of neonatal TLR-mediated cytokine responses.

In summary, neonatal innate immune cells demonstrate immunologic responses distinct from those of adults. In general, Th1-polarizing responses to pure TLR agonists are impaired at birth, whereas production of Th2-polarizing and anti-inflammatory cytokines is preserved. However, it should be noted that under certain circumstances, such as in-vitro stimulation with whole group B streptococci and *Mycobacterium bovis* bacillus Calmette–Guérin (BCG), human neonatal monocytes and APCs are capable of mounting adult-level responses [28,29]. Thus, although responses to pure TLR agonists are polarized, neonatal mononuclear cells can mount robust inflammatory/Th1-polarizing responses to certain microbial particles. We will next discuss how innate immune stimulation in the early postnatal period may induce permanent changes that determine immune function in later life and protect from or predispose to asthma and allergies.

Evidence that neonatal innate immunity protects against allergy development

Epidemiological studies have suggested that early life exposure to innate immune stimuli, such as endotoxin (TLR4), reduces the risk of allergy development, leading to the hygiene hypothesis [30]. This could already occur prenatally, as several studies suggest that atopic sensitization occurs prenatally [31,32]. Many allergens can be transferred transplacentally, and antigen-specific T cells can be detected in neonatal cord blood. *In utero*, the presence of high concentrations of sCD14 in amniotic fluid decreases the risk of atopic dermatitis in the newborn [33]. A European study in 922 women living in rural areas showed that maternal exposure to animal sheds

during pregnancy decreased the risk of specific immunoglobulin E (IgE) in cord blood [31], possibly by Th1 polarization in the neonate [34 $\bullet$]. After birth, children exposed to house dust endotoxin, mould or farm animals have decreased risk of allergic airway disease [31,35,36]. Breastfeeding protects against atopic disease [37]. Children fed breast milk that contains low levels of sCD14 have a higher risk of subsequent allergy [33], indicating that the protective effect of breast milk may be mediated through innate immune mechanisms. Day care attendance, which is related to microbial exposure during early childhood, is associated with decreased total IgE at age of 3 years and decreased risk of asthma at school age [38,39]. The protective effect of farm living is strongly dependent on the genetic variation in CD14 and TLRs [40], stressing the role of the innate immune system in early life immune polarization. An Australian healthy birth cohort study [41 $\bullet\bullet$] showed that cord blood mononuclear cells from infants born to allergic mothers have increased Th1-driving responses, such as IL-12 and IFN γ production, to different TLR agonists. Increased IL-6 and TNF α production to lipoteichoic acid (TLR2), LPS (TLR4) and flagellin (TLR5) at birth was associated with an increased risk of allergic disease at the age of 1 year. Taken together, epidemiological data suggest that high exposure to microbial products before or after birth protects against the risk of allergic diseases (Table 2) [31,35,36,42 $\bullet\bullet$, 43–46,47 $\bullet\bullet$ –49 $\bullet\bullet$,50–56]. However, the role of timing, dose and interaction with susceptibility genes requires further study.

Basic studies have added to the evidence that stimulation of the innate immune system may protect against allergic responses. In a murine model of allergic asthma, the role of synthetic TLR3 or TLR7 agonists during sensitization has been studied. Systemic treatment with poly I:C (TLR3) or R-848 (TLR7) 1 day before intraperitoneal sensitization with ovalbumin (OVA) prevented eosinophilic airway inflammation, histopathological changes and airway hyper-responsiveness (AHR) upon subsequent allergen challenge [51,57]. Most of these effects, except bronchoalveolar lavage (BAL) eosinophilia, were dependent on IL-12. The effect of TLR9 ligation on subsequent allergen challenge was tested in an OVA murine model [48 $\bullet$]. Subcutaneous injection of CpG–oligodeoxynucleotide (ODN) (TLR9) reduced BAL eosinophilia and AHR by an IL-12-dependent mechanism. In addition, in human adult volunteers with established house dust mite (HDM) allergy, TLR2 stimulation during antigen exposure decreased the production of Th2-polarizing cytokines [58]. The role of innate immune stimulation in established allergy may be different from the role in allergic sensitization. In allergen-sensitized mice, continuous exposure to LPS (TLR4) decreases allergic airway responses upon allergen challenge [43]. Similar effects on allergen challenge were seen using sterile house dust extracts, although

Table 2 Role of innate immune stimulation in allergy development in mice and humans

Type of studies	Allergy promotion	Ref.	Allergy prevention	Ref.
Murine	Allergen may signal through TLR by molecular mimicry	[42**]	Continuous local innate immune stimulation decreases response to allergen	[43]
	Local TLR stimulation enhances concomitant response to allergen	[44,45]	Repeated nebulization of CpG–ragweed compound reduces airway symptoms by enhancing Th1 response	[46]
	TLR4 on airway epithelium is required for house dust mite allergic responses	[47**]	Systemic administration of TLR3, 4, 7, 8, 9 agonists prevents exaggerated Th2 response to allergen	[43,48*,49**,50–52]
	NKT cells in the airways promote asthmatic airway inflammation	[52]		
Human	Loss-of-function TLR mutations are associated with risk of asthma	[53,54]	Epidemiological negative association between infant endotoxin exposure and asthma risk	[31,35,36]
			Perinatal use of probiotics protects against atopic eczema	[55,56]

Comparison of evidence that innate immune responses promote or prevent the development of allergy. NKT, natural killer T cell; Th1, T-helper cell 1; Th2, T-helper cell 2; TLR, toll-like receptor.

the effects of house dust were only partially TLR4-mediated. In a recent study, the concept that TLR stimulation during sensitization may decrease the eosinophilic airway response during challenge was expanded to a viral model. Footpad inoculation with poly I:C (TLR3), LPS (TLR4) or PolyU (TLR8) during vaccination with formalin-inactivated respiratory syncytial virus (RSV) prevented against eosinophilic airway inflammation and AHR during challenge [49**]. It was shown that TLR ligation exerted its effect by increasing maturation of antibody affinity. Although this is not an allergic model, it further supports the concept that systemic use of TLR agonists may modulate the immune response to protect against subsequent allergic sensitization at distant sites.

Evidence that neonatal innate immunity promotes allergy development

Epidemiologic studies [59,60] have demonstrated that infections with certain pathogens, including *Chlamydo-phila pneumoniae*, *Mycoplasma pneumoniae*, RSV and influenza virus, may predispose to asthma or exacerbate pre-existing asthma. In addition, several genetic studies report associations between loss-of-function variants in innate immune genes and asthma risk. Polymorphisms inhibiting the function of CD14 or TLR4 have been associated with decreased asthma incidence and disease severity [54]. Inhibitory polymorphisms in TLR1 and TLR6, which form heterodimers with TLR2 to induce cytokine production, also protect against asthma development. This is consistent with experimental data showing that activation of APC by Pam3Cys induces high levels of Th2-polarizing effector molecules, including IL-13 and IL-1 β , but low levels of IL-12, IFN α , IL-18 and IL-27 [61]. Finally, mutations that decrease the TLR-inhibitory effect of IL-1 receptor kinase M (IRAK-M) were associated with early-onset asthma

[53]. Thus, epidemiologic and genetic studies support a role for innate immune stimulation in promoting allergy development (Table 2).

Multiple experimental studies have attempted to clarify the mechanisms by which innate immune stimulation promotes allergy development. Studies using purified TLR agonists demonstrate a key role for TLR stimulation in atopic sensitization. Mice deficient in MyD88, a key adapter for TLR signaling, do not develop a specific IgE response, eosinophil airway inflammation and AHR in a HDM allergy model that uses intranasal sensitization [62*]. OVA-sensitized mice that lack functional TLR4 exhibit less pronounced airway inflammation upon OVA challenge compared with wild-type mice [63]. Another study confirmed that LPS, administered intranasally during OVA sensitization, can function as an adjuvant to promote OVA-induced airway sensitization [64]. This study reports a dose-dependent effect of LPS, with low doses promoting Th2-type responses, whereas high doses induce a Th1-type response. In this and other studies, LPS-dependent sensitization to intranasal antigen did not occur in TLR4-deficient mice and depended on MyD88-dependent maturation of pulmonary dendritic cells [45,64]. HDM allergen Der p 2 has homology to myeloid-differentiation protein-2 (MD-2), a component of the TLR4 receptor complex that binds LPS. It was shown that Der p 2 signals through MD-2/TLR4 as a result of molecular mimicry [42**]. This study shows that allergen can use TLRs to initiate a robust innate immune response. The role of TLR4 signaling in HDM allergy is also supported by the finding that murine airway epithelial TLR4 expression is required for allergic airway inflammation [47**]. Double-stranded RNA (dsRNA), a TLR3 agonist, may also protect against allergy development. In a murine model, concomitant mucosal administration of OVA and low-dose dsRNA enhanced allergen-induced lung inflammation in wild-type mice but not

in mice deficient for TLR3, IL-4 or signal transducer and activator of transcription 6 (STAT6) [44]. Rat pups have altered pulmonary response to macrophage-activating lipopeptide 2 (MALP-2) (TLR2/6) with increased tissue and decreased bronchoalveolar mononuclear cells [65]. Finally, TLRs may modulate direct effects of allergen on nonimmune cells such as smooth muscle or the neuro-endocrine system. It was shown that TLR2 signaling is required for in-vitro rat smooth muscle contraction to HDM [66]. Interestingly, the effects of TLR agonists on atopic sensitization appear to be dose-dependent, with low doses of dsRNA and LPS inducing a Th2-type response, whereas high doses promote Th1-biased responses [44,67]. Of note, most studies used adult mice, and the effect of TLR agonists in neonatal mice remains to be determined.

Non-dendritic cell, non-TLR-mediated mechanisms that affect the development of allergy have also been described. Natural killer (NK) cells interact with dendritic cells to produce IFN γ and to promote Th1-type responses [68]. Adult asthma patients have lower numbers of IFN γ -producing CD56⁺/CD16⁺ NK cells, indicating that NK cells may predispose to asthma or exacerbate existing asthma. As neonatal NK cells are phenotypically and functionally mature [69], we hypothesize that NK cells may modulate the asthmatic response in established asthma. The role of NKT cells in asthmatic airway inflammation was demonstrated by showing specific increase in numbers of CD4⁺ NKT cells in the lungs of asthmatic patients but not in those with sarcoidosis [52]. In Th1-deficient (T-bet^{-/-}) mice, which spontaneously develop allergic airway inflammation, the essential role of NKT cells was confirmed [70]. Most CD4⁺ NKT cells produce IL-4, and a subset expresses the IL-25 receptor IL-17RB. This subset of NKT was shown as required for the induction of allergic airway inflammation [71^{*}]. As neonatal NKT cells are distinct and are virtually all CD4⁺ cells, it is conceivable that the neonate is more susceptible to NKT cell-driven allergic responses [6].

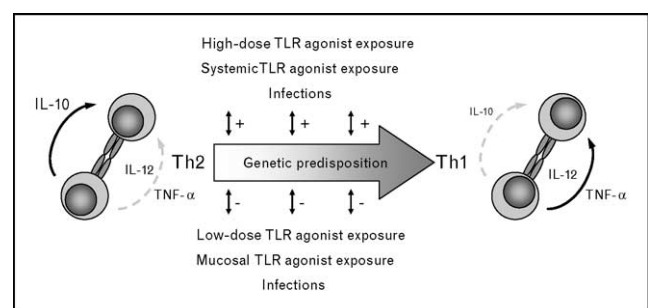
In summary, there is evidence that innate immune stimulation of mucosal cells may be a causative factor in the development of asthma. This appears to be a global effect because similar effects are observed following different agonists of the innate immune apparatus. However, intraspecies differences in the innate immune system prevent direct extrapolation of results from murine studies to humans. In addition, the exact underlying mechanism remains to be determined. Further translational studies on the effect of mucosal immune activation on allergy development will be important. In addition, it is not yet clear to what extent the effect of local innate immune activation on Th1 or Th2 polarization and allergy development is determined by the immunological maturational status of the child.

Protection or promotion: can both be true?

How can we reconcile apparent contradictory evidence that stimulation of the innate immune response can both prevent and promote allergic sensitization? Clearly, it is difficult to compare the level of evidence for both hypotheses. Whereas clinical evidence mainly points to a protective effect, experimental studies showing the opposite are quite convincing. Murine studies need to be interpreted with caution, as accumulating evidence indicates important differences between the murine and human TLR systems [72].

Nevertheless, different response patterns upon innate immune stimulation can be distinguished, leading to either protection or promotion (Fig. 1). The effect of innate immune responses on the development of allergy is determined by the timing, dose and site of stimulation [60]. Both experimental and human observational studies [44,67] have shown that the magnitude of mucosal innate immune stimulation determines whether innate responses prevent or enhance allergy development. Repeated high-dose administration of TLR agonist in the airways induces a Th1 response upon allergen exposure, whereas exposure to low-dose TLR agonists is associated with a Th2-like immune response. The site of innate immune triggering appears crucial for the effect on allergic sensitization. Mucosal exposure to TLR agonists enhances concomitant or subsequent responses to aeroallergens. Apparently, innate immune agonists prime the mucosal APCs to respond more vigorously to subsequent stimuli. It has been postulated that innate

Figure 1 Effects of allergen exposure on development of allergy: a model



Neonatal innate immune responses at birth polarize towards a Th2 response, characterized by decreased TLR agonist-induced production of Th1 cytokines such as IL-12 and TNF α , and increased agonist-induced production of IL-10. After birth, there is a gradual maturation towards Th1-polarizing responses. The degree and speed of this maturational process determine the risk of subsequent allergy development. Exposure to innate immune stimuli generally enhances innate immune maturation. However, the effects of innate immune stimulation are not universal and depend on host factors such as genetic predisposition and maturational state of the innate immune system, as well as on the site, dose and timing of exposure. IL, interleukin; Th1, T-helper cell 1; Th2, T-helper cell 2; TLR, toll-like receptor; TNF α , tumor necrosis factor alpha.

stimuli represent danger signals, which in turn may trigger Th2 responses [73]. This is advantageous in the case of pathogen encounter but may enhance IgE response upon concomitant allergen exposure. Systemic innate immune activation appears to have a global protective effect on allergy development, which is mediated by Th1-driving signals. To our knowledge, no reports exist that systemic innate immune activation has a deleterious effect on allergen exposure at distant sites.

Targets for intervention

Probiotics have been used in an attempt to reduce the development of allergic disease. A small randomized controlled trial (RCT) in 2001 showed that treatment of pregnant women during late pregnancy with *Lactobacillus rhamnosus* GG (LGG) reduced the development of atopic dermatitis in the neonates [74]. Since that publication, many RCTs have been reported with conflicting results. The largest published RCT ($n = 925$) confirmed that treatment with a mixture of Bifidobacterium and Propionibacterium of pregnant women 2–4 weeks before delivery and of their children during the first 6 months of life prevented the development of eczema (odds ratio 0.74, $P = 0.04$) [55]. However, meta-analyses of this and other studies were inconclusive [56,75]. Taken together, we conclude that the large differences in RCT results do not yet support large-scale supplementation of probiotics to infant formula milk in the general population.

Synthetic TLR agonists are being developed for treatment of oncologic, autoimmune and allergic diseases [76]. In addition, TLR agonists are under biopharmaceutical development as vaccine adjuvants and as immunomodulators in cancer and allergy. Synthetic TLR9 ligands are being considered in the treatment of allergic diseases. Although synthetic TLR ligands used in RCTs have not always proven effective in decreasing symptoms, they are well tolerated in general. ISS 1018 (Dynavax Technologies, Berkeley, California, USA) is a short, synthetic, unmethylated CpG motif-based ODN, which induces cell activation through TLR9. Repeated nebulization of this compound in stable mild asthmatics resulted in an increased local expression of IFN γ but did not affect the expression of Th2 cytokines, airway eosinophilia or AHR upon experimental allergen challenge [77]. Subsequently, this compound was conjugated to ragweed. In an RCT with 25 patients with allergic rhinitis, increasing doses of this allergen–TLR9 agonist conjugate injected for 6 consecutive weeks prior to the ragweed season were well tolerated [46]. In the intervention group, a two-thirds reduction of symptoms was observed compared with placebo-treated patients. Apparently, concomitant administration of TLR9 agonist and allergen can induce tolerance in allergic individuals.

However, the precise mechanism remains to be defined. Whether this strategy may be used for primary prevention is not known. Recently, TLR4 agonists have been used to treat allergic patients. A single dose of the chemical compound CRX-675 was well tolerated at different doses in patients with allergic rhinitis 1 day before allergen challenge, although no clear benefit was demonstrated in this phase 1 trial [78]. In two ongoing RCTs, it is being investigated whether the genetic background of patients determines the response to LPS. The inflammatory response in airway tissue in atopic and nonatopic asthmatic patients is measured 4 h after instillation of LPS in the lower airways (ClinicalTrials.gov NCT00644514). The effect of repeated LPS nebulization in healthy volunteers is measured in relation to their TLR4 genotype (ClinicalTrials.gov NCT00671892). In healthy volunteers, the effect of low versus high-dose LPS inhalation on exhaled nitric oxide concentration is being assessed (ClinicalTrials.gov NCT00643058). Finally, administration of a combination of TLR agonists has a synergistic effect on in-vitro maturation of human neonatal dendritic cells, suggesting that targeting a combination of TLRs may be a powerful approach to instruct the neonatal immune system towards a protective Th1 response [10]. Taken together, the synthetic TLR agonist may be a novel tool to prevent or treat allergic disease. However, most studies have been performed in adults with established allergy. More information is needed with respect to the effect of these compounds on allergy prevention in newborns.

Finally, the NKT cell is a promising target for immunomodulation. In an allergic mouse model, a single intraperitoneal injection of α -galactosylceramide during allergen challenge prevents eosinophilic airway inflammation and AHR [79]. In humans, α -galactosylceramide is well tolerated [80], but RCTs have not yet been performed in atopic individuals.

Conclusion

The role of innate immune responses in the development of allergy is controversial. Recent literature has provided the fundamental insight that magnitude and direction of the effect of innate immune responses are unequivocal. Timing, dose, site of activation and host genetic background are clearly crucial to understanding the interaction between innate immune stimulation and allergy development. Initially, clinical and basic studies showed that repeated systemic innate immune stimulation before birth or during the newborn period redirects the Th1–Th2 balance. However, increasing evidence argues against the general benefit of innate immune stimulation on allergy development, suggesting that host factors, site, dose and timing of exposure play an essential role. Host genetics may modulate the effects of TLR activation on

allergy development. Low-dose innate immune stimulation of mucosal cells enhances concomitant sensitization to allergen. Various environmental influences, including cigarette smoke and respiratory viruses, trigger the innate immune system, by which they may enhance responses upon allergen exposure.

On the basis of epidemiologic data, synthetic innate immune stimuli are being studied as a strategy to prevent allergy. A better understanding of the complex interaction between these stimuli and the developing neonatal immune system will be essential to determine the optimal use of these compounds.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

- 1 Adkins B, Leclerc C, Marshall-Clarke S. Neonatal adaptive immunity comes of age. *Nat Rev Immunol* 2004; 4:553–564.
- 2 Levy O. Innate immunity of the newborn: basic mechanisms and clinical correlates. *Nat Rev Immunol* 2007; 7:379–390.
- 3 Drohan L, Harding JJ, Holm B, *et al.* Selective developmental defects of cord blood antigen-presenting cell subsets. *Hum Immunol* 2004; 65:1356–1369.
- 4 Belderbos ME, Bleek G, Levy O. Skewed pattern of toll-like receptor 4-mediated cytokine production in human neonatal blood: low LPS-induced IL-12p70 and high IL-10 persist throughout the first month of life. *Clin Immunol* 2009 [Epub ahead of print]
- The first study to determine development of innate immune responses in healthy human newborns during the first month of life.
- 5 Novak N, Bieber T. 2. Dendritic cells as regulators of immunity and tolerance. *J Allergy Clin Immunol* 2008; 121:S370–S374.
- 6 Eger KA, Sundrud MS, Motsinger AA, *et al.* Human natural killer T cells are heterogeneous in their capacity to reprogram their effector functions. *PLoS One* 2006; 1:e50.
- 7 Levy O, Zarembek KA, Roy RM, *et al.* Selective impairment of TLR-mediated innate immunity in human newborns: neonatal blood plasma reduces monocyte TNF- α induction by bacterial lipopeptides, lipopolysaccharide, and imiquimod, but preserves the response to R-848. *J Immunol* 2004; 173:4627–4634.
- 8 Levy O, Coughlin M, Cronstein BN, *et al.* The adenosine system selectively inhibits TLR-mediated TNF- α production in the human newborn. *J Immunol* 2006; 177:1956–1966.
- 9 Vanden ES, Goriely S, De WD, *et al.* Preferential production of the IL-12(p40)/IL-23(p19) heterodimer by dendritic cells from human newborns. *Eur J Immunol* 2006; 36:21–26.
- 10 Krumbiegel D, Zepp F, Meyer CU. Combined toll-like receptor agonists synergistically increase production of inflammatory cytokines in human neonatal dendritic cells. *Hum Immunol* 2007; 68:813–822.
- 11 Goriely S, Van LC, Dadkhah R, *et al.* A defect in nucleosome remodeling prevents IL-12(p35) gene transcription in neonatal dendritic cells. *J Exp Med* 2004; 199:1011–1016.
- 12 Yan SR, Qing G, Byers DM, *et al.* Role of MyD88 in diminished tumor necrosis factor α production by newborn mononuclear cells in response to lipopolysaccharide. *Infect Immun* 2004; 72:1223–1229.
- 13 Lappalainen M, Roponen M, Pekkanen J, *et al.* Maturation of cytokine-producing capacity from birth to 1 yr of age. *Pediatr Allergy Immunol* 2009 [Epub ahead of print]
- A birth cohort study that incorporates maternal and neonatal innate immune function at birth and at the age of 1 year to predict the development of asthma.
- 14 De WD, Tonon S, Orlislagers V, *et al.* Impaired responses to toll-like receptor 4 and toll-like receptor 3 ligands in human cord blood. *J Autoimmun* 2003; 21:277–281.
- 15 Aksoy E, Albarani V, Nguyen M, *et al.* Interferon regulatory factor 3-dependent responses to lipopolysaccharide are selectively blunted in cord blood cells. *Blood* 2007; 109:2887–2893.
- 16 Levy O, Suter EE, Miller RL, Wessels MR. Unique efficacy of toll-like receptor 8 agonists in activating human neonatal antigen-presenting cells. *Blood* 2006; 108:1284–1290.
- 17 Kaisho T, Akira S. Toll-like receptor function and signaling. *J Allergy Clin Immunol* 2006; 117:979–987.
- 18 Kaiko GE, Horvat JC, Beagley KW, Hansbro PM. Immunological decision-making: how does the immune system decide to mount a helper T-cell response? *Immunology* 2008; 123:326–338.
- 19 De WD, Orlislagers V, Goriely S, *et al.* Blood plasmacytoid dendritic cell responses to CpG oligodeoxynucleotides are impaired in human newborns. *Blood* 2004; 103:1030–1032.
- 20 Forster-Waldl E, Sadeghi K, Tamandl D, *et al.* Monocyte toll-like receptor 4 expression and LPS-induced cytokine production increase during gestational aging. *Pediatr Res* 2005; 58:121–124.
- 21 Yerkovich ST, Wikstrom ME, Suriyaarachchi D, *et al.* Postnatal development of monocyte cytokine responses to bacterial lipopolysaccharide. *Pediatr Res* 2007; 62:547–552.
- 22 Sadeghi K, Berger A, Langgartner M, *et al.* Immaturity of infection control in preterm and term newborns is associated with impaired toll-like receptor signaling. *J Infect Dis* 2007; 195:296–302.
- 23 Goriely S, Molle C, Nguyen M, *et al.* Interferon regulatory factor 3 is involved in toll-like receptor 4 (TLR4)- and TLR3-induced IL-12p35 gene activation. *Blood* 2006; 107:1078–1084.
- 24 LeBouder E, Rey-Nores JE, Raby AC, *et al.* Modulation of neonatal microbial recognition: TLR-mediated innate immune responses are specifically and differentially modulated by human milk. *J Immunol* 2006; 176:3742–3752.
- 25 Wills-Karp M. Complement activation pathways: a bridge between innate and adaptive immune responses in asthma. *Proc Am Thorac Soc* 2007; 4:247–251.
- 26 Drew JH, Arroyave CM. The complement system of the newborn infant. *Biol Neonate* 1980; 37:209–217.
- 27 Schnurr M, Toy T, Shin A, *et al.* Extracellular nucleotide signaling by P2 receptors inhibits IL-12 and enhances IL-23 expression in human dendritic cells: a novel role for the cAMP pathway. *Blood* 2005; 105:1582–1589.
- 28 Levy O, Jean-Jacques RM, Cywes C, *et al.* Critical role of the complement system in group B streptococcus-induced tumor necrosis factor α release. *Infect Immun* 2003; 71:6344–6353.
- 29 Vekemans J, Amedei A, Ota MO, *et al.* Neonatal bacillus Calmette-Guerin vaccination induces adult-like IFN- γ production by CD4⁺ T lymphocytes. *Eur J Immunol* 2001; 31:1531–1535.
- 30 Tse K, Horner AA. Allergen tolerance versus the allergic march: the hygiene hypothesis revisited. *Curr Allergy Asthma Rep* 2008; 8:475–483.
- 31 Ege MJ, Bieli C, Frei R, *et al.* Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children. *J Allergy Clin Immunol* 2006; 117:817–823.
- 32 Miller RL, Chew GL, Bell CA, *et al.* Prenatal exposure, maternal sensitization, and sensitization in utero to indoor allergens in an inner-city cohort. *Am J Respir Crit Care Med* 2001; 164:995–1001.
- 33 Jones CA, Holloway JA, Popplewell EJ, *et al.* Reduced soluble CD14 levels in amniotic fluid and breast milk are associated with the subsequent development of atopy, eczema, or both. *J Allergy Clin Immunol* 2002; 109:858–866.
- 34 Schaub B, Liu J, Hoppler S, *et al.* Maternal farm exposure modulates neonatal immune mechanisms through regulatory T cells. *J Allergy Clin Immunol* 2009; 123:774–782.
- This study relates maternal farm exposure during pregnancy to a higher number of regulatory T (Treg) cells, with decreased Th2-polarizing cytokine secretion in cord blood.
- 35 Gereda JE, Leung DY, Thatayatikom A, *et al.* Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitization in infants at high risk of asthma. *Lancet* 2000; 355:1680–1683.
- 36 Schram-Bijkerk D, Doekes G, Douwes J, *et al.* Bacterial and fungal agents in house dust and wheeze in children: the PARSIFAL study. *Clin Exp Allergy* 2005; 35:1272–1278.
- 37 Oddy WH, Peat JK. Breastfeeding, asthma, and atopic disease: an epidemiological review of the literature. *J Hum Lact* 2003; 19:250–261.
- 38 Ball TM, Castro-Rodriguez JA, Griffith KA, *et al.* Siblings, day-care attendance, and the risk of asthma and wheezing during childhood. *N Engl J Med* 2000; 343:538–543.
- 39 Rothers J, Stern DA, Spangenberg A, *et al.* Influence of early day-care exposure on total IgE levels through age 3 years. *J Allergy Clin Immunol* 2007; 120:1201–1207.

- 40 Smit LA, Siroux V, Bouzigon E, *et al*. CD14 and toll-like receptor gene polymorphisms, country living, and asthma in adults. *Am J Respir Crit Care Med* 2009; 179:363–368.
- 41 Prescott SL, Noakes P, Chow BW, *et al*. Presymptomatic differences in toll-like receptor function in infants who have allergy. *J Allergy Clin Immunol* 2008; 122:391–399; 399.e1–5.
- This is the first prospective study to demonstrate that differences in innate immune function at birth predispose to the development of atopy.
- 42 Trompette A, Divanovic S, Visintin A, *et al*. Allergenicity resulting from functional mimicry of a toll-like receptor complex protein. *Nature* 2009; 457:585–588.
- This study proposes an interesting novel mechanism by which common allergens may induce innate immune responses and allergy.
- 43 Lam D, Ng N, Lee S, *et al*. Airway house dust extract exposures modify allergen-induced airway hypersensitivity responses by TLR4-dependent and independent pathways. *J Immunol* 2008; 181:2925–2932.
- 44 Jeon SG, Oh SY, Park HK, *et al*. TH2 and TH1 lung inflammation induced by airway allergen sensitization with low and high doses of double-stranded RNA. *J Allergy Clin Immunol* 2007; 120:803–812.
- 45 Piggott DA, Eisenbarth SC, Xu L, *et al*. MyD88-dependent induction of allergic Th2 responses to intranasal antigen. *J Clin Invest* 2005; 115: 459–467.
- 46 Creticos PS, Schroeder JT, Hamilton RG, *et al*. Immunotherapy with a ragweed-toll-like receptor 9 agonist vaccine for allergic rhinitis. *N Engl J Med* 2006; 355:1445–1455.
- 47 Hammad H, Chieppa M, Perros F, *et al*. House dust mite allergen induces asthma via toll-like receptor 4 triggering of airway structural cells. *Nat Med* 2009; 15:410–416.
- A hallmark study that identifies airway epithelial cells as key cells in HDM-induced allergic airway responses in mice.
- 48 Ashino S, Wakita D, Zhang Y, *et al*. CpG-ODN inhibits airway inflammation at effector phase through down-regulation of antigen-specific Th2-cell migration into lung. *Int Immunol* 2008; 20:259–266.
- This study gives insight into how synthetic TLR9 agonists may inhibit allergic airway inflammation.
- 49 Delgado MF, Coviello S, Monsalvo AC, *et al*. Lack of antibody affinity maturation due to poor toll-like receptor stimulation leads to enhanced respiratory syncytial virus disease. *Nat Med* 2009; 15:34–41.
- This study demonstrates that immature neonatal TLR responses result in low-affinity antibodies upon vaccination, resulting in enhanced RSV disease. Because of the role of RSV in asthma pathogenesis, TLR-modifying agents may be of use to promote vaccine responses that prevent asthma.
- 50 Kneyber MC, van Woensel JB, Uijtendaal E, *et al*. Azithromycin does not improve disease course in hospitalized infants with respiratory syncytial virus (RSV) lower respiratory tract disease: a randomized equivalence trial. *Pediatr Pulmonol* 2008; 43:142–149.
- 51 Moisan J, Camateros P, Thuraingam T, *et al*. TLR7 ligand prevents allergen-induced airway hyperresponsiveness and eosinophilia in allergic asthma by a MYD88-dependent and MK2-independent pathway. *Am J Physiol Lung Cell Mol Physiol* 2006; 290:L987–L995.
- 52 Akbari O, Faul JL, Hoyte EG, *et al*. CD4⁺ invariant T-cell-receptor⁺ natural killer T cells in bronchial asthma. *N Engl J Med* 2006; 354:1117–1129.
- 53 Balaci L, Spada MC, Olla N, *et al*. IRAK-M is involved in the pathogenesis of early-onset persistent asthma. *Am J Hum Genet* 2007; 80:1103–1114.
- 54 Senthilselvan A, Rennie D, Chenard L, *et al*. Association of polymorphisms of toll-like receptor 4 with a reduced prevalence of hay fever and atopy. *Ann Allergy Asthma Immunol* 2008; 100:463–468.
- 55 Kukkonen K, Savilahti E, Hahtela T, *et al*. Probiotics and prebiotic galactooligosaccharides in the prevention of allergic diseases: a randomized, double-blind, placebo-controlled trial. *J Allergy Clin Immunol* 2007; 119:192–198.
- 56 Lee J, Seto D, Bielory L. Meta-analysis of clinical trials of probiotics for prevention and treatment of pediatric atopic dermatitis. *J Allergy Clin Immunol* 2008; 121:116–121.
- 57 Sel S, Wegmann M, Sel S, *et al*. Immunomodulatory effects of viral TLR ligands on experimental asthma depend on the additive effects of IL-12 and IL-10. *J Immunol* 2007; 178:7805–7813.
- 58 Taylor RC, Richmond P, Upham JW. Toll-like receptor 2 ligands inhibit TH2 responses to mite allergen. *J Allergy Clin Immunol* 2006; 117:1148–1154.
- 59 Newcomb DC, Peebles RS Jr. Bugs and asthma: a different disease? *Proc Am Thorac Soc* 2009; 6:266–271.
- 60 Schroder NW, Arditi M. The role of innate immunity in the pathogenesis of asthma: evidence for the involvement of toll-like receptor signaling. *J Endotoxin Res* 2007; 13:305–312.
- 61 Redecke V, Hacker H, Datta SK, *et al*. Cutting edge: activation of toll-like receptor 2 induces a Th2 immune response and promotes experimental asthma. *J Immunol* 2004; 172:2739–2743.
- 62 Phipps S, Lam CE, Kaiko GE, *et al*. Toll/IL-1 signaling is critical for house dust mite-specific Th1 and Th2 responses. *Am J Respir Crit Care Med* 2009; 179:883–893.
- A murine study showing a differential role for TLR2 and TLR4 triggering in regulation of HDM-induced airway inflammation.
- 63 Dabbagh K, Dahl ME, Stepick-Biek P, Lewis DB. Toll-like receptor 4 is required for optimal development of Th2 immune responses: role of dendritic cells. *J Immunol* 2002; 168:4524–4530.
- 64 Eisenbarth SC, Piggott DA, Huleatt JW, *et al*. Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J Exp Med* 2002; 196:1645–1651.
- 65 Luhmann A, Grote K, Stephan M, *et al*. Local pulmonary immune stimulation by the toll-like receptor 2 and 6 ligand MALP-2 in rats is age dependent. *Immunol Lett* 2007; 108:167–173.
- 66 Chiou YL, Lin CY. Der p2 activates airway smooth muscle cells in a TLR2/MyD88-dependent manner to induce an inflammatory response. *J Cell Physiol* 2009; 220:311–318.
- 67 Kim YK, Oh SY, Jeon SG, *et al*. Airway exposure levels of lipopolysaccharide determine type 1 versus type 2 experimental asthma. *J Immunol* 2007; 178:5375–5382.
- 68 Scordamaglia F, Balsamo M, Scordamaglia A, *et al*. Perturbations of natural killer cell regulatory functions in respiratory allergic diseases. *J Allergy Clin Immunol* 2008; 121:479–485.
- 69 Dalle JH, Menezes J, Wagner E, *et al*. Characterization of cord blood natural killer cells: implications for transplantation and neonatal infections. *Pediatr Res* 2005; 57:649–655.
- 70 Kim HY, Pichavant M, Matangkasombut P, *et al*. The development of airway hyperreactivity in T-bet-deficient mice requires CD1d-restricted NKT cells. *J Immunol* 2009; 182:3252–3261.
- 71 Stock P, Lombardi V, Kohlraut V, Akbari O. Induction of airway hyperreactivity by IL-25 is dependent on a subset of invariant NKT cells expressing IL-17RB. *J Immunol* 2009; 182:5116–5122.
- One of the first studies identifying the role of NKT cells in AHR development.
- 72 Werling D, Jann OC, Offord V, *et al*. Variation matters: TLR structure and species-specific pathogen recognition. *Trends Immunol* 2009; 30:124–130.
- 73 Willart MA, Lambrecht BN. The danger within: endogenous danger signals, atopy and asthma. *Clin Exp Allergy* 2009; 39:12–19.
- 74 Kalliomaki M, Salminen S, Arvilommi H, *et al*. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* 2001; 357:1076–1079.
- 75 Osborn DA, Sinn JK. Probiotics in infants for prevention of allergic disease and food hypersensitivity. *Cochrane Database Syst Rev* 2007:CD006475.
- 76 Kanzler H, Barrat FJ, Hessel EM, Coffman RL. Therapeutic targeting of innate immunity with toll-like receptor agonists and antagonists. *Nat Med* 2007; 13:552–559.
- 77 Gauvreau GM, Hessel EM, Boulet LP, *et al*. Immunostimulatory sequences regulate interferon-inducible genes but not allergic airway responses. *Am J Respir Crit Care Med* 2006; 174:15–20.
- 78 Casale TB, Kessler J, Romero FA. Safety of the intranasal toll-like receptor 4 agonist CRX-675 in allergic rhinitis. *Ann Allergy Asthma Immunol* 2006; 97:454–456.
- 79 Morishima Y, Ishii Y, Kimura T, *et al*. Suppression of eosinophilic airway inflammation by treatment with alpha-galactosylceramide. *Eur J Immunol* 2005; 35:2803–2814.
- 80 Giaccone G, Punt CJ, Ando Y, *et al*. A phase I study of the natural killer T-cell ligand alpha-galactosylceramide (KRN7000) in patients with solid tumors. *Clin Cancer Res* 2002; 8:3702–3709.