

REVIEW ARTICLE

Perspectives on immunomodulation early in life

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Abstract

The immune system early in life is characterized by immature activation and function of immune cells and a preponderance of Th2 cytokines. Together with other factors such as genetics and epigenetics, these immature immune responses might prone newborns susceptible to severe infections as well as allergic diseases. Immunomodulation therapy may have potential as therapeutic strategy against those disorders and might have implication in early-life interventions in the future. In this review, we will focus on two immunomodulatory substance classes, Toll-like receptor (TLR) ligands and sphingolipids, which are the focus of extensive research to date. Both TLRs and sphingolipid receptors have a very distinct distribution pattern and function on immune cells. Therefore, they can potentially modulate and balance immune responses, which might be in particular beneficial for the immaturity of the immune response early in life.

Immunomodulation is a promising novel therapeutic strategy and may have future implications in a broad range of disorders affecting the immune system such as autoimmune dis-

eases, cancer, allergy, or functional immunodeficiencies. The latter two are of importance, especially early in life because of the imbalanced immune system with a Th2-dominated cytokine response. The setting of immunologic immaturity has impact on both the innate and the adaptive immune system. The functional impairment of the immature immune system, together with additional factors, is believed to prone newborns to develop severe infections in the first year of life and for the dominance of allergic disorders in early childhood. Of the large number of immunomodulators, we focus in this review on TLR ligands and sphingolipids as two promising novel modulatory candidates. Both have the ability to counteract the Th2 predominance and to balance the immune system and might, thus, find application as therapeutic agents in immune-mediated disorders of early life in the future.

Abbreviations

TLR, Toll-like receptor; Th, T-helper cell; HLA-DR, human leukocyte antigen – DR; TGF- β , transforming growth factor beta; APC, antigen-presenting cell; DC, dendritic cell; pDC, plasmacytoid dendritic cell; IFN, interferon; RSV, *respiratory syncytial virus*; TNF, tumor necrosis factor; IL, interleukin; Ig, immunoglobulin; CRP, C-reactive protein; LBP, LPS-binding protein; PAMP, pathogen-associated molecular pattern; CpG-ODN, CpG oligodeoxynucleotides; GM-CSF, granulocyte macrophage colony-stimulating factor; FEV, forced expiratory volume; AHR, airway hyper-responsiveness; S1P, sphingosine-1 phosphate; S1PR, sphingosine-1 phosphate receptor; SphK, sphingosine kinase; BAL, bronchoalveolar lavage; SNP, single nucleotide polymorphism; DMS, *N,N*-dimethylsphingosine; LPS, lipopolysaccharide; tBLP, triacylated bacterial lipopeptides; IDO, indoleamine 2,3-dioxygenase; PBMCs, peripheral blood mononuclear cells; DMSO, dimethyl sulfoxide; CCR, chemokine receptor; RANTES, regulated upon activation, normal T-cell expressed, and secreted.

The imbalance of the immune system early in life

Early in life, the immune response is characterized by immature cellular and humoral functions. This impairment in combination with the first-time exposure to a wide variety of

microbes, antigens and allergens might favor the occurrence of infections and allergies. The major characteristics of the newborns' adaptive immune system are deficient activation as well as function of memory and effector cells. As a consequence, a preponderance of the Th2 responses can be observed and this Th2 skewing is perhaps one of the best characterized immunologic differences compared to adulthood (1). Physiologically, higher amounts of Th2 cytokines are present during pregnancy and are considered as a protective factor against abortion (2). Functional structures behind this Th2 bias are localized in the feto-placental tissue and are most likely to influence not only the fetal but subsequently also the newborn's immune system. Besides the production of adenosine, an immunosuppressive purine metabolite in neonatal blood plasma, placental tissues also release transforming growth factor beta (TGF- β) and prostaglandin E2, which together with progesterone from corpus luteum enhance Th2 cytokine production (3).

After birth, antigen-presenting cells (APCs) are critical for balancing the postnatal Th2 preponderance towards Th1 immune responses. However, the costimulatory signals responsible for the induction of Th1 cells are defective at this stage of life. A relative absence of interleukin (IL)-12 production, which does not reach adult levels until late childhood (4), has been suggested to favor Th2 cytokine responses. Thus, in early life, APCs release little IL-12 and only inefficiently drive the immune response toward Th1. Without the up-regulation of MHC-II and costimulatory molecules such as CD86 or CD80 and CD28, in combination with diminished IL-12 secretion, neonatal naïve T-cells are even primed for increased IL-4 production *in vitro* (4). In addition, other Th2 cytokines (IL-6 and IL-10) are produced at similar or even elevated levels upon TLR stimulation of neonatal cord blood-derived monocytes and APCs (5). Thus, when antigens are presented to naïve T cells, APCs initiate a Th2 response because of the absent Th1 stimuli (Fig. 1). Interestingly, the poor proliferation and activation of neonatal T cells by neonatal dendritic cells (DCs) can be overcome by T-cell priming with adult DCs, which induce strong interferon (IFN)- γ responses (6). At the age of 3 months, PMA/Con-A stimulation of whole blood of children with high endotoxin exposure early in life was associated with significantly higher IFN- γ production. This pointed toward a contribution of environmental factors to the maturation of immune system during infancy (7). Additionally, plasmacytoid DCs (pDCs), which are potent producers of the pro-inflammatory cytokine type I IFN, can influence the imbalanced Th1-Th2 environment early in life. Although IFN- α secreted by pDCs can counteract Th2 responses, it has recently been postulated that the pDCs influence on the immune response is dependent on the surrounding Th1 or Th2 cytokine environment. Upon Toll-like receptor-7 (TLR7)-specific stimulation, pDCs are even potent producers of the Th2-modulating cytokine IL-4, however, only in a preexisting Th2 milieu (8). Thus, pDCs might contribute to the Th2 preponderance in early life. It has to be kept in mind that pDCs *per se* are altered in neonates. Although recent data reported an increased proportion of pDCs in newborn babies compared with adults (9), we have

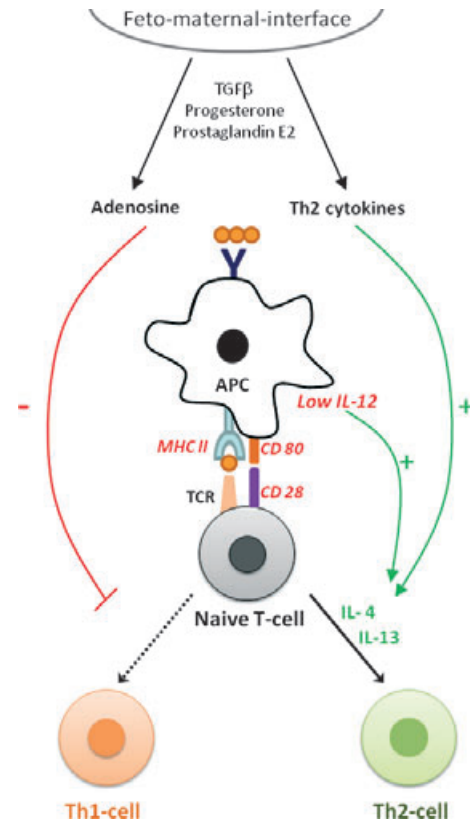


Figure 1 Immature function of antigen-presenting cells early in life shift naïve T cells toward Th2 effector cells. Secreted factors from the fetal-maternal interface directly influence the maturation of naïve T cells. Adenosine of placental tissue inhibits the Th1-cell maturation, while a defective activation (indicated by red-colored italic style) by MHC-II, CD80, and CD28, together with impaired IL12 responses and a predominant Th2 cytokine environment drives the immune response towards Th2.

revealed that the functionality of pDCs upon TLR9 stimulation differs, as pDCs of preterm and term neonates produced significantly lower levels of IFN- α compared with adult pDCs (Schüller S, Sadeghi K, Diesner S, Prusa A, Klebermasz-Schrehof K, Neumüller J, Helmer H, Husslein P, Pollak A, Spittler A, Förster-Waldl E, manuscript submitted).

In addition to the preponderance of Th2 responses, other factors contribute to common early-life diseases, such as allergies and infections. T-regulatory (T-reg) cells are one of the key players for preventing misguided immune responses. However, at this age, T-reg cells are inefficiently stimulated and lack their inhibitory functional signals, such as CTLA-4 (cytotoxic T-lymphocyte antigen 4). Very recently, Th17 cells, which are discussed to contribute to chronic inflammatory conditions (10), were ascribed to have regulatory properties early in life (11). However, both cells might feature impaired activation and functionality early in life and might therefore only inefficiently modulate the immune system.

Implications of Th2 cytokine milieu on infectious diseases

Epidemiologic data have confirmed that neonates are highly susceptible for infections as corroborated by 4 million deaths per year of infants younger than 6 months of age worldwide. Under physiologic adult conditions, the innate and adaptive immune system counteract in concert infections by inducing humoral and cellular immune response mechanisms. However, with regards to the immaturity of the immune system after birth and to the aforementioned malfunctioning T-cell responses, defense mechanisms might be inefficient and prone newborns especially susceptible to infections by intracellular microorganisms (1).

The presentation of pathogenic material by APCs is a key requisite to induce a prominent and long-lasting adaptive immune response against pathogens. However, upon activation of cord blood DCs, neither the expression of DC costimulatory molecules (CD80 and CD86) and activation markers (CD83), nor the production of pro-inflammatory and anti-inflammatory cytokines, such as tumor necrosis factor alpha (TNF- α) and (IL-10) were induced (12), making it impossible to stimulate naïve T cells adequately. Also antigen-presenting B cells lack costimulatory signals leading to inefficient B-cell and T-cell interactions, as well as antibody isotype switch, being evidenced by lower IgG2 and higher IgM titers (13). Lower antibody titers, which do not reach adult levels until adolescence, and limited proliferative responses of B-cells are a common feature of the newborns' immune response. Furthermore, IgM of low affinity additionally recognize only a limited number of antigens. Interestingly, early in life, IgM is produced in response to 100–1000-fold lower antigen concentrations than dosages required for antibody responses in adults. The immaturity of B-cells is also reflected by the failure of potent vaccines to trigger an adequate antibody response early in life (14). Interestingly, the extent of the Th2-biased immune response is associated with the severity of certain infections (14). Infants with severe *respiratory syncytial virus* (RSV) bronchiolitis have higher levels of the Th2 cytokine IL-9 in their airways (15). Additionally, infections with certain viruses (e.g. RSV or *pneumonia virus* in mice) *per se* can promote a Th2-like response accompanied by accumulation of eosinophils at the site of inflammation (16).

In summary, the quality and quantity of immunologic responses are immature early in life leading to inefficient defense mechanisms against numerous pathogens underlining the urgent need for immunomodulatory strategies to prevent severe infections and sepsis in this age group.

Th2 dominance as a risk factor for allergy induction early in life

Allergies are typically characterized by production of Th2 cytokines IL-4, IL-13, and IL-5, allergen-specific IgE, accumulation of effector cells such as eosinophils and mast cells, and, if persisting, cellular-mediated chronic inflammation. In contrast, non-atopic individuals have higher levels of the Th1 cytokine IFN- γ , that inhibits the predominance of Th2 cells.

Recently, the complexity of immune responses underlying allergic diseases has increased as other Th-cell subtypes, such as Th17 and Th9 cells, were reported to substantially contribute to the pathophysiology (17). Additionally, it has become apparent that genetic and epigenetic factors have a paramount influence on the induction of allergic diseases early in life (18).

Genetic factors contributing to allergic diseases have been especially characterized for asthmatic disorders. Allergic asthma is characterized by chronic airway inflammation, bronchial hyperreactivity, mucus hypersecretion, and increased Th2 cytokine production. In meta-analyses of asthma genome-wide association studies, single nucleotide polymorphisms (SNPs) in several loci, such as 17q21 (which will be discussed in more detail below), IL1RL1/IL18R1 (which encodes for ST2, the receptor for IL-33) TSLP and IL-33 (both of them promote differentiation and activation of Th2 cells) were considered to be significantly associated with asthma susceptibility (19). Recently, polymorphisms of the IL-9 gene have been linked to the development of atopy and bronchial hyperreactivity, as reviewed by Soroosh (20). Furthermore, IL-9 might impact on airway remodeling by the induction of mucus production, even without the occurrence of inflammation (21). Additionally, also Th17 cells were associated with an asthmatic phenotype and with disease severity, as elevated IL-17 levels were found in bronchoalveolar lavage (BAL) fluid of asthmatic patients and might contribute to local accumulation of neutrophils (22). Interestingly, a coding region variant (H161R) of the IL-17F gene, which stands for an IL-17F antagonist, was found to be inversely correlated with asthma (23). Although these two novel Th-cell subtypes might influence asthma also early in life, data on their functionality in asthmatic children are scarce. Another influence on diseases associated with the Th2 bias in childhood might be generated by epigenetic factors, which are heritable changes in gene expression, being caused by chemical modifications, such as histone modification and DNA methylation (24). In response to environmental changes, epigenetic factors are responsible for rapid adaptations across generations (24). Also the increase in allergic diseases in early life was hypothesized to be correlated with environmentally induced changes of epigenetic effects on gene expression already beginning in fetal life. Environmental prenatal exposures, such as maternal diet, microbial infections, and tobacco smoke, can epigenetically activate genes and, thus, regulate the immune development. It might be speculated that owing to epigenetic modification, silencing of genes for Th1 and T-reg differentiation contribute to the preponderance of the Th2 milieu (25). Furthermore, also early in life, epigenetic effects might be induced by viral respiratory infections, probably leading to the onset of airway hyperreactivity (24).

Essentially, a delayed Th1 maturation in early life predisposes susceptible infants to allergic diseases (26). Physiologically, the profound Th2 response after birth is suppressed during the first years of life. In atopic children, the Th2 environment persists and is accompanied by a defective production of IFN- γ . Especially high-risk children of families with atopic history have less IFN- γ responses in the neonatal

period compared with low-risk children with negative allergy family history (27).

Another factor that may predict the development of allergies within the first 2 yr of life is human leukocyte antigen-DR (HLA-DR) expression on cord blood monocytes. Lower expression of HLA-DR was associated with allergic diseases because of a reduced capacity to produce IL-12p70 and higher production of IL-13 (28). In addition, maturation of APCs in early childhood is crucial for establishing a Th1 cytokine response and, thus, for protecting against sensitization (26). IFN- γ produced by CD8+ Tc1 cells and NK-1 cells in childhood stimulates APCs and balances the immune response by skewing it toward a Th1 phenotype (26). The most potent stimulator of DCs is lipopolysaccharide (LPS), which may be necessary for the postnatal maturation of APCs (26). The interaction of LPS and DCs is mainly mediated by CD14 (29). Genetic polymorphism of the CD14 gene impacts on concentrations of circulating soluble CD14 (sCD14), which together with LPS is a potent activator of mature DCs. Levels of sCD14 are directly correlated with IFN- γ concentrations and inversely linked with IL-4 production by PBMCs (peripheral blood mononuclear cells) upon mitogens stimulation. Thus, sCD14 modulates Th1/Th2 balance and IgE synthesis (26). Additionally, SNPs of TLR itself, especially of TLR1, TLR6, and TLR10, have been associated with protective effects on atopic asthma by the induction of Th1 cytokine expression, while Th2-associated IL-4 production was markedly reduced (30). The potential of LPS and other TLR ligands to stimulate APCs' maturation and consequently influence the development of allergy is determined by timing, dose, and site of stimulation. High doses of LPS as TLR4 ligand (31) have been shown to protect against allergy induction by promoting Th1 responses, while low doses of this TLR ligand favors a Th2 environment. Therefore, low-level stimulation of mucosal cells with TLR ligands early in life may enhance concomitant sensitization to allergens (9).

Food allergy is more frequent in infants and children than in adults. In addition to the aforementioned Th2-biased immune responses, the functionality of the gastrointestinal mucosal immune system plays a decisive role in food allergy or tolerance induction. It might even be hypothesized that the intestinal mucosal immune homeostasis is a prerequisite for the development of a balanced immune system. Birth and weaning, two important timepoints of microbial colonization, are especially crucial for the development of an appropriate response to pathogens and dietary antigens as the antigenic composition of the intestinal content suddenly changes (32). A functional mucosal barrier, which is the prerequisite for mucosal defense, is poorly developed for a variable period after birth. The intestinal surface barrier may remain immature up to 2 yr of age leading to enhanced uptake of intact dietary antigens, which might favor sensitization (33).

The colonization by commensal microbial flora after birth is crucial for the establishment of an adequate mucosal immune homeostasis by inducing oral tolerance and IgA plasma cells in the gut lamina propria (34). An indirect association of reduced or aberrant microbial exposure in infancy

and high incidence of allergies has first been postulated in the hygiene hypothesis (35). In atopic children, a delayed postnatal IgA induction and reduced numbers of IgA plasma cells in jejunal mucosa were found. While newborns are exposed to regulatory factors such as TGF- β or IgA via maternal milk, these factors are absent after weaning, which may disturb the mucosal balance (34). The transient lack of these immunomodulating factors may under certain environmental or genetic conditions become permanent and predispose to the development of allergies by favoring excessive effector functions (34). Besides, colonization can affect the expansion of either effector or regulatory cells. These cells can either activate the immune system or tolerize it by discriminating between harmless and harmful antigens (34). In line with the hygiene hypothesis, the reduced microbial load in early life results in an insufficient Th1-cell activity and low IFN- γ levels to counterbalance Th2-cell responses and might additionally explain the increasing incidences of food allergy especially in Westernized countries (36). Furthermore, commensal bacteria can activate epithelial cells via TLRs, which may lead to an interaction with lamina propria cells, including DCs, macrophages, and T-cells (37). Under normal conditions, a balanced cytokine secretion by DCs and macrophages favors the induction of T-reg cells (33). The exposure of newborns to LPS and major dietary allergens leads to the generation of T-reg cells and the up-regulation of the suppressive surface molecule of T cells, CTLA-4, which contributes to the suppressive effects on CD4+ T-cells. Although regulatory cells might exert their suppressive functions also early in life, the functional capacity of T-reg cells was found to be impaired in newborns with a positive atopic family history (38). Additionally, also in cord blood-derived mononuclear cells (CBMCs), the regulatory capacity of T-reg cells was reduced independently of atopic background probably due to a lower expression of CD4+CD25+ cells and Foxp3 (11). Recently, Foxp3+ cells have even been found in fetal placental tissue, and their expression levels were lower in infants who developed allergic diseases (39). Interestingly, Schaub et al. (11) reported that the secretion of IL-17, present already at low amounts in CBMCs, was restricted to innate stimulation with peptidoglycan being a TLR2 ligand and lipid A as TLR4 ligand. They hypothesized that besides T-reg cells, Th17 cells in cord blood may contribute to early immune regulation and maturation by interference with balanced T-cell responses.

Altogether, the data support the concept that the first year of life may be crucial in programming the immune system as colonization with commensal bacteria shifts the T-cell response toward a Th1 profile and stimulates the balanced induction of T-reg cells and sIgA, while in atopic infants, the Th2-dominated cytokine milieu will continue and might predispose to allergy (33).

Strategies for immunomodulation of Th2-biased diseases

Immunomodulation is a therapeutic intervention intended to either stimulate or suppress a particular immune response; it

ranges from vaccination against common infectious diseases to immunotherapy for allergy and cancer. Especially in early life, vaccines do not always induce effective and long-lasting immunity because of an immature immune response. Furthermore, some of the adjuvants used in vaccine development, for example aluminum salts, bias the immune system and may even enhance the already existing Th2 environment (40). Therefore, infants may substantially benefit from the development of novel immunomodulatory adjuvants, for both vaccination and balancing immune responses during diseases.

With regard to pediatric allergy treatment, hyposensitization is the only accepted causative therapy, where repeated subcutaneous injections of allergen extracts adsorbed to an adjuvant are necessary. Although the efficacy has especially been proven for venom and inhalative allergy, such as allergic rhinitis, side effects such as an initial induction of a Th2 response accompanied by enhanced allergic reactions may occur (41). Alternative treatment approaches, however, currently not being standard practice, include sublingual immunotherapy, which has especially been propagated for the treatment of food allergies, and efficacy has been proven on an immunologic level by the enhancement of allergen-specific IgG, and by the reduction in allergen-specific IgE as well as in the Th2 cytokine IL-5 (42). According to recommendations of the World Allergy Organization in 2009, sublingual immunotherapy might only be considered as initial treatment for a carefully selected patient cohort (43) and is currently not recommended as the first-line therapeutic approach for allergic children (43). Both immunotherapeutic strategies may induce various side effects including even life-threatening anaphylactic reactions. Therefore, it is of great interest and importance to develop novel and safe treatment options especially tailored to the particular needs of pediatric allergic patients.

Why are TLRs of interest early in life?

An important first strike mechanism of the innate immune system against pathogens is the recognition of pathogen-associated molecular patterns (PAMPs) by TLRs on immune and non-immune cells. In humans, up to eleven members of TLRs have been identified (44) showing substantial differences in distribution patterns among adults and neonates. Common peri- and postnatal pathogens such as gram-positive bacteria (*Streptococcus pneumoniae*), gram-negative bacteria (*Escherichia coli*), RSV, rotavirus, cytomegalovirus (CMV), herpes simplex virus express TLR ligands that can be recognized via TLR receptors (45, 46). Therefore, the focus of many studies on the stimulation of neonatal cells with TLR ligands may not only mimic the immune stimulating capacity of pathogens, but may also be physiologically relevant in neonatal infections (47). Upon stimulation of TLRs, inflammatory cytokines are produced to directly induce an anti-infectious environment and to activate the adaptive immune system. The expression and function of TLRs on immune cells of neonates were reported to differ from those of adults. Monocytes of preterm neonates express less TLR4 but normal TLR2 compared with adults, whereas the TLR expression of term-born babies is comparable with adult

levels (48). In addition to the quantitative differences of various TLRs in newborns, the function upon TLR-specific stimulation is impaired. Whole cord blood stimulation with the TLR4 ligand LPS showed diminished TNF- α production compared with adult peripheral blood, and agonists of TLR 1-7 did not induce high TNF- α levels in newborns (47). Neonatal pDCs being potent producers of type I IFN reveal an impaired production of IFN- α after TLR3- or TLR9-specific stimulation (49) (Schüller et al. manuscript submitted). Neonatal monocyte-derived DCs produce less IFN- β after, for example, LPS stimulation (50). Investigations of the downstream signaling pathways revealed that LPS-induced phosphorylation of the MAPK p38 is decreased in whole cord blood, and the adaptor molecule MyD88 is diminished in cord blood monocytes (48). However, also the MyD88-independent signaling pathway is impaired in neonates. These data have led to the concept that stimulation of the innate immune system via TLRs is deficient in newborns because of changes in receptor expression and/or diminished downstream signaling.

The response of innate immune cells toward PAMPs may be partially responsible for the impaired immune functions as neonatal immune cells were also reported to produce significantly less IL-12p70, IFN- α , and IFN- γ when compared with adult cells while the capacity to generate IL-6, IL-23, IL-1 β and especially IL-10 was even enhanced (47, 51). In the same context, the capacity of neonatal immune cells to produce two or more diverse cytokines was less efficiently developed compared with adults (51). As IL-1 β , IL-6, and IL-23 are important signals for the differentiation towards Th17 cells, it was suggested that the innate immune response was not only biased towards Th2 responses but that TLR stimulation even favored Th17 production over Th1 cell development (51). Th17 cells promote the production of antimicrobial peptides at mucosal and epithelial surfaces as well as in blood plasma (52), indicating an important role in the defense against extracellular pathogens. Th1 cells on the other hand are mainly involved in systemic defense against intracellular pathogens. High levels of IL-6 and a high ratio of IL-6/TNF- α are not only found in cord blood, but are even more profound in the first days of life, correlating with elevated CRP (C-reactive protein) and LBP (LPS-binding protein) levels after birth (47). IL-6 is not only an anti-inflammatory cytokine but additionally has Th2-polarizing properties contributing to the overall Th2 milieu and to the higher susceptibility of newborns to microbial infections (47).

Therefore, by bridging innate and adaptive immune responses, TLRs are important especially early in life, when the immune system encounters new antigens and microbes. However, strong stimulation of the immune system via TLRs might be inefficient, especially in this period of life.

TLR agonists for supportive treatment of infections in early life

Owing to the high prevalence of infectious diseases in the first 6 months of life, the improvement in neonatal immune responses by stimulation with immunomodulating substances

became a research focus to protect against infection and reduce the mortality of sepsis in early life.

TLR agonists are among the most promising candidates as novel treatment strategy against newborn infections and have been the focus of extensive research in animal and human studies. Before selecting a TLR as target for immunomodulating therapy in early life, the expression pattern of TLRs, the kind of immune cells that carry the targeted TLR on their surface, and the quantity of induced TLR response have to be taken into consideration.

Despite the impaired production of Th1 cytokines and low up-regulation of costimulatory molecules in response to TLR1-7 agonists, recent data revealed TLR8 as a strong activator of immune responses in human newborns (48). A recent murine study investigated the effects of a TLR3, TLR4 and TLR7/8 agonist pretreatment on the outcome of neonatal sepsis in a murine model. Survival of neonatal mice was increased by 21% with a TLR7/8 ligand (resiquimod, R-848) and by even 40% with a TLR4 agonist (LPS) being associated with substantially reduced bacteremia (53). The TLR3 agonist (poly I:C) had no effect on survival or bacteremia. These beneficial effects were caused by induction of increased phagocytic ability by TLR7/8 agonist and enhanced neutrophil recruitment with elevated oxidative burst production upon TLR4 ligands encounter. However, the effects were shown to be type I IFN independent but more likely through a MyD88-dependent response (53). Ligands for TLR1/2, TLR2/6, TLR4, and TLR7 induced lower TNF- α levels (10–1000-fold lower) in cord blood of newborns compared with peripheral blood of adults despite normal basal expression levels of TLRs (54). This impaired response appeared to be caused by high levels of plasma adenosine, which increase cAMP and thereby inhibiting the production of Th1 cytokines (3). In contrast, the TLR7/8 ligand resiquimod or agonists of TLR8 alone elevated the release of the inflammatory marker TNF- α from newborn cord blood cells in a similar fashion as in cells from adult peripheral blood (54). Therefore, TLR8 ligands may represent unique and efficacious immunomodulators of acute infections or vaccine adjuvants in newborns by inducing the expression of IFN- α , IL-6, IL8, granulocyte macrophage colony-stimulating factor (GM-CSF), and TNF- α through the activation of monocytes and DCs and by promoting Th1 responses by APCs activation (54).

Another promising candidate for TLR-mediated immunomodulation appears to be TLR9-binding CpG motifs contained in oligodeoxynucleotides (ODN). Although many studies focused on the immunomodulatory Th1-favoring effects of TLR9 ligands in adult mice (55), recent data indicated that activation of TLR9 in neonatal animals improved the survival because of accelerated production of antibodies directed toward a *neurotropic virus* (56). In a murine *Listeria monocytogenes* model, CpG oligodeoxynucleotides (CpG-ODN) treatment induced protection of neonatal mice against *Listeria* challenges and increased the survival by induction of TNF- α , IL-12, and NO (57). Therefore, further investigations on the potential of TLR9 ligands for the prevention of infectious diseases are required.

Considering the fact that TLRs are differentially distributed on immune cells and act upon distinct pathways, a combination of TLR agonists may strongly activate immune responses. A combined binding to TLR4 and TLR8 triggered neonatal DCs to produce IL-12, which induced the production of IFN- γ by neonatal T-cells (58). Thus, a synergistic stimulation of neonatal DCs may be postulated to overcome the impaired function and induce strong Th1 cytokine responses counteracting insufficient responses of neonates to infectious agents and vaccines.

TLR ligands: promising candidates for allergy therapy?

The most prominent effects of natural TLR ligands were detected in the farming environment following the idea of the hygiene hypothesis. In the *Allergen and Endotoxin study*, children being exposed to animal sheds, and thus to higher endotoxin concentrations, and the consumption of unprocessed milk within the first year of life, were protected from the development of atopic disorders such as asthma and hay fever. Furthermore, even the exposure of the mother to the farming environment during pregnancy significantly influenced the development of allergies in the child. By the exposure to endotoxin from gram-negative bacteria, gene expression for TLR2, TLR4, and CD14 was significantly increased in children living on a farm, as recently reviewed by von Mutius (35).

Synthetic TLR ligands may not only be a novel therapeutic approach in infectious diseases but are also promising candidates for treatment strategies of allergy diseases in early life (Table 1). CpG-ODNs may play a significant role in suppression of Th2 cytokine responses. In a mouse model of ragweed-induced allergic airway inflammation, the protective effects of CpG-ODNs were mediated by the increasing levels of IFN- γ (59). CpG-ODN motifs triggered Th1 cytokine production by the up-regulation of the regulatory transcription factor T-bet (60) and the suppression of IL-5 in a dose-dependent manner, the latter being significantly correlated with IL-10 release (61). The higher expression of T-bet was associated with elevated allergen-specific IgG2a levels and a suppression of IgG1 and IgE titers (60). CpG-ODNs further stimulated the maturation of human DCs being associated with enhanced MHC-II and costimulatory molecules expression, higher Th1 cytokine secretion (62) and induced indoleamine 2,3-dioxygenase (IDO)-dependent T-cell regulatory functions (63).

The efficacy of CpG-ODNs has been mostly investigated in asthma treatment. A combination of hyposensitization and CpG-ODN as adjuvant significantly reduced airway eosinophilia and bronchial hyperresponsiveness in asthmatic mice (64). Furthermore, CpG-ODN as monotherapy together with ambient levels of allergen may be sufficient to beneficially balance immune responses (65). Also in human studies, a combined treatment of CpG-ODN with ragweed immunotherapy revealed reduced eosinophils counts after allergen challenge in nasal biopsies and an increase in IFN- γ /IL-4 mRNA ratio after the first ragweed pollen season being accompanied by ameliorated clinical symptoms during the second ragweed season compared with placebo controls.

Table 1 Beneficial effects of TLR ligands as immunomodulators

TLR	TLR ligand	Infection	Allergy	References
TLR1/2	tBLP	TNF- α ↓		(54)
TLR2/6	MALP	TNF- α ↓		(54)
TLR3	Poly I:C	Survival ↔ Bacteremia ↔	Th1 cytokines ↑	(53)
TLR4	LPS	Bacteremia ↓ Survival 40% ↑ Neutrophil recruitment ↑ Oxidative burst ↑	Th1 cytokines ↑	(53, 54)
TLR7	Imiquimod		AHR ↓ Th2 (IL-4, IL-5, IL-6, IL-13) ↓ Th1 cytokines ↑ Eosinophilia ↓, IgE ↓ Leukocyte infiltration	(70, 71)
TLR7/8	Resiquimod	Survival 21% ↑ Phagocytic ability ↑ Th1 cytokines (TNF- α) ↑	AHR ↓ Th2 ↓ Th1 (IL-12, IFN- α) ↑	(53, 54)
TLR8	ssRNA	↑TNF- α , IL-12 ↑, CD40 ↑ APC activation ↑ of IFN- α , IL-6, IL-8, GM-CSF, TNF- α		(54)
TLR9	CpG	Survival ↑, antibody production ↑ ↑ of IFN- α , TNF- α , IL-12, NO	Th2 cytokines (IL-5) ↓ IL10 ↑, IFN- γ ↑, Tbet ↑ IgG2a ↑, IgG1 ↓, IgE ↓ MHC-II ↑, DC maturation Th1 secretion ↑ Allergic rhinitis: eosinophilia ↓ Asthma: Th1 ↑, FEV ↔	(55, 56, 59–62, 64, 66–68)

TLR, Toll-like receptor; tBLP, triacylated bacterial lipopeptide; MALP, macrophage-activating lipopeptide; Poly I:C, polyinosinic:polycytidylic acid; LPS, lipopolysaccharide; ssRNA, single-stranded RNA; CpG, cytosine-phosphate diester-guanine; GM-CSF, granulocyte macrophage colony-stimulating factor; FEV, forced expiratory volume; AHR, airway hyper-responsiveness; DC, dendritic cell; IFN, interferon; IL, interleukin.

These findings indicate beneficial effects of CpG-ODN on allergic rhinitis (66). However, CpG-ODN treatment for asthma in human patients is discussed controversially. Although CpG-ODN induced an elevation of the Th1 cytokine IFN- γ , there were no differences in allergic airway responses including FEV1 or sputum eosinophils (67). These inconsistencies with other studies may be explained by different CpG-ODN application routes and differing dosing regimens indicating the urgent need of further control studies to evaluate the efficacy of CpG-ODN for treatment of inhalative allergies (65). As most of the studies focus on the effects of CpG-ODN on allergic diseases in adults, data on the implication of TLR9 ligands as treatment approach in early life are rare. In a recent murine study, the effects of CpG-ODN administration on sensitization have been compared in neonatal and adult animals. CpG-ODN treatment significantly decreased anti-OVA IgE titers, which was less pronounced in neonates than in adults. Although CpG-ODN failed to inhibit anaphylactic anti-OVA IgG1 antibodies, the protective IgG2a levels were enhanced in neonates (68). Interestingly, the cytokine response upon CpG-ODN stimulation varied between neonates and adults. While adult B cells produced more IL-6 after CpG-ODN stimulation, neonatal cells showed a predominant IL-10 cytokine production, which may be explained by the impaired TLR9 up-regulation in

early life (68). Additionally, it should be noted that animal studies investigating the potential of TLR9 ligands as immunomodulators might not necessarily reflect the situations in human patients, as it is known that TLR9 is diversely expressed on various murine immune cells but is restricted to pDCs and B cells in humans (69). TLR4 and TLR7 ligands have been investigated as therapeutic approaches for allergy. In an asthma mouse model, TLR7 ligand therapy ameliorated airway hyperresponsiveness (AHR) upon allergen challenge, inhibited both Th2 (IL-4, IL-5, IL-6 and IL-13) and Th1 (IL-12 and IFN- γ) cytokines and prevented eosinophilic infiltration to the lungs. Furthermore, TLR7 agonist treatment was associated with a reduction in IgE levels and favored the production of IgG2a (70). These data are in line with a recent murine study investigating the effect of the TLR 7/8 ligand resiquimod on Th2-mediated airway hyper-reactivity. A single application of resiquimod significantly reduced lung infiltration of inflammatory cells, decreased airway hyper-reactivity and Th2 cytokines, whereas the Th1 cytokines IL-12 and IFN- γ were increased (71).

The suitability of synthetic TLR ligands as treatment approaches for food allergies have not been investigated yet. A recent study reported that consumption of the probiotic *Bifidobacterium longum* AH1206 reduced gene expression of Peyer patches being associated with antigen presentation,

TLR signaling, and cytokine production. Furthermore, this microbe blocked the production of IgE in an oral OVA sensitization model and induced Foxp3⁺ T-reg cells, which may be responsible for the protective effects in this food allergy model (72). Thus, TLR ligands, especially agonists of TLR9 and TLR7/8, may be promising candidates for allergy treatment in the future. However, data on TLR stimulation as treatment approaches in food allergy are missing and have to be focus of future research.

In conclusion, several TLR ligands have been reported as potent modulators of immune responses. However, it has to be kept in mind that most of the data were generated in animal studies and TLR expression pattern cannot be directly compared between animals and men. Therefore, general conclusions have to be drawn very carefully. Furthermore, one has to keep in mind that stimulation of TLRs might lead to diverse side effects as a strong pro-inflammatory response might be induced. Therefore, TLR ligands as immunomodulators might be applicable for a very specific patient population, which has to be in-depth characterized in future. Whether TLR ligands are suitable for the specific requirements of the pediatric population, where immature immune responses might only hardly be overcome by TLR stimulation, has to be subject of further research.

The potential of sphingolipids as immunomodulators

The second immunomodulator to be discussed in this review, sphingolipids and their metabolites, are a large family of lipids characterized by a broad range of bioactive functions. In particular, the metabolites of the sphingolipid catabolism, sphingosine and sphingosine-1 phosphate (S1P), and the enzymes involved in their synthesis and degradation [sphingosine kinase (SphK) and S1P lyase], directly affect cell motility, calcium homeostasis, cell survival and differentiation and modulate immune-cell egress and functions (73).

On the one hand, S1P can be introduced exogenously by dietary intake of sphingolipids such as sphingomyelin, which is highly present in egg and milk products (Fig. 2). Upon ingestion, sphingomyelin can then be metabolized in the small intestine to ceramide by sphingomyelinases, which is secreted by pancreas and bile. Ceramide can be further catabolized by ceramidases to sphingosine, which is then phosphorylated by SphKs to S1P. The majority of sphingosine is immediately taken up by intestinal cells and is either degraded into fatty acids or metabolized to complex structures (74). A small amount of sphingolipid metabolites, especially S1P, can be transported to the blood stream through the mucosa (74). On the other hand, S1P can be produced endogenously by the two kinases SphK1 and SphK2 that phosphorylate sphingosine intracellularly. Both SphKs have diverse tissue distribution and functions. In mouse tissue, a high SphK activity was found in lung and small intestine. In the small intestine and spleen, the contribution of SphK1 to total SphK activity was up to 70%, while SphK2 played only a minor role (75). Upon activation of the cell, S1P can be secreted and act in an autocrine/paracrine manner via five S1P receptors (S1PRs), orchestrating a panel of immunologic responses.

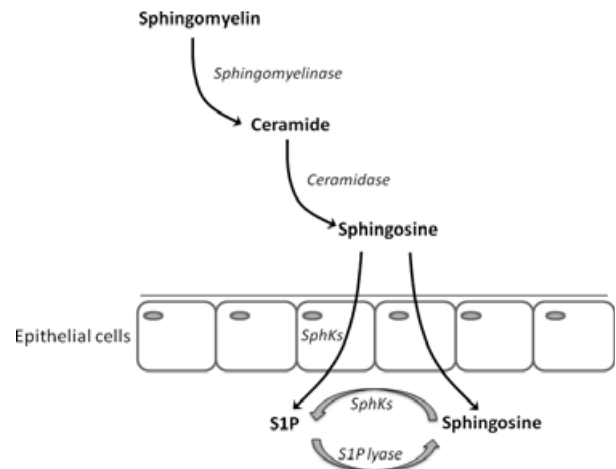


Figure 2 Intestinal sphingolipid metabolisms in the intestine. Sphingomyelin from dietary sources is metabolized by sphingomyelinase to ceramide, which is further degraded to sphingosine by ceramidase. Sphingosine can either be directly transported through the intestinal mucosa or be metabolized intracellularly to sphingosine-1 phosphate (S1P) by intestinal cells. The S1P homeostasis is mediated by sphingosine kinases and S1P lyase, which regulate the balance between S1P and sphingosine.

The potential of sphingolipids as immunomodulators in inflammation

Data on the impact of sphingolipids on immunologic defense properties during inflammatory processes are scarce. Recently, sphingolipid metabolism was linked to host defense and immunity against virus infections. Intervention of the S1P homeostasis by either a reduction in SphK1 activity or an over-expression of S1P lyase was described to suppress *influenza virus* replication and virus-induced cytopathogenicity while activating JAK/STAT signaling (76). However, extracellularly administered S1P did not result in enhanced virus replication, suggesting that the observed effects were independent of membrane S1PRs (76). One has to keep in mind that modulation of the S1P-metabolizing enzymes does not only change S1P concentrations but also affects the intracellular balance of other sphingolipids such as ceramide, which could also be involved in antiviral mechanisms against *influenza virus* (76). Another aspect to take into consideration is that SphK, S1P and S1PRs in locations other than the immune system may also affect the pathologic inflammation in response to a highly virulent infection. Thus, a recent study describes a key role for the lung endothelium in the regulation of innate immune cells infiltration and the early cytokine and chemokine production, the 'cytokine storm', whose magnitude correlates with poor outcomes in patients suffering from influenza infections (77). This study shows that activation of S1PR1 present in endothelial cells inhibits endothelial IFN- α production and results in the dampening of pro-inflammatory early cytokine responses in response to influenza infection using H1N1 2009 Swine influenza virus.

As a consequence, administration of S1PR1 agonists dramatically improves survival to a lethal infection of influenza in mice (77). Furthermore, SIP signaling may have impact on type I IFN-induced lymphocyte egress and DC modulation upon virus infection (76). It has been reported that the S1P-producing enzyme SphK1 negatively regulates the production of pro-inflammatory cytokines and chemokines by Th1 cells and reduces inflammatory Th1 responses (78). S1PRs may also regulate cytokine production of T-cells and DCs. S1PR1 stimulation induced the production of the Th2 cytokine IL-4 in S1PR1 transgenic mice (79), while in T-cell lines overexpressing S1PR4, S1P inhibited IL-4, IL-2, and IFN- γ cytokine expression and enhanced the immunosuppressive cytokine IL-10 (80). However, T-cells deficient in S1PR4 showed no major functional defects; instead, DCs lacking S1PR4 were affected in their migratory and cytokine production patterns, showing an impairment in their ability to drive Th-17 differentiation of T-cells (81). Therefore, targeting specific S1PRs or S1P metabolizing enzymes may be promising novel immunomodulatory treatment strategies in infectious diseases early in life, where the immune response is often impaired with a resulting need for stimulatory agents.

The impact of sphingolipids on allergic diseases

S1P receptors are expressed in important allergy effector cells, such as T-cells, eosinophils and mast cells and regulate cell trafficking and cellular functions in allergic immune responses (82). Mast cells, as main effector cells in allergy, express both SphKs and can secrete S1P when stimulated with an allergen (83). S1P generated by mast cells can act in an autocrine manner on mast cells via S1PR1, which is responsible for cytoskeletal rearrangements and migration of mast cells toward the antigen, and via S1PR2, which is required for degranulation (82). Although the paracrine effects of the S1P released by mast cells into the tissue environment have not been demonstrated, it may potentially participate in many aspects of the allergic response. For example, S1P may recruit and alter the responses of other inflammatory cells such as eosinophils, Th2 cells, and neutrophils (84). In addition, increased levels of S1P were found in BAL fluids of asthmatic patients after allergen challenge and these levels significantly correlated with higher eosinophil counts in BAL fluid, suggesting a role for locally produced S1P in the asthmatic response. Interestingly, in a recent genome-wide association study, genetic variants on chromosome 17q21 have correlated asthmatic disease with orosomucoid 1-like 3 (ORMDL3) gene expression already early in life (85). SNPs near the ORMDL3 locus have been identified to be associated with an increased risk of early-onset childhood and adolescent asthma and were also linked to disease severity (86). These ORM proteins were described to strongly regulate the sphingolipid metabolism, which may have a direct causative role in the development of asthma (87). ORM proteins inhibit serine palmitoyltransferase, which are responsible for *de novo* synthesis of sphingolipids from palmitoyl-CoA and L-serine. As sphingolipids and especially the homeostasis between sphingosine, ceramide and S1P are

essential for diverse immune responses, including cell apoptosis or survival, cytokine induction or effector cell recruitment, it might be hypothesized that the polymorphism of ORMDL3 favors an asthmatic phenotype by the impact on immune cell function and recruitment. Targeting the sphingolipid metabolism may possibly counteract the potential effects of alterations in ORMDL3 activity (87). 17q21 polymorphisms were further correlated with an enhanced IL-17 production (85), the main cytokine of Th17 cells, which are postulated to have a relevant role in asthma development (88).

Influencing the S1P homeostasis by SphK inhibitors, specific S1P receptor agonists or S1P analogs have been the focus of extensive research (Table 2). Non-specific sphingosine agonist prodrugs such as FTY720, which can be phosphorylated by SphKs and in their phosphorylated form are analogs of S1P, bind to four of the five S1PRs. The phosphorylated FTY720 is not only an agonist for S1PRs, but the effects of this drug can also be attributed to functional antagonism of S1PR1 because after binding to this receptor, it induces a rapid internalization and degradation of S1PR1 (89, 90). FTY720 treatment inhibited Th1-induced neutrophilic and Th2-induced eosinophilic airway inflammation, suppressed T-cell infiltration into airways, reduced Th2-specific cytokines and affected bronchial hyper-responsiveness and goblet cell hyperplasia in a murine asthma model (91). However, these data are in apparent contrast to the suggested role of S1P in bronchial inflammation and airway remodeling in asthmatic patients (92). It is important to note that the down-regulation of S1PR1 after activation by the S1P analog FTY720 is more potent and longer lasting than the one induced by S1P itself, and systemic application of this drug may have more complex effects on the immune and vascular systems than a local production of S1P in the lung. Thus, the exact contribution of S1P in asthma remains to be elucidated. Agonists of the S1PR1 may be beneficial by regulating lymphocyte trafficking from lymphoid organs and blood, endothelial barrier integrity (93) and release of pro-inflammatory cytokines, like TNF- α (94). Most S1PR1 agonists, such as SEW2871 or AUY954, are poorly water soluble but need to be dissolved in DMSO (dimethyl sulfoxide) or methanol, which limits their suitability for therapeutic applications. In mouse model of allergic airway inflammation local airway application of the recently described water-soluble S1PR1-specific agonist, the tartrate salt of CYM-5442 inhibited the release of chemokines, impaired the recruitment of CD4⁺ T cells and eosinophilic accumulation (90). As S1P was suggested to be involved in the pathogenesis of asthma, the inhibition of the S1P production by blocking of SphKs may be beneficial for disease progression. In an asthma mouse model, nebulization treatment with SphK inhibitors, DMS (*N,N*-dimethylsphingosine) or SK-I [2-(*p*-hydroxyanilino)-4-(*p*-chlorophenyl) thiazole], reduced S1P levels in BAL fluids, decreased eosinophil infiltration, AHR, and goblet cell hyperplasia in pre-existing airway inflammation (95). Nishiuma et al. (95) stated that this inhalation treatment protocol is superior over the aforementioned oral application of FTY720 because it only inhibits local airway inflammation, while the

Table 2 Sphingolipid targets and their impact on the immune response

Sphingolipid target	Active agent/model of modulation	Immunologic impact	References
S1P analog, S1PR agonist	FTY 720	Binding to S1PR1, S1PR3, S1PR4, S1PR4 ↓ egress of lymphocytes from secondary lymphoid organs ↓ S1PR1 ↓ Th1-induced neutrophilic and Th2-induced eosinophilic airway inflammation ↓ T-cell infiltration ↓ Th2 cytokines ↓ bronchial hyperresponsiveness ↓ goblet cell hyperplasia	(89–91, 100)
SphK1 antagonist		↑ pro-inflammatory cytokines and chemokines by Th1 cells	(78)
SphK1	siRNA	↓ pro-inflammatory cytokines IL-6, TNF- α , IFN- γ ↓ Th2 cytokines in BAL fluids ↓ serum IgE ↓ eosinophilic airway inflammation	(79, 109)
SphK2	siRNA	↑ pro-inflammatory cytokines by Th1	(109)
SphK inhibitors	DMS, SK-I	In asthma: ↓ S1P in BAL fluids ↓ eosinophils infiltration ↓ airway hyperresponsiveness ↓ goblet cell hyperplasia	(95)
SphK1 inhibitor		↓ mucin production	(96)
SphK1 inhibition	Knockout mouse	↓ circulatory S1P ↓ production of IgE in food allergy model	(83, 103)
SphK2 inhibition	Knockout mouse	↓ S1P production in mast cells ↓ IgE-mediated degranulation of mast cells ↓ production of IL-6, IL-13, TNF- α of mast cells ↓ production of IgE in food allergy model	(83, 103)
S1PR1 stimulation		↑ IL-4	(79)
S1PR4 stimulation		↓ IL-4, IL-2, IFN- γ ↑ IL-10	(80)
S1PR4 inhibition	Knockout mouse	↓ Th17 differentiation ↑ Th2 dominated immune response Effect on DC migration and cytokine secretion	(81)
S1PR1 agonist	SEW2871, AUY954, CYM-5442	Regulation of lymphocyte trafficking, endothelial barrier integrity and release of pro-inflammatory TNF- α ↓ T cells and eosinophils	(90, 93, 94)

S1P, sphingosine-1 phosphate; SphK, sphingosine kinase; S1PR, S1P receptor, siRNA, small interfering RNA; DMS, *N,N*-dimethylsphingosine; SK-I, 2-(*p*-hydroxyanilino)-4-(*p*-chlorophenyl) thiazole; BAL, bronchoalveolar lavage; DC, dendritic cell; IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

systemic immune response remained unaffected. As SphK1 is also involved in mucin production (96), blocking of this SphK may ameliorate the pathophysiologic asthma feature. The use of SphK1 small interfering RNA (siRNA) as an alternative inhibition approach has been reported to suppress eosinophilic airway inflammation and reduce Th2 cytokines in BAL fluids and serum IgE levels (97). Although SphK1-deficient mice had a decreased allergen-evoked acute lung inflammation (i.e. reduction in AHR and reduced eosinophilia), they did not show reduced Th2 cytokine levels (98). Furthermore, after long-term allergen exposure, pulmonary arterial hyper-responsiveness was actually increased in

SphK1-deficient mice, suggesting a dissociation between the function of SphK1 in allergen-induced airway pathology and pulmonary vascular pathology (98). Thus, the idea of targeting SphKs for the treatment of asthma is still in a preliminary phase because the role of SphK/S1P signaling in airway inflammation as well as the development of pulmonary vascular pathogenesis has not been studied in-depth.

Mast cell responsiveness and therefore, the onset of the allergic response can be influenced by S1P homeostasis inside the mast cell as well as in the environment the mast cell populates. Genetic deletion of SphK1 or 2 revealed very distinct roles for these kinases on the functionality of mast cells.

While SphK1 affects mast cell function by regulating the circulatory levels of S1P *in vivo* through a mast-cell-independent mechanism, SphK2 is responsible for S1P production within the mast cells, mediating degranulation and production of cytokines like IL-6, IL-13, and TNF- α after Fc ϵ RI engagement (83). Although both SphK1 and SphK2 can modify mast cell immediate responses and, thus, the onset of the allergic response, recent studies on systemic anaphylaxis have indicated that the role of SphK1/S1P on allergy extends beyond the influence on mast cells. Although the lack of SphK1 results in a reduction in the levels of S1P *in vivo* and a reduced mast cell response to a systemic challenge, the absence of SphK1 can also influence the severity of the response by affecting the vascular system. Histamine released during systemic anaphylaxis drives S1P production by activating SphK1. This is required for the engagement of S1PR2, which plays an important role in counteracting the vasodilator actions of histamine and therefore the hypotension associated with systemic anaphylaxis (99).

To date reports on the impact of S1P alterations on food allergy are limited. Recently, it was described that S1P affects migration of CD4⁺ T-cells from spleen to the intestine resulting in antigen-specific intestinal allergic hypersensitivity (100). FTY720 treatment of OVA-sensitized mice inhibited T-cell and mast cell migration into the large intestine, suppressed allergic diarrhea and reduced the Th2 cytokines IL-4 and IL-5 without affecting IgE levels (100). Further, intraperitoneal injections of S1P induced eosinophilic infiltration into the peritoneal cavity in a CCR3 (chemokine receptor)-RANTES (regulated upon activation, normal T-cell expressed, and secreted)-dependent manner (101). In our recent study, we have investigated the influence of SphK1 or SphK2 genetic deletion on the induction of food allergy in a mouse model. Feedings of food allergens under gastric acid suppression was repeatedly shown to mediate food allergy including elevated levels of allergen-specific IgE, Th2 cytokine responses, and anaphylactic symptoms after oral allergen provocation (102). However, we found that deletion of both, SphK1 and SphK2, impaired the production of OVA-specific IgE via the oral route and inhibited mast cell migration after oral ovalbumin provocation into the gastric mucosa, indicating a crucial role of both SphKs in food allergy. Additionally, S1P affects the gastrointestinal permeability in a dose-dependent manner by influencing tight-junction integrity. Our data suggest that S1P opens tight junctions in CaCo2 uptake experiments and favors the transmucosal allergen passage (103). Thus, S1P may substantially affect the uptake of food allergens through the intestinal mucosa. In line with these results, previous studies have correlated the destabilization of tight junction of epithelial cells with sphingolipids (104). The breakdown of epithelial barrier represents a well-known risk factor for many diseases, such as food allergy and inflammatory bowel diseases in intestine (105). A disrupted epithelial barrier in lung, which may be substantially mediated by S1PR3 (106), was even correlated with respiratory lung failure associated with formation of lung edema and inflammatory cell migration (107). Besides the enhanced

passage of allergens, opened epithelial tight junctions are also an entry site for bacteria or virus, representing a substantial risk for infections. In asthmatic individuals, a deficient airway epithelial barrier increases the risk for severe viral infections and, thus, of disease exacerbations (108).

Inhibitors of SphKs that block S1P production may inhibit IgE-mediated mast cell degranulation and cytokine production. Specific SphK inhibitors with improved pharmaceutical properties such as aqueous solubility may find therapeutic use in allergic disorders in the future (84). Alternatively, antagonists of S1PRs or anti-S1P antibodies could provide interesting treatment options for allergic diseases; however, their suitability needs to be experimentally proven.

In summary, sphingolipids might be very promising targets for immunomodulation of diverse diseases in future. Owing to their distinct receptor expression and function on immune cells, the immune system might be modulated differently. However, sphingolipids are a very complex class of lipids, and the interactions of their metabolites have not been fully understood yet. Therefore, before sphingolipids can be applied as immunomodulatory therapeutics in human patients, this complex field has to be investigated in more detail, especially when aiming to apply them in such a vulnerable patient group as children.

Conclusions

In conclusion, early-life immunity is characterized by impaired functions of innate and adaptive immune responses including a predominance of the Th2 cytokine branch. This immunologic status may prone newborns and children for a higher susceptibility to infections and allergic diseases, especially when additional contributors such as genetical or epigenetical factors are present. Targeting the immune system by immunomodulating substances may beneficially influence some immunologic disorders of early life. TLR ligands and sphingolipids have been the focus of extensive research in the last few years and are promising novel treatment strategies for a wide range of immunologically mediated disorders. Besides their effects on immune cell activation and recruitment, TLR ligands and the therapeutic targeting of the sphingolipid metabolism might modulate the immune system toward a strong induction of Th1 cytokines, which, if unbalanced, might also be detrimental because of the induction of possible autoimmune phenomena. Therefore, it should be the focus of research to characterize those immunomodulators within TLR ligands and sphingolipids, which may not only induce a shift toward Th1 but which rather balance the immune response. As both TLRs and receptors involved in the sphingolipid pathway display a very distinct distribution and function on immune cells, it is possible to modulate the immune response in one or the other direction. Specific ligands have the potential to beneficially influence the immunologic environment and possibly enhance immune responses of early life. Of course it has to be kept in mind that interfering with the immune system might also include disadvantages. As TLRs and sphingolipid receptors are ubiquitously expressed also on non-immune cells, side effects on diverse

other organ systems might occur. Therefore, without any doubt, these substances need to be in-depth evaluated for their suitability and efficacy, especially for application in the pediatric population.

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