

# Fetal Epigenetic Mechanisms and Innate Immunity in Asthma

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**Abstract** Allergy and asthma are chronic inflammatory diseases that result from complex gene–environment interactions. Recent evidence points to the importance of prenatal and postnatal developmental processes in the maturation of balanced immune responses. Novel data indicate that epigenetic mechanisms contribute to the development of T-helper–cell function. Environmental factors, including diesel exhaust particles, vitamins, and tobacco smoke, operate through such mechanisms. Furthermore, the role of environmental microbes provides another—and maybe an even more important—group of exogenous exposures that operate in this critical time frame. A better understanding of fetal immuno-maturation conditions will provide the basis for the development of novel allergo-protective clinical strategies.

**Keywords** Allergy · Asthma · Toll-like-receptors · Environmental exposures · Microbial stimuli · Innate immune system · Allergy protection · Prenatal · Fetal · Animal model

## Introduction

Allergic asthma as a complex outcome of pathogenic, immunologic, cellular, and functional modifications of the airways is initiated by allergic reactions as a response mostly to inhalant allergens. Dysregulation of innate and adaptive immune functions contributes to the pathogenesis

of the disease. A hallmark is the development of a T-helper type 2 (Th2)-driven inflammatory response in the airways. The underlying Th2-skewed balance is the result of a multifunctional process in which genetic predisposition and environmental exposures interact as major players. Maturation of the immune system starts in utero, during the most critical phase in the ontogenetic programming of the offspring. Endogenous and exogenous exposures may influence the maturation and differentiation of immune cells of the fetus and may thereby contribute to disorders such as allergies and asthma later in life. Epigenetic mechanisms are proposed to mediate these effects. Within this review, we provide an overview on the interaction of fetal exposures and the developing immune system that may contribute to or protect the progeny against the development of asthma. The new and exciting field of epigenetics is highlighted with respect to T-cell differentiation and early allergic disease development. Furthermore, we emphasize new investigations that aimed to analyze fetal host innate immune responses to environmental microbial microorganisms and their possible future application in asthma protection.

## Fetal Development: A Critical Time Frame for Immune Maturation

The fetal phase of life is characterized by proliferation and differentiation processes and is thereby determined by sophisticated, regulated DNA activity. These initial steps in the shaping of life are not only governed by the genetic program of the progeny but also by the maternal environment that is provided and controlled by the placenta. Although the fetomaternal barrier protects the fetus from hazardous influences, it allows a cross-talk between maternal stimuli

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and responses of the offspring, potentially mediated by factors of the innate immune system. With the onset of immune maturation in the second trimester of gestation, environmental factors mediated or transferred by the mother may interfere with this process and may set a direction in immune cell lineage commitment. One concept of how these environmental effects may act in progeny is the mechanism of epigenetic regulation [1•].

### Key Mechanisms in Epigenetic Regulation

Epigenetic mechanisms are proposed to play a major role in the orchestration of prenatal ontogenetic differentiation and regulate metabolic and mitotic cycles on the transcriptional level. Based on a genetic blueprint, epigenetic reactions alter the phenotype without modifying the genetic sequence. Thus, epigenetic modifications result in activation or complete/partial silencing of genes. This is accomplished by histone modification and DNA methylation (Fig. 1a) [2]. Epigenetic modifications contribute to nearly all pathogenetic processes in which environmental and genetic factors are involved and therefore may contribute to a better understanding of the mechanisms involved in asthma development [3].

### Epigenetic Regulation of Th1 and Th2 Development

The development of allergic phenotypes at the skin, lung, and gastrointestinal mucosal tissue is closely linked to the generation of Th2 cells, which produce a characteristic cytokine pattern including interleukin (IL)-4, IL-5, and IL-13. IL-4 has been considered as a key cytokine that plays an important role in initiating Th2 development. Furthermore, IL-4 is required to maintain a pool of Th2 cells, as it promotes proliferation of these cells. On the other end of the spectrum, the Th1-related cytokine interferon (IFN)- $\gamma$  has a broad variety of Th2-counteracting activities, including the suppression of Th2 responses. Therefore, regulation of IL-4 and IFN- $\gamma$  production are critical events in allergic conditions.

Recent data indicate that expression of both the IL-4 and the IFN- $\gamma$  gene remain under close epigenetic regulatory control. In the naïve, not-yet-committed T cell, the cytokine genes are only partially silenced because the baseline levels of IFN- $\gamma$  and IL-4 transcription are evident within a very short time frame after cell activation. This is independent of T-box transcription factor (T-bet) or GATA-3 expression, two transcription factors that are critically involved in Th-cell differentiation. To progress in the Th-cell differentiation pathway, it is important to sustain expression of one cytokine and repress the other cytokine in these particular cells,

respectively. This polarizing process results from the interplay of transcriptional activation and repression that is characterized by a reinforcement of a mutually negative feedback of the above-mentioned transcription factors. The epigenetic remodelling of cytokine gene loci seems to be strongly involved in T-cell differentiation, as it governs the accessibility of these gene loci for the respective transcription factors [4].

GATA-3 and T-bet mediate many of the structural changes of the chromatin that occur during T-cell differentiation. Overexpression of T-bet induces the DNase I hypersensitivity region of the IFN- $\gamma$  gene locus and enhances IFN gene transcription. T-bet induces IFN- $\gamma$  by inducing a co-factor, HLX, which together with T-bet appears to activate the IFN- $\gamma$  locus synergistically to promote remodelling of the chromatin structure [5]. A recently published study conducted by Thomas et al. [6•] pointed out the critical role of Ikaros in Th2 polarization. By binding at the endogenous *tbx21* promoter, Ikaros silences T-bet expression and thereby suppresses IFN production. Inhibition of Ikaros DNA binding activity during Th2 polarization resulted in a loss of Ikaros promoter occupancy and increased T-bet expression and T-bet-dependent production of IFN- $\gamma$ . Ikaros was also required for epigenetic imprinting of the IFN- $\gamma$  locus during Th2 polarization because Ikaros DNA binding resulted in alterations of the DNA methylation pattern in the IFN- $\gamma$  promoter region, with significant changes in the methylation status at the CpG site<sup>-53</sup> [6•]. The CpG site<sup>-53</sup> of the IFN- $\gamma$  promoter seems to be critically involved in the regulation of IFN- $\gamma$  gene expression. CpG<sup>-53</sup> resided in the proximal activator of protein 1 (AP1) binding site and when methylated can change transcription factor binding. A subsequent study demonstrated that methylation of the same CpG site significantly inhibited cyclic adenosine monophosphate response element-binding protein (CREB) and activating transcription factor-2/c-Jun (ATF2/c-Jun) transcription factor binding to the CpG-containing AP1 site [7, 8].

Th1 polarization also closely involves the IL-4 genes, as methylation of the highly conserved DNase I hypersensitivity region at the 3' end of the IL-4 gene is critically associated with Th1 differentiation [9]. Increased expression of GATA-3 is closely associated with changes occurring within the IL-4 gene locus during Th2 development. This includes DNA demethylation, the appearance of DNase I hypersensitivity sites, and histone acetylation within the same locus [10]. All these events result in increased accessibility of the regulatory enzymes and transcriptional factors within the IL-4 locus, which results in increased IL-4 gene transcription. IFN- $\gamma$  silencing occurs simultaneously and is associated with DNA methylation/histone deacetylation of the T-bet and IFN- $\gamma$  genes [11]. Demethylation along the IL-4 gene tends to progress in sequential order during

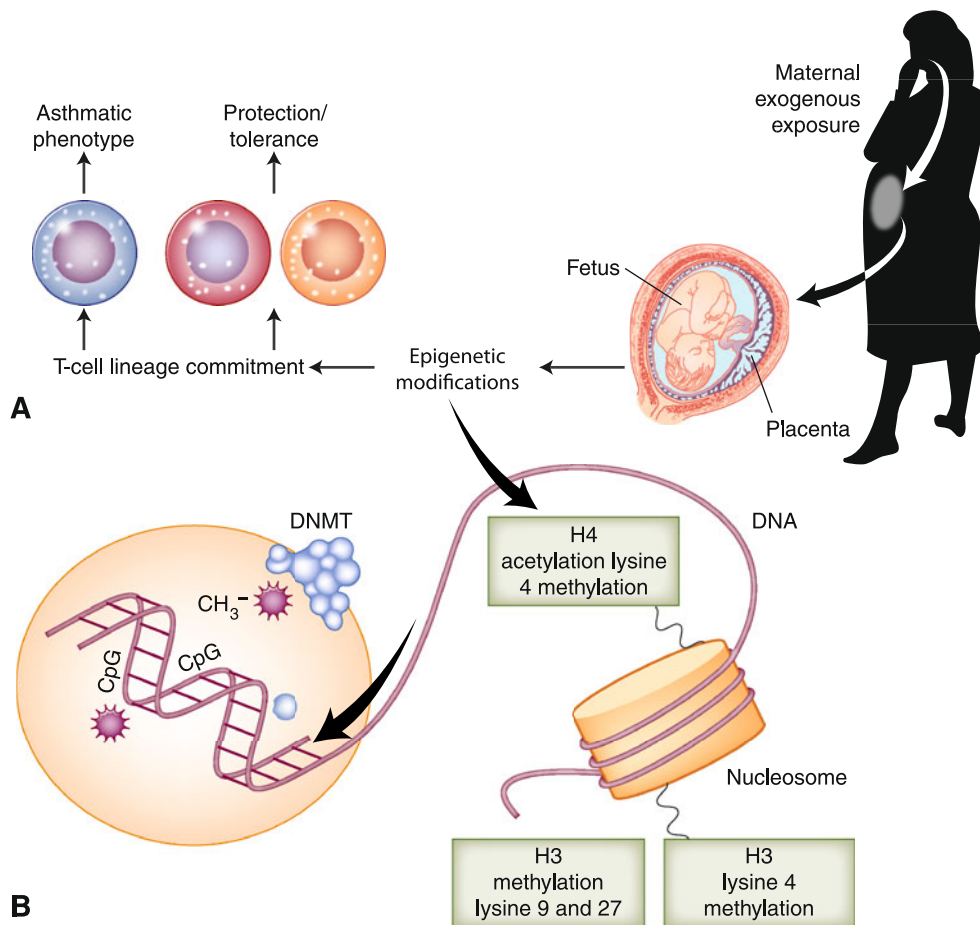


**Fig. 1** Epigenetic pathways in fetal immune modulation. **a**, A model of prenatal modulation of the fetal immune system by exogenous maternal exposures. Exogenous exposures incorporated by the mother during pregnancy might activate the innate immune system. Thus, initiated maternal signals may be transferred through the fetomaternal interface. A strong, long-lasting, and early stimulus mediated by the mother may result in epigenetic changes that influence T-cell lineage commitment in the progeny. Depending on the character of the maternal exposure, the developing fetal adaptive immune system is committed to an allergy-favoring direction or balanced toward tolerance and protection against allergies by a T-helper type 1 (Th1)/regulatory T cell (Treg)-dominated direction. **b**, Epigenetic modifications in mammalian cells. Histone modifications and DNA methylation are two major epigenetic mecha-

nisms that govern gene expression in mammalian cells. DNA methylation is the covalent addition of a methyl group at a dinucleotide sequence CpG. When the gene is expressed within a cell, these islands are maintained in an unmethylated state. Methylation of those islands through the action of DNA methyl transferases (DNMT) inhibits gene transcription. Histones consist of eight subunits that wrap the DNA around. If these histones are tightly packed, the transcription apparatus cannot access the DNA, and the gene is therefore repressed. If the histones are located at some distance from each other, the DNA is “open” for transcription. This is achieved by acetylation or lysine 4 methylation of histone H3 and H4, while methylation of lysine 9 and 27 are well-known markers of silent or inactive gene regulation

Th2 differentiation from the 5' to the 3' end. It was shown that demethylation begins at CpG<sup>-408</sup>. This CpG site has been identified as a putative transcription factor AP2 binding site, although it is not associated with IL-4 gene transcription to date [12].

In conclusion, these data strongly indicate that epigenetic mechanisms operate in the differentiation process of a naïve T cell into Th1/Th2 effector status. This operation includes epigenetic chromatin remodelling at many genes, including IL-4 and IFN- $\gamma$  as prominent examples. However, it must be noted that epigenetic regulation may not be restricted to these two genes alone, as many genes, including the genes

of transcription factors themselves, are under tight epigenetic control.

### Experimental Models for Epigenetic Regulation of Allergic Phenotypes

#### Diesel Exhaust Particles

Epidemiologic studies have identified low-income minority children who are more likely to reside near heavy traffic-related air pollution sites as high-asthma risk individuals

[13]. In a recent study using the murine *Aspergillus fumigatus* allergen model, it was shown that inhaled diesel exhaust particles induced augmentation of the allergic phenotype, which was associated with epigenetic changes [14]. Chronic diesel exhaust particle inhalation induced hypermethylation at the CpG<sup>-45</sup>, CpG<sup>-53</sup>, and CpG<sup>-205</sup> sites of the IFN- $\gamma$  promoter. This was associated with higher IgE production. Conversely, hypomethylation at CpG<sup>-408</sup> in the proximal IL-4 promoter in CD4<sup>+</sup> cells was also detected and was associated with changes in IgE levels. Although this study had several limitations, it was the first to demonstrate a link between environmental exposure, epigenetic regulation, and altered phenotype development.

#### Vitamin B12/Folic Acid

In a recent hallmark experiment, it was demonstrated that dietary methyl supplementation during pregnancy may alter heritable phenotype development in the offspring. The example used by the investigators was development of coat-color distribution, which is regulated by the agouti gene. Methyl supplementation can be achieved with folic acid, vitamin B12, and other agents. It was shown that such supplementation altered coat-color expression via increased CpG methylation of the region upstream (promoter region) of the agouti gene [15]. The relevance of a methyl-rich diet during pregnancy to development of an allergic phenotype was demonstrated in a prenatal murine model. Feeding mother mice with a methyl donor-rich diet results in enhanced severity of the asthmatic phenotype. The increment of airway inflammation was associated with an increase in DNA methylation in transcription factors that balances inflammation on the bronchus [16••].

#### Polycyclic Aromatic Hydrocarbons

The traffic-related increased asthma risk, particularly in the inner-city population, might not be related only to diesel exhaust particles but also to polycyclic aromatic hydrocarbons (PAHs). In a recent study, transplacental exposure to traffic-related PAHs was studied with regard to childhood asthma development and changes in DNA methylation pattern. The investigators monitored maternal PAH exposure and studied the methylation status in umbilical leukocytes. They identified more than 30 DNA sequences whose methylation status was dependent on the level of maternal PAH exposure. The highest concordance between extent of methylation and the level of gene expression in matched fetal placental tissues was found for acyl-CoA-synthetase long-chain family member 3 (*ACSL3*) [17•]. This gene belongs to a family that encodes key enzymes in fatty acid metabolism. It is expressed in lung and thymic tissue and is responsible for the intracellular conversion of long-chain fatty acids. Thus, hypermethylation

of this gene is expected to diminish fatty acid utilization and may also influence membrane phospholipid composition. Whether these functional changes directly affect the development of the asthmatic phenotype, however, remains unknown. However, several epidemiologic studies have shown that fatty acid composition in milk, diet, and umbilical cord blood affects the development of allergy and asthma. This has been attributed to the anti-inflammatory and immune-modulating effects of omega-3 (n-3) fatty acids [18]. It is interesting to note that *ACSL3* is located in the chromosomal region 2q36.1. This region was recently shown to be associated with asthma susceptibility [19]. In conclusion, transplacental PAH exposure provides another model situation to underscore the importance of epigenetic regulation in terms of phenotype development, although the detailed course-effect relationship between PAH exposure, hypermethylation of the *ACSL3* gene, and asthma development remains to be fully established.

#### Gestational Smoke Exposure

Most of the convincing data indicating that prenatal environmental exposures can influence the risk of subsequent asthma development originate from research on environmental tobacco smoke (ETS) exposure. Overwhelming evidence indicates that prenatal exposure to ETS is associated with several hallmark parameters of asthma, including impaired respiratory function, wheezing, respiratory infections, and altered airway structure [20]. Recent data now suggest that the risk of ETS exposure on asthma development may be transmitted across two generations [21]. The investigators conducted a case-control study including 338 children with asthma and 570 control participants. They used an innovative sampling design to efficiently investigate the associations of in utero exposure to maternal smoking with asthma occurrence. The study confirmed that in utero exposure to maternal smoking was associated with increased risk of asthma diagnosed in the first 5 years of life. In addition, it is most remarkable that only grandmaternal smoking during the mother's fetal period may present a risk factor for asthma in the grandchildren generation. Furthermore, this was independent of maternal smoking. The risk of asthma in grandchildren was highest when both the grandmother and the mother smoked during pregnancy.

Recently, maternal smoking has been associated with stronger neonatal T-cell proliferation [22]. However, the effect on cytokine responses was not investigated. Another study provided evidence that maternal smoking in pregnancy is associated with lower Th1 responses to mitogenic stimulation as measured on the mRNA expression level [23]. Whether this is directly epigenetically regulated has not been investigated thus far. However, it must be noted that tobacco smoke was recently shown to modify gene expression by promoter hypermethylation associated with

downregulation of gene transcription in the lung [24]. These data indicate that tobacco smoke in principle has the capacity to act on the level of epigenetic regulation; however, whether the observed increased risk of asthma development is also related to epigenetic events remains to be investigated. The epidemiologic data, particularly on grandmaternal exposures, at least point in this direction.

### Fetal Innate Immune Responses to Microbial Stimuli

#### Evidence from Epidemiologic Studies

The traditional farming environment provides allergy and asthma protection via contact of farm children with a wide range of naturally prevalent microbes in barns and hay lofts as well as in the domestic environment. The so-called “farming effect” was first described in epidemiologic studies conducted in the Alpine region and confirmed in other regions around the world. Many cross-sectional studies confirmed that the early contact with animals and consumption of farm-derived products may protect against allergies and asthma later in life. In this context, it was shown that the farming environment may provide protection against allergic disorders in utero. Maternal exposure to livestock and consumption of farm-produced milk during pregnancy were associated with a reduced risk of allergies in the offspring [25•]. Data from the ongoing birth cohort PASTURE (Protection Against Allergy Study in Rural Environments) study confirmed the prenatal modulating influence of maternal farming exposure on the developing immune system of the offspring. Cord blood samples from neonates born to farming families showed substantially more IFN- $\gamma$  and tumor necrosis factor (TNF)- $\alpha$  production after mitogen stimulation than samples from babies born to nonfarming families. This may indicate that the innate immune system is involved in interactions between environmental factors and the developing immune system [26•].

#### Early Responses of the Innate Immune System

The first response of an organism to a pathogen is mediated by antigen-presenting cells of the innate immune system. Dendritic cells (DCs), as the main antigen-presenting cell population, are critical to the fate of Th cells and, subsequently, to the development of asthma. Depletion of CD11<sup>+</sup> DCs leads to significant abrogation of characteristic features of experimental asthma, indicating that these cells are necessary and sufficient for the induction of a Th2-driven inflammatory allergic response [27]. The activation status of DCs plays an important role in this regard, as the expression profile of DCs is affected by the interaction of their so-called pattern-recognition receptors (PRRs) with

pathogen-associated molecular patterns (PAMPs) from a broad range of microbes. The interaction between PAMPs and PRRs is in contrast to the highly specific recognition of antigens by T-cell receptors. PAMP signaling initiates selective effector cascades (e.g., the release of antimicrobial defensins and the signaling to the adaptive immune response) [28]. The family of Toll-like receptors (TLRs) represents the best-characterized class of PRRs. TLRs represent sensors for microorganisms and microbial components. TLR1/TLR6 and TLR1/TLR2 heterodimers are PRRs for gram-positive bacteria such as mycobacteria or lactic acid bacteria. TLR4 recognizes lipopolysaccharide (LPS) from gram-negative bacteria. TLR5 serves as a receptor for flagellin, the main protein in bacterial flagella. TLR3, TLR7, and TLR9 are endosome-associated TLRs that recognize bacterial and viral DNA. Double-stranded DNA from bacteria and viruses are recognized by TLR3 and TLR9. TLR9 can also detect CpG domains in double-stranded DNA, while TLR7 detects bacterial and virus single-stranded DNA. Ligand binding on TLRs leads to an activation of several intracellular signaling pathways, including the nuclear factor- $\kappa$ B pathway, via direct or indirect interaction of TLR with MyD88 (except TLR3) and other cytosolic factors. As a result of a successful TLR-mediated activation, expression of co-stimulatory molecules (CD80 and CD86) and proinflammatory cytokines (TNF- $\alpha$ , IFNs, IL-1, IL-6, IL-10, and IL-12) is induced [29]. By this, DCs play a key role in linking innate and adaptive immunity. In close contact with naïve T cells, they orchestrate the polarization of these T-cell populations toward a Th1-, Th2-, Th17-, or T-regulatory subset differentiation [30]. In addition to their function in the defense of infectious agents, TLR responses become involved in the onset of immune-modulated diseases such as allergies and asthma by delivering appropriate signals to the T-cell compartment. Thereby, TLRs contribute to T-effector-cell commitment (Fig. 1b).

Little is known about the TLR development in ontogenesis. A murine study of changes in TLR2 and TLR4 expression in several organs during prenatal development reported that in the placenta, the mRNA expression levels of TLR4 during mid-pregnancy were twice as high as those of TLR2. In the lung, TLR2 and TLR4 expression levels were low during the first trimester of gestation and increased several fold during prenatal development, and then further after birth [31].

### Experimental Models of Fetal Immune Modulation by Microbial Stimuli

Several prenatal murine models of experimental asthma have demonstrated that innate immune responses via TLRs

play a major role in asthma protection. In these models, microbial TLR-activating stimuli were exposed to pregnant mice, and the offspring were sensitized and challenged with ovalbumin (OVA) to provoke the development of an asthmatic phenotype. On the basis of the epidemiologic findings that prenatal maternal exposure to LPS may protect the offspring against allergic disorders, prenatal murine experimental models were created to prove this concept. Recently, we have examined the effects of prenatal exposure to LPS on mice in allergic airway responses and found a reduction in allergen-induced airway eosinophilia and increased IFN- $\gamma$  production by splenocytes, but no changes in the airway hyperreactivity [32]. In a recently published report using a rat model, the reduction in allergic airway inflammation and Th2 bias in cytokines from bronchoalveolar lavage was confirmed. In addition and in contrast to the above-mentioned murine experiments, airway hyperresponsiveness was normalized in offspring from LPS-treated mothers [33]. This discrepant result in terms of altered lung functions may be explained by species-specific effects or methodologic differences in lung function measurements.

Bacteria isolated from the farming environment were shown to be protective against the development of an allergic phenotype when administered prior to sensitization [34]. Furthermore, *Acinetobacter lwoffii* F78, a stable-derived, gram-negative bacterium, showed antiallergic properties in a prenatal murine model. In contrast to the prenatally tested *Lactobacillus rhamnosus* GG and LPS, intranasal maternal application of *A. lwoffii* F78 resulted in a reduction in all features of airway inflammation and in normalization of the airway hyperresponsiveness in OVA-sensitized offspring. For OVA-specific IgE, a trend toward lower antibody formation was observed. We recently investigated the mechanisms by which protection might be transferred from the mother to the progeny [35•]. Intranasal exposure of the dams resulted in low but stable IL-6 production locally in the lung and systemically in the peripheral blood. TLR expression and cytokine production in the placenta were suppressed. By using TLR2/TLR3/TLR4/TLR5/TLR7/TLR9<sup>-/-</sup> females mated with wild-type males, the *A. lwoffii* F78-triggered protective effect was abolished, although the heterozygous offspring were able to signal via TLR. This indicates that a functional TLR signaling of the mother is required to mediate protective effects from environmental stimuli in pregnancy (Fig. 2).

### Probiotic Bacteria as Early Modulators of Innate Immune Responses

As a substantial part of a healthy gut microflora, probiotic bacteria were suggested to modulate the intestinal immune

functions and may therefore also act as protective factors against atopy and asthma [36]. The colonization of the neonatal gut by microbes from the neonatal environment leads to microbial stimulation of the intestinal immune system toward a more Th1-biased direction to compensate for the Th2 bias established in utero in order to prevent rejection of the fetus. Shaping the intestinal immune system by modulation of human colonic DCs, mostly via TLR2 signaling toward a Th1 direction, probiotic bacteria are involved in the stimulation of transforming growth factor- $\beta$ -producing Th cells to release Th1 cytokines. These cytokines act on B cells, inducing them to switch the IgA isotype, a mode to reduce inflammation, and to establish clinical tolerance against common antigens. The lack of such microbial action may give rise to elevated IgE production by B cells, with an increased risk of allergic reactions due to the subsequent activation of mast cells [37]. Experiments in a perinatal animal model of allergic asthma revealed promising results concerning the prevention of allergic airway inflammation. In a perinatal murine model of asthma, we applied *L. rhamnosus* GG orally to mother mice and observed a significant reduction in airway eosinophilia and inflammation in the lung but a failure to normalize lung function in OVA-sensitized offspring [38].

Nevertheless, benefits from probiotic bacteria in the prevention of allergic diseases are controversially discussed, as results from clinical trials focusing on the primary prevention of pediatric atopic dermatitis by prenatal application of probiotic bacteria are contradictory. A meta-analysis conducted by Lee et al. [39] revealed overall ORs below 1, indicating preventive effects of lactic acid bacteria on pediatric atopic dermatitis. These results were challenged by Salfeld and Kopp [40], who argued that these calculations included studies referring to the same study populations. However, comparative data for prenatal asthma prevention by probiotics are not available.

### Immune Modulation via TLR9 Ligands

Kitagaki et al. [41] demonstrated that application of TLR9 ligand CpG DNA prior to sensitization prevents Th2 inflammatory responses and effectively interacts with the development of atopic airway diseases in a murine model of experimental asthma. Moreover, when administered in combination with an experimental allergen, CpG promotes the reversal of established eosinophilic inflammation [41]. We recently found that TLR3 or TLR7 activation by viral TLR ligands has both preventive and suppressive effects on experimental asthma that are mediated by the additive effects of IL-12 and IL-10;

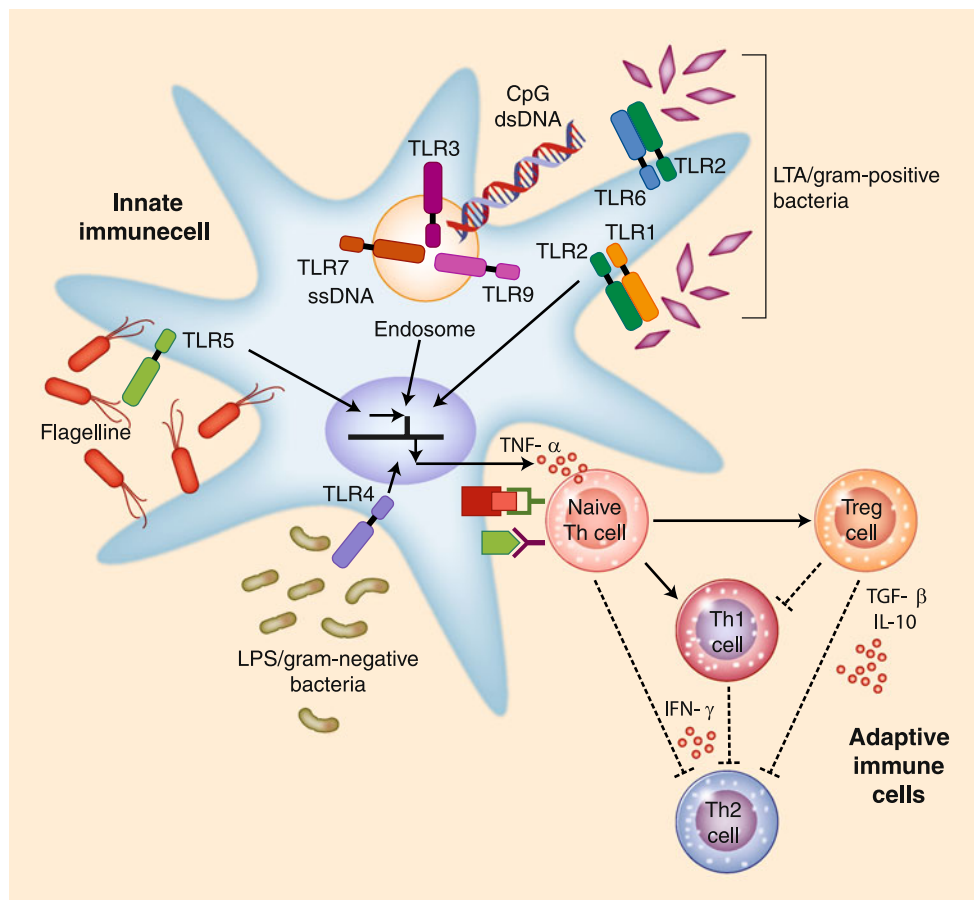


**Fig. 2** Activation of the innate immune system by environmental microbes/cross-talk between dendritic and T-helper (Th) cells as a result of Toll-like receptor (TLR) activation by microbial stimuli. Dendritic cells are at the front line of different environmental exposures. TLRs represent sensors for microorganisms and microbial components. TLR1/TLR6 and TLR1/TLR2 complexes are pathogen-associated molecular patterns for gram-positive bacteria such as mycobacteria or lactic acid (LTA) bacteria. TLR4 recognizes lipopolysaccharides (LPS) from gram-negative bacteria. TLR5 serves as a receptor for flagellin, the main protein in bacterial flagella. TLR3, TLR7, and TLR9 are endosome-associated TLRs that recognize bacterial and viral DNA. Double-stranded (dsDNA) from bacteria

and viruses is recognized by TLR3 and TLR9. TLR9 is able to detect CpG domains in dsDNA, while TLR7 detects bacterial and virus single-stranded DNA (ssDNA). TLR signaling results in a cytosolic cascade for transcription factors such as tumor necrosis factor (TNF)- $\alpha$ . In parallel, antigen presentation via major histocompatibility complex II complexes (green-orange) and notch signaling (yellow) primes naive Th cells toward Th1 and in a regulatory T cell (Treg) direction. Tregs establish a moderate Th1-cell development and suppress generation of Th2 cells via transforming growth factor (TGF)- $\beta$  and interleukin (IL)-10 production. Inhibition of Th2-cell development is supported by release of interferon (IFN)- $\gamma$  by Th1 cells

however, this concept was not proven in a prenatal experimental set-up [42].

### Helminth Infections as Perinatal Allergy Protective Factors

Both helminth infections and allergic diseases are strong inducers of Th2 responses. The upregulation of the Th2 cytokines, total IgE production, and eosinophilia are hallmarks of the host immune response against helminth infections as well as in atopic disease. Asymptomatic sustainment of parasites as a feature of helminth infections in endemic regions seemed to be achieved by

activation of regulatory T cells and systemically elevated levels of IL-10 and possibly transforming growth factor- $\beta$ . By this, helminth infections may provide a downregulatory effect on the risk of developing allergic disease [43].

Recent studies pointed out that helminth infections could affect the early development of immunity, as fetal exposure to helminth antigens can occur in utero via maternal infection. Exposure to helminths and protozoan infection in the mother may alter TLR expression in cells of the innate immune system and subsequently influence cytokine production in cord blood mononuclear cells [44, 45]. Experimental set-ups mainly use the model of schistosomiasis to investigate allergy-diminishing effects and related mecha-

nisms on allergic outcomes, but none of them proved the hypothesis of in utero protection.

### Stimulation of Innate Immune Responses by Other Environmental Exposures

Stimulation of the innate immune system through microbial exposure, such as activation of the innate TLRs, may reduce the development of allergy in childhood. In addition to microbial exposures, other factors may affect neonatal TLR stimulation and may therefore modulate the capacity of TLRs to respond to microbial stimuli. In a study comparing the cytokine production and gene expression profile in cord blood cells after stimulation with the TLR2 agonist peptidoglycan, samples from neonates born to nonatopic mothers showed higher levels of IL-10 production and forkhead box P3 expression than those born to atopic mothers [46]. A study conducted in cord blood cells revealed associations between maternal allergy and significantly higher neonatal IL-12 and IFN- $\gamma$  responses to TLR2, TLR3, and TLR4 activation, whereas TNF- $\alpha$  and IL-6 responses to TLR2, TLR4, and TLR5 activation were significantly higher in newborns who subsequently had allergic disease. Newborns who developed an allergic disease later in life had lower Th1/IFN- $\gamma$  responses to mitogens [47]. Data from a US inner-city cohort pointed out that prenatal stress affects innate immunity, indicating that higher prenatal stress scores were related to increased IL-8 production after microbial stimulation of TLRs and increased TNF- $\alpha$  production in cord blood cells [48]. Other factors that may modulate TLR-mediated innate cytokine responses include parental allergic and airway diseases, somatic fetal growth, ethnicity, and season of birth [49]. Common environmental toxic chemicals also may alter TLR signaling, as was reported in a prenatal murine model in which mother mice were exposed to low-level toluene concentration during pregnancy [50]. Collectively, these findings suggest that urban prenatal exposures and genetic factors affect the development of the fetal innate immune system.

### Conclusions

Epidemiologic, clinical, and experimental evidence accumulating during the past few years provides compelling new insight into the critical role played by the prenatal and postnatal environment in terms of immuno-programming, immuno-education, and subsequent allergy and asthma protection. These studies now provide the foundation on which underlying mechanisms will be elucidated and discovered. A better understanding of the contributing

cellular and molecular network will be the prerequisite for the development of novel allergy protective and preventive strategies. This work will require a better understanding of the relationship between the genome, epigenome, and transcriptome that eventually leads to phenotype development. In this regard, it is now well-established that environmental factors contribute to the epigenome and, therefore, the transcriptional and translational machinery of specific cell types. Understanding how the environment connects to cell biology and phenotype development will be the key to developing novel clinical approaches in this field.

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