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REVIEW

Maternal signals for progeny prevention against allergy and asthma

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Abstract Allergy and asthma are chronic inflammatory diseases which result from complex gene–environment interactions. Recent evidence indicates the importance of prenatal and postnatal developmental processes in terms of maturation of balanced immune responses. According to the current view, gene–environment interactions during a restricted time frame are responsible for programming of the immune system in favor of allergic immune mechanisms later in life. The interaction between genes and environment is complex and only partially understood; however, heritable epigenetic modifications including chemical additions in and alternative packaging of the DNA have been shown to play a crucial role in this context. Novel data indicate that epigenetic mechanisms contribute to the development of T-helper cell function. Environmental factors, including diesel exhaust particles (DEP), vitamins and tobacco smoke, operate through such mechanisms. Furthermore, the role of environmental microbes provides another and maybe even more important group of exogenous exposures which operates in this critical time frame.

Keywords Allergy · Asthma · Toll-like receptors · Innate immune system · Allergy protection · Prenatal · Animal model

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Introduction

Allergies and bronchial asthma are chronic inflammatory diseases that arise from a complex dysregulated interaction between the innate and adaptive immune responses. This results in a predominant activation of T-helper 2 (Th2) cells, leading to the development of a Th2-driven inflammation in response to a respective allergen [1]. It is now well established that maturation of the adaptive immune system and development of functionally active T cell subsets already starts prenatally. Furthermore, it has been proposed that immune programming by environmental influences may also occur at this early developmental stage. Indeed, studies have demonstrated that many factors affecting the initiation and course of allergies and asthma appear to act within a narrow window of opportunity, either prenatally or early in life [2, 3]. The maturation of the immune system already starts in utero, the most critical phase in the ontogenetic programming of the offspring. Endogenous as well as 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. However, it is still unresolved how the protective signals are transferred from the mother to the developing fetus. Epigenetic mechanisms are proposed to mediate these effects [4]. 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 allergies and asthma. The new and exciting field of epigenetics will be highlighted with respect to T cell differentiation and the development of early allergic disease. Furthermore, we emphasize new investigations that aim to analyze fetal–host innate immune responses to environmental microbial microorganisms and their possible future application in asthma protection.

Fetal development and immune maturation

The fetal phase of life is characterized by cellular proliferation and differentiation processes, and is thereby determined by a sophisticated regulation of gene expression. These initial steps in shaping life are not only governed by the genetic program of the progeny but also by the maternal environment which is controlled and transferred through the placenta. Although the placenta separates the fetus from hazardous influences, it allows crosstalk between maternal stimuli 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 meditated or transferred by the mother may interfere with this process and may define a direction in immune cell lineage commitment. One concept of how these environmental effects may act in the progeny is via epigenetic regulation [5].

Key mechanisms in epigenetic regulation

Epigenetics is the study of heritable changes in gene expression that occur without directly altering the DNA sequence. Epigenetic mechanisms are proposed to play a major role in orchestrating prenatal ontogenetic differentiation, and in regulating metabolic and mitotic cycles on transcriptional and translational levels. Based on the genetic blueprint, epigenetic mechanisms alter the phenotype without modifying the genetic sequence. This can result in activation or complete/partial silencing of gene expression.

The development of allergic phenotypes may involve environmentally induced epigenetic mechanisms, which could be triggered by diet, microbial components, aging or pharmacological agents [5–9]. Epigenetic regulation of gene expression operates not only during early development but also during post-developmental differentiation of mature cells. These mechanisms include genomic imprinting, histone modification, altered DNA methylation and regulation by microRNA.

Genomic imprinting

Under many circumstances, it has been observed that there is unequal expression of the maternal and paternal alleles. This is presumably due to reversible modification of gene activity associated with the sex of the parent. Imprinting may be regulated via differential DNA methylation in the promoter regions of reprogrammable genes [7]. For example, infections during pregnancy have been hypothesized to transmit trans-generationally the imprints of infections and inflammations. This may reduce the offspring's ability to withstand environmental pathogens and might lead to a higher morbidity and mortality later in life [10]. However, this mechanism has not yet been identified in allergy and asthma.

DNA methylation/demethylation

DNA methylation is the covalent addition of the methyl group to the C5 position of cytosine that is followed by a guanine in the dinucleotide sequence CpG. In general, DNA in higher eucariods is devoid of the CpG sequence; however, some clustering is observed near gene promoters and these regions are referred to as CpG islands. The CpG islands are defined as regions of approximately 500 base pairs that contain greater than 55% GC content. When the gene is expressed within a cell, these islands are maintained in an unmethylated state (see Fig. 1)

When the island is methylated, the binding of the transcriptional complex to the gene promoter is inhibited and gene transcription prevented. Gene repression can be then passed on to subsequent cell generations without any changes occurring in the DNA sequence itself. Methylation of CpG islands is performed through the action of a set of DNA methyl transferases. Many gene silencing events occur during early development. However, the further a cell progresses into the end-stage of cell differentiation (specialization), the further de novo methylation decreases. In contrast, de novo methylation occurs frequently in cell lines, clones, and cells that become malignant [11–14].

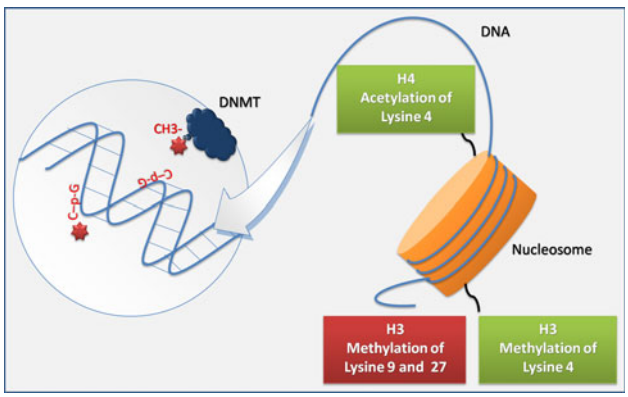

Fig. 1 DNA methylation and histone modification during transcriptional processes. Methylation of CpG islands at promoter regions of target genes by DNA methyltransferases (*DNMT*) may contribute to gene silencing. Methylation of H3 residues at lysine 8 and 27 reduce the accessibility of the respective DNA segment, while methylation of lysine 4 at the same residue or acetylation of the H4 residue at lysine 4 increases the accessibility of the DNA

Histone acetylation/deacetylation

Histones consist of eight subunits and act as spools for the DNA molecule. If the histones are tightly packed, then the transcription apparatus of the cell is unable to access the DNA and the gene is repressed. Conversely, if the histones are in a loose formation and remain, "open", this particular DNA structure is accessible for the DNA transcription apparatus. This latter state is achieved when the histones are modified. These modifications occur post-translationally and include acetylation, methylation, phosphorylation and ubiquitylation of certain residues in the histones. One important mechanism in this regard is acetylation and is performed by enzymes termed histone acetyl transferases (HAT). Conversely, deacetylation is catalyzed by histone deacetylases (HDAC). Histone methylation is regulated via histone methyl transferase at specific residues of the H3 subunit [15–18]. All of the above-mentioned mechanisms (acetylation, methylation, etc.) can occur simultaneously and create a complex pattern on each histone, sometimes referred to as the histone code.

MicroRNA

In addition to modifying transcription, epigenetics can also affect the translation of mRNA. A recently emerging field of epigenetic research is the role of micro (mi)RNAs. miRNAs belong to a class of small non-coding regulatory RNAs that act through repression of protein expression at the post-transcriptional level. To date, more than 300 miRNAs have been identified in humans. Each miRNA can regulate up to several hundred target genes by blocking translation. These small molecules are believed to regulate up to one-third of all human genes by promoting the degradation of target mRNAs. Expression of miRNAs may therefore contribute to the pathogenesis of many human diseases, and some of which have been recently implied to be novel valuable diagnostic or even prognostic disease markers [19–21].

Epigenetic regulation of Th1 and Th2 development

The development of allergic phenotypes in the skin, lung or the gastrointestinal mucosal tissue is closely linked to the generation of Th2 T cells. Th2 cells produce a characteristic cytokine pattern including IL-4, IL-5 and IL-13. IL-4 has been identified as a key cytokine which plays an important role in the initiation of Th2 development. Furthermore, IL-4 is required to maintain a pool of Th2 cells as it promotes Th2 cell proliferation. At the other end of the spectrum, the Th1-related cytokine IFN-γ has a broad spectrum of Th2 counteracting activities, including the suppression of Th2 T cell responses. Therefore, the regulation of IL-4 and IFN-γ production are critical events in allergic conditions.

Recent data indicate that both IL-4 and IFN-γ gene expression are under close epigenetic regulation. In naïve (not yet committed) T cells, cytokine genes are only partially silenced because the baseline level of IFN-γ and Il-4 transcription is evident within a very short time frame after cell activation. Expression is independent of T-bet or GATA-3 expression, transcription factors that are critically involved in Th1 and Th2 cell differentiation, respectively [22]. In order to progress along the T-helper cell differentiation pathway, it is important to sustain expression of one cytokine and repress the other. Cytokine expression is closely dependent on the expression of the transcription factors, T-bet and GATA-3. T cell differentiation depends on cell division, it has been suggested that time was required for one cytokine locus to become completely accessible while the others to be completely silenced [23]. Epigenetic modification of cytokine gene expression was proposed as a mechanism to promote cytokine gene accessibility for one cytokine locus over the other as a naïve T cell differentiates into a Th1 or Th2 cell [24–26].

GATA-3 and T-bet mediate many of the chromatin structural changes that occur during T cell differentiation. As a result, either the IFN-γ or the IL-4 locus becomes accessible to regulatory enzymes and transcription factors [24, 27, 28]. Overexpression of T-bet induces DNase I hypersensitivity of the IFN-γ gene locus and enhances transcription of the interferon gene. T-bet induces and interacts with its co-factor, HLX. Together, these proteins activate the IFN-γ locus synergistically by promoting remodeling of the chromatin structure [25, 29, 30].

One important mechanism to alter accessibility of the IFN-γ gene is DNA methylation and demethylation. Increased IFN-gene expression is observed in T cells activated in the presence of DNA methylation inhibitors and also in T cells from DNA methyltransferase-knockout mice [31–33]. This is further supported by the finding of decreased DNA methylation in the IFN-γ promoter region of Th1 cells [24, 31]. Conversely, reduced expression of IFN-γ has been associated with increase in de novo methylation in such cells [34].

Recently, new data have started to reveal how these methylation patterns may modulate downstream molecular signaling pathways. The CpG^{-53} site of the IFN-γ promoter seems to be critically involved in regulating IFN-γ gene expression. CpG^{-53} resides in a proximal activator protein 1 (AP1) binding site and when methylated alters transcription factor binding [32, 35, 36]. A subsequent study demonstrated that methylation of CpG^{-53} significantly inhibited

binding of cAMP response element binding protein (CREB) and ATF2/c-Jun transcription factor to the CpG containing AP1 site, and leads to an augmented pro-allergic Th2 polarization [35]. In addition, it was shown in a Th1 cell line that methylation of CpG^{-53} was sufficient to inhibit the IFN-γ promoter-driven reporter gene expression [35]. Another important CpG site in this gene is CpG^{-190}, which also interacts with AP1-CREB DNA binding complexes [36, 37]. An important aspect is that many CpG sites found in T helper-associated genes are highly conserved across species. Both CpG^{-53} and CpG^{-190} of the IFN-γ promoter are highly conserved in the rats, dogs, chimpanzees and humans [35, 36]. A recent study by Thomas et al. demonstrated the critical role of the Ikaros protein in Th2 polarization. The binding of Ikaros to the endogenous T-bet promoter (*tbx21*) silences T-bet expression and, thereby, suppresses interferon production. Inhibition of Ikaros DNA binding activity during Th2 polarization resulted in dissociation from the *tbx21* promoter, increased T-bet expression and, consequently, IFN-γ production. During Th2 polarization, the binding of Ikaros to the IFN-γ promoter significantly alters the methylation state of CpG^{-53} resulting in epigenetic imprinting by altering DNA methylation patterns [38].

Th1 polarization also integrally involves the IL-4 gene. Methylation of the highly conserved DNase I-hypersensitivity region at the 3′-end of the IL-4 gene is critically associated with Th1 differentiation [39, 40]. 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 [41–45]. All these events result in increased accessibility of regulatory enzymes and transcriptional factors to the IL-4 locus and, therefore, increased IL-4 gene transcription. Simultaneous silencing of IFN-γ occurs and is associated with DNA-methylation/histone deacetylation of the T-bet and IFN-γ genes [46–48]. It is important to note that, during Th2 differentiation, demethylation along the entire IL-4 gene tends to progress sequentially. Demethylation first begins at CpG^{-408} and advances to the 3′-end. Interestingly, CpG^{-408} has been identified as a putative binding site for the transcription factor AP, although hitherto AP2 has not been associated with IL-4 gene transcription [49–51].

In conclusion, these data strongly indicate that epigenetic mechanisms operate in the differentiation of naïve T cells to a Th1/Th2 effector status. This includes epigenetic chromatin remodeling of many genes, including Il-4 and IFN-γ as prominent examples. However, it must be noted that epigenetic regulation might not be restricted to these two genes alone, but that many genes including the genes of transcription factors themselves may be under epigenetic control.

The role of miRNAs in Th1 and Th2 development

A number of studies have demonstrated the modulating role of miRNAs in the regulation of humoral and cell-dependent immune responses (reviewed in [52]). miRNAs are important regulators of T cell development. The elimination of Dicer, the RNAse III enzyme that generates functional miRNAs, results in a reduction of regulatory T cell (T-reg) numbers and immune pathology [53]. Additionally, the use of Dicer-deficient mice demonstrated that miRNAs have an important role in Th1/Th2 differentiation. The analysis of isolated Dicer-deficient $CD4^+$ T lymphocytes revealed that the proliferation and differentiation into Th1/Th2 lineages was dysregulated in the absence of miRNA. Although Dicer-deficient T cells were viable, proliferation was significantly reduced and cytokine expression appeared to be modified during differentiation. Under Th2 polarizing conditions, repression of IFN-γ expression was impaired and accompanied by lower expression of GATA-3 [54]. Although these data indicate that translational regulation by miRNAs is involved in Th1/Th2-development, the responsible miRNAs are still to be elucidated.

Experimental models for epigenetic regulation of allergic phenotypes

Diesel exhaust particles

Epidemiological studies have identified low-income minority children, who are more likely to reside near traffic-related air pollution sites, as asthma-high-risk individuals [55–57]. In a recent study utilizing the murine *Aspergillus fumigatus* allergen model, it was shown that inhaled-DEP augmented the allergic phenotype and was associated with epigenetic changes [58]. Chronic DEP inhalation induced hyper-methylation at the CpG^{-45}, CpG^{-53} and CpG^{-205} sites of the IFN-γ promoter in $CD4^+$-cells and was associated with higher IgE production. Conversely, hypomethylation at CpG^{-408} in the proximal IL-4 promoter in $CD4^+$-cells was associated with changes in IgE levels. Although this study has several limitations, this is the first instance demonstrating a link between environmental exposure, epigenetic regulation and altered phenotype development.

Vitamin B_{12}/folic acid

In a recent hallmark experiment, it was demonstrated that dietary supplementation during pregnancy can alter heritable phenotype in the offspring. The investigators demonstrated that supplementing the diet with folic acid,

vitamin B_{12} or other agents can alter mouse coat color. The increased abundance of methyl donors in the diet enhanced CpG methylation in the promoter of the agouti gene and therefore altered coat color [57, 59]. The relevance of a methyl-rich diet during pregnancy on development of an allergic phenotype has been shown in a prenatal mouse model. Mothers fed with a donor-rich diet increased the severity of the asthmatic phenotype. Increased airway inflammation was associated with enhanced DNA methylation of transcription factors that balance inflammation of the bronchus [60].

Policyclic aromatic hydrocarbons

The traffic-related increase in asthma development, particularly in the inner city population, might not only be related to DEP but also to polycyclic aromatic hydrocarbons (PAH). In a recent study, the relationship of transplacental exposure to traffic-related PAH and the development of childhood asthma was explored. The investigators monitored maternal PAH exposure and studied the methylation status in umbilical cord white blood cells. Over 30 DNA sequences were identified whose methylation status was dependent on the level of maternal PAH exposure. The highest concordance between the levels of methylation and gene expression in matched fetal placental tissues was found for acyl-coa-synthetase long-chain family member 3 (ACSL3) [61]. This gene belongs to a family of genes that encodes key enzymes in fatty acid metabolism [62]. ACSL3 is expressed in lung and thymic tissue and is responsible for the intracellular conversion of long-chain fatty acids [63, 64]. Thus, hypermethylation of this gene is expected to diminish fatty acid utilization and may also possibly influence membrane phospholipid composition. Whether these functional changes directly affect the development of the asthmatic phenotype is unknown. However, several epidemiological studies have shown that the fatty acid composition in milk and other nutrients affect the development of allergy and asthma. This effect has been attributed to the anti-inflammatory and immune-modulating effects of omega-3 (n-3) fatty acids [65–69]. It is interesting to note that ACSL3 is located in 2q36.1, a region that has been recently shown to be associated with asthma susceptibility [70, 71]. In conclusion, the transplacental PAH exposure provides another model situation that underlies the importance of epigenetic regulation in terms of phenotype development, although the detailed cause and effect relationship between PAH exposure, hypermethylation of the ACSL3 gene and asthma development still remains to be fully established.

Gestational smoke exposure

The most convincing data that prenatal environmental exposure can influence the risk for subsequent asthma development originate from the work on environmental tobacco smoke (ETS) exposure. There is overwhelming evidence that prenatal exposure to ETS is associated with a number of asthma hallmarks, including impaired respiratory function, wheezing, respiratory infections, and altered airway structure [72–74]. Most recently, the adverse impact of smoking has also been demonstrated to significantly increase the risk of developing atopic dermatitis [75]. Recent data suggest that the effect of ETS exposure on asthma development can be transmitted across two generations [76]. The investigators conducted a case–control study that included 338 children with asthma and 570 control subjects and used an innovative sampling design to efficiently investigate the association between in utero exposure to maternal smoking and asthma occurrence. The study confirmed that in utero exposure was associated with increased risk for asthma diagnosis in the first 5 years of life. Additionally, it is most remarkable that even grandmaternal smoking during the mother's fetal period may present a risk factor for asthma in the grandchild's generation. Furthermore, this was independent of maternal smoking. The risk for asthma in grandchildren was highest if both the grandmother and the mother smoked during pregnancy.

From a mechanistic point of view, parental smoking is associated with higher cord blood IgE levels [77]. Since IgE does not cross the placental barrier, this provides evidence of a direct effect of maternal smoking on fetal immune functions. Recently, maternal smoking has been associated with stronger neonatal T cell proliferation [78]. However, the effects on cytokine responses were not investigated. Another study has provided evidence that maternal smoking in pregnancy is associated with lower Th1 responses to mitogenic stimulation as measured by mRNA expression levels [79]. Whether this is directly regulated epigenetically has not so far been investigated, but it must be noted that tobacco smoking has recently been shown to modify gene expression by promoter hypermethylation associated with down-regulation of gene transcription in the lung [80]. These data indicate that, in principle, tobacco smoke has the capacity to act on the level of epigenetic regulation. Whether the observed increased risk of asthma development is also related to epigenetic events remains to be investigated. The epidemiological data, particularly on grandmaternal exposures, at least point in this direction.

Fetal innate immune responses to microbial stimuli

Evidence from epidemiological studies

The traditional farming environment confers protection to allergy and asthma by the 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 epidemiological studies conducted in the Alpine region and then confirmed for other regions all over the world. Numerous cross-sectional studies supported the observations that 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 already 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 (reviewed in [81]). Data from the ongoing birth-cohort PASTURE-Study supported the modulating influence of prenatal maternal farming exposure on the developing immune system of the offspring. Cord blood samples from neonates born to farming families were shown to produce substantially more IFN-γ and TNF-α after cytokine stimulation compared to samples from babies born to non-farming families. This may indicate that the innate immune system might be involved in interactions between environmental factors and the developing immune system [82].

Early responses of the innate immune system

The innate immune response to a pathogen is coordinated by antigen-presenting cells (APC). The main APC population is dominated by dendritic cells (DCs), cells that are crucial for determining T-helper cell fate and, subsequently, for the development of asthma. Depletion of $CD11c^{+}$–DCs leads to significant abrogation of the characteristic features of experimental asthma. This indicates that these cells are necessary and sufficient for the induction of a Th2-driven inflammatory allergic response [83]. The activation status of DCs has an important role in this regard. The expression profile of DCs is affected by the interaction of their so-called pattern recognition receptors (PRRs) with the microbes' pathogen-associated molecular patterns (PAMPs). In contrast to the highly-specific antigen recognition of the T cell receptors, the interaction between PAMPs and PRRs is rather unspecific. PAMP activation initiates different effector cascades, e.g., the release of antimicrobial defensins or signaling the adaptive immune response [84]. The Toll-like receptor (TLR) family represents the best-characterized class of PRRs. TLRs represent sensors for microorganisms and microbial components: TLR 1/6 and TLR 1/2 complexes are PRRs that predominantly recognize Gram-positive bacteria such as mycobacteria or lactic acid bacteria; TLR4 recognizes LPS from Gram-negative bacteria, TLR5 serves as the receptor for flagellin, the main protein in bacterial flagella; TLR3,7 and 9 are endosomal-associated TLRs that recognize foreign DNA: double-stranded (ds)DNA from bacteria and viruses is recognized by TLR3 and 9, TLR9 is able to detect CpG domains in dsDNA, while TLR7 detects bacterial and virus single-stranded DNA. Ligand-binding to the TLR activates several intracellular signaling pathways including the NF-κB pathway. Intracellular TLR signaling (with the exception of TLR3) occurs via MyD88-dependent or -independent pathways. TLR-mediated activation of DCs induces expression of co-stimulatory molecules (CD80 and CD86) as well as of inflammatory cytokines (TNF-α, INFs, IL-1, IL-6, IL-10, and IL-12) [85]. By this, DCs play a key role in linking innate and adaptive immunity. Due to the close contact with naïve T cells, DCs orchestrate T cell polarization towards a Th1, Th2, Th17 or T-reg phenotype [86]. In addition to their role in the defense against infectious agents, TLR-responses are involved in the onset of immune-modulated diseases such as allergies and asthma via delivering appropriate signals to T cells. Thereby, TLRs contribute to T-effector cell commitment (see Fig. 2)

Little is known about the TLR development in ontogeny. During prenatal development in mice, changes in TLR2 and TLR4 expression are observed in several organs. In the placenta, the mRNA level of TLR4 during mid-pregnancy was twice as high as those of TLR2. While in the lung, TLR2 and TLR4 expression was low in first third of gestation but increased several-fold during prenatal development and still further after birth [87].

Experimental models of fetal immune modulation by microbial stimuli

Epidemiological studies have revealed that prenatal maternal exposure to LPS may protect the offspring against allergic disorders. On this basis, a number of prenatal murine models of experimental asthma have been conducted. In these models, offspring from pregnant mice treated with microbial TLR-activating stimuli were protected from ovalbumin (OVA)-induced asthma. Recently, we have examined the effects of prenatal exposure to LPS in mice on the allergic airway response. A reduction in allergen-induced airway eosinophilia, a diminished IL-5 production in the bronchoalveolar lavage, and increased IFN-γ production by splenic lymphocytes were observed, but mice were not protected against decreased lung

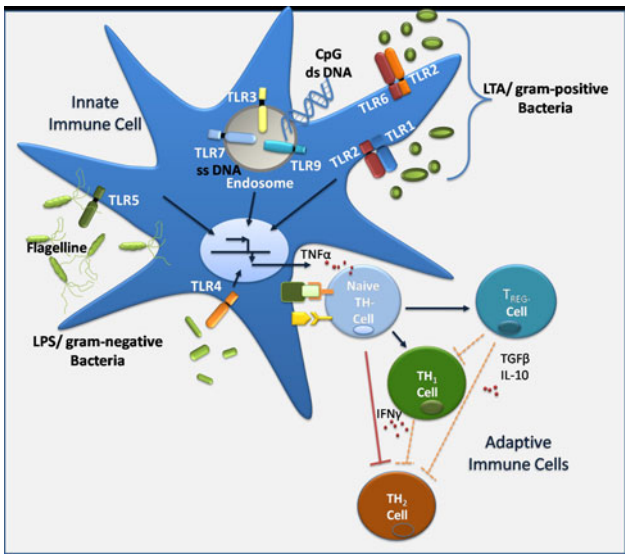

Fig. 2 Crosstalk between dendritic and T helper cells as a result of TLR activation by microbial stimuli. Dendritic cells are at frontline to different environmental exposures. TLR represent sensors for micro-organisms and microbial components: TLR 1/6 and TLR 1/2 complexes are pattern recognition receptors for Gram-positive bacteria such as mycobacteria or lactic acid bacteria; TLR4 recognizes LPS from gram-negative bacteria, TLR5 serves as receptors for flagellin, the main protein in bacterial flagella; TLR3, 7 and 9 are endosome associated TLRs that detect foreign DNA: double-stranded (ds)DNA from bacteria and virus is recognized by TLR3 and 9, TLR9 is able to detect CpG domains in dsDNA while TLR7 detects bacterial and virus single-stranded (ss)DNA. TLR signaling results in a cytosolic cascade for transcription factors such as TNF-α. In parallel, antigen presentation via MHC II complexes (*green–orange*) and notch signaling (*yellow*) primes naïve Th-cells towards a Th1 or T-reg direction. T-regs moderate Th1 cell development and suppress generation of Th2 cells via TGF-β and IL-10 production. Inhibition of Th1 development is supported by release of IFNγ by Th1 cells

function [88]. In a recently published report that describes LPS application in a rat model of allergic airway inflammation, the reduction in Th2 cytokine production and cell recruitment in the bronchoalveolar lavage was confirmed. However, in contrast to the above mentioned mouse experiments, airway hyperresponsiveness was normalized in offspring from LPS-treated mothers [89]. The reasons for these discrepant results in terms of altered lung functions are still unclear, but may be explained by species-specific effects or methodological differences in lung function measurements.

Bacteria isolated from the farming environment have been shown to confer protection against the development of an allergic phenotype when administered prior to sensitization [90]. Furthermore, *Acinetobacter lwoffii* F78, a stable-derived Gram-negative bacterium, exhibits anti-allergic properties in a prenatal mouse model. In contrast to the prenatally tested *Lactobacillus rhamnosus* GG and LPS, intranasal maternal application of *A. lwoffii F78* resulted in a reduction of all features of airway inflammation and normalized airway hyperresponsiveness in OVA-sensitized offspring. Additionally, a trend towards lower OVA-specific IgE formation was observed. Recently, we investigated the mechanisms by which protection might be transferred from the mother to the progeny [91]. Intranasal exposure of the mother mice triggered low but stable IL-6 production in the lung, which could also be detected systemically in the peripheral blood. TLR-expression and cytokine production in the placenta was suppressed. In offspring from TLR 2/3/4/5/7/9 knockout females mated with wild-type males, the protective effect of prenatal *A. lwoffii* application was abolished, although the heterozygous offspring were able to signal via TLRs. This indicates that a functional TLR-signaling of the mother is necessary to mediate protective effects from environmental stimuli during pregnancy (see Fig. 3).

Pro-biotic bacteria as early modulators of innate immune responses

In addition to farm-derived microbes, pro-biotic Gram-positive bacteria and other food-additive microorganisms that provide a benefit to human health have been described as helpful in the prevention of allergic diseases. These bacteria, as a substantial part of a healthy gut micro-flora, are able to modulate the intestinal immune functions and may also confer protection against atopy and asthma [92]. The colonization of the neonatal gut by microbes from the

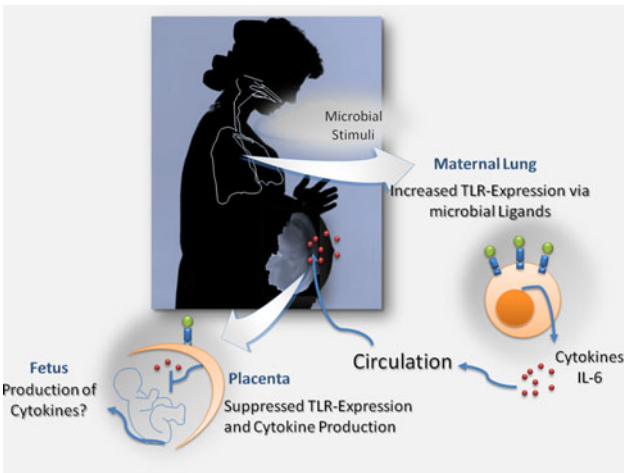

Fig. 3 Epigenetic pathways in fetal immune modulation—a model of prenatal modulation of the fetal immune system by exogenous maternal exposures. Exogenous exposures incorporated by the mother during pregnancy might activate the innate immune system. Thereby, maternal signals may be transferred through the feto–maternal interface. A long-lasting and early stimulus mediated by the mother may result in epigenetic changes that influence T cell lineage commitment in the progeny. The nature of the maternal exposure directs the developing fetal adaptive immune system to an allergy-favoring direction (Th2) or towards tolerance and protection against allergies (Th1/T-regulatory dominated)

neonate environment stimulates intestinal immune system towards a Th1 phenotype compensating the Th2-bias established in utero that prevents rejection of the fetus [93]. The shaping of the intestinal immune system by human colonic DCs occurs predominantly via TLR2 signaling. Pro-biotic bacteria also induce the production of transforming growth factor (TGF)-β, which in turn stimulates T-helper cells to release Th1 cytokines. These cytokines act on B cells by inducing a switch to IgA-production; a mechanism that reduces inflammation and establishes clinical tolerance against common antigens. The lack of microbial stimuli may lead to an elevated IgE production by B cells and an increased risk of allergic reactions due to the subsequent activation of mast cells [94].

A large group of bacteria with pro-biotic properties is represented by the genus *Lactobacillus*. *Lactobacilli* were shown to have immune-modulating capabilities by altering the cytokine pattern of the host. Reviewing clinical trials of the last decade with regard to preventive properties of *Lactobacilli*, two clinical research groups have provided contradictory results. The group of Kalliomäki and Isolauri from Finland reported protective effects on the development of atopic eczema in pre- and postnatally-treated high-risk infants with sustained effects in follow-up studies after 7 years [95]. However, Taylor et al. did not obtain any significant findings [96]. Another trial in which pro-biotic substances were applied to high-risk neonates showed protective effects on atopic eczema [97]. A meta-analysis done by Lee et al. revealed an overall Odds Ratios below 1 indicating preventive effects of lactic acid bacteria on pediatric atopic dermatitis [98]. These results were challenged by Salfeld and Kopp arguing that these calculations included studies referring to the same study populations [99]. Unfortunately, comparative data for prenatal asthma prevention by pro-biotics are currently not available.

Experiments in acute and perinatal animal models of experimental allergic asthma revealed more consistent results [100]. In a perinatal murine model of asthma, we applied *L. rhamnosus GG* orally to mother mice. This resulted in a significant reduction of airway eosinophilia and inflammation in the lung, but failed to normalize lung function in OVA-sensitized offspring [101].

Immune modulation via TLR9 ligands

Kitagaki et al. demonstrated that application of the TLR9 ligand, CpG containing DNA, prior to sensitization prevented the Th2 inflammatory response and effectively interferes with the development of atopic airway disease in a murine model of experimental asthma. Moreover, when administered in combination with an experimental allergen, CpG promoted the reversal of established eosinophilic inflammation [102]. Recently, we found that activation of TLR3 and 7 by viral TLR ligands has both preventive as well as suppressive effects on experimental asthma and is mediated by the additive effects of IL-12 and IL-10. However, this concept is still to be tested in a prenatal experimental set-up [103].

Helminth infections as perinatal allergy protective factors

Both helminth infections and allergic diseases are strong inducers of Th2 responses. Similar to atopic disease, helminth infections also up-regulate Th2 cytokines, IgE-production and induce eosinophilia. Asymptomatic persistence of helminth infections in endemic regions appear to be achieved by activation of regulatory T cells and systemically elevated levels of IL-10 and possibly of TGF-β. By this mechanism, helminth infections may have a down-regulatory effect on the risk of developing allergic disease [104].

Recent studies have indicated that helminth infections could affect the early development of immunity. 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 [105, 106]. Experimental set-ups mainly use the model of schistosomiasis to investigate allergy-diminishing effects and related mechanisms on allergic outcomes, but none have yet proved the hypothesis of in utero protection.

Stimulation of innate immune response by other environmental exposures

Stimulation of the innate immune system by microbial exposure may reduce the development of childhood allergy. Additionally, other environmental factors such as fatty acids may affect neonatal TLR stimulation and, therefore, modulate the capacity of TLR to respond to microbial stimuli. Cord blood cells from neonates born to non-atopic mothers showed higher levels of IL-10 production and FOXP3-expression when stimulated with the TLR2 agonist peptidoglycan than those born to atopic mothers [107]. A similar study conducted with cord blood cells revealed associations between maternal allergy and significantly higher neonatal IL-12 and IFN-γ responses to TLR2, 3, and 4 activation. TNF-α- and IL-6 responses to TLR2, 4, and 5 activation were significantly higher in newborns that later developed allergic disease. Newborns that developed an allergic disease later on in life had lower Th1-IFN-γ responses to mitogens [108]. Data from a United States inner city cohort revealed that prenatal stress affects the innate immunity. Higher prenatal maternal stress scores were related to an increased IL-8 production

after microbial stimulation of TLR and increased TNF-α production in cord blood cells [109]. Other factors that may modulate TLR-mediated innate cytokine responses include parental allergic and airway diseases, somatic fetal growth, ethnicity, and season of birth [110]. Common environmental toxic chemicals may also alter TLR signaling. Decreased expression of transcription factors GATA-3, T-bet and Foxp3 was observed in offspring from a prenatal murine model, where mother mice were exposed to a low-level toluene concentration and the TLR2 ligand peptidoglucan during pregnancy [111]. Collectively, these findings suggest that urban prenatal exposures and familial genetic factors affect the development of the fetal innate immune system.

TLR gene polymorphisms

TLR gene polymorphisms as heritable factors may be relevant in prenatal development. Epidemiological association studies have identified certain TLR gene polymorphisms that are associated with the increased prevalence of allergic diseases [112]. For example, allergic diseases in farmers' children could have contributed to a significantly elevated prevalence of a polymorphism found at the TLR2/-16934 locus [113]. Results from a Swedish study indicated that a polymorphism in the TLR4-gene is associated with asthma characterized by a decreased IL-12 production by APCs after LPS stimulation. On the other hand, TLR1/6 heterodimer polymorphisms are associated with an elevated protection against childhood asthma [114]. Gene–environment interactions may also modify TLR2- and CD14-mediated responses. Individuals growing up in a rural environment are more susceptible to allergies and asthma when carrying polymorphisms in the respective TLR genes [115]. A mutation in the TLR2/R753Q region was shown to be associated with a modified cytokine production and TLR expression in patients with atopic dermatitis [116].

Conclusion

Epidemiological, clinical and experimental evidence provides compelling new insights into the critical role of the pre- and postnatal environment in terms of immuno-programming, immuno-education and subsequent allergy and asthma protection. These studies provided the basis on which underlying mechanisms will be elucidated and discovered. An increased understanding of the underlying cellular and molecular networks will be the prerequisite for the development of novel allergy-protective and -preventive strategies. Further investigations on the relationship between the genome, epigenome and transcriptome may lead to a better understanding of the phenotype development. Similarly, the understanding of how exogenous exposures initiate gene–environment interactions on the transcriptional and translational level in specific cell types and thereby modify the phenotype may hold the key for novel clinical approaches in allergy and asthma.

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