

REVIEW

The role of innate immunity in asthma development and protection: Lessons from the environment

Vadim Pivniouk^{1,2}  | Joao Antonio Gimenes Junior¹ | Linnea K. Honeker¹  | Donata Vercelli^{1,2} 

¹Asthma and Airway Disease Research Center, University of Arizona, Tucson, AZ, USA

²Department of Cellular and Molecular Medicine, University of Arizona, Tucson, AZ, USA

Correspondence

Donata Vercelli, The University of Arizona, The BIO5 Institute, Rm. 339, 1657 E. Helen Street, Tucson, AZ 85721, USA.
Email: donata@email.arizona.edu

Abstract

Asthma, a complex, chronic disease characterized by airway inflammation, hyper-responsiveness and remodelling, affects over 300 million people worldwide. While the disease is typically associated with exaggerated allergen-induced type 2 immune responses, these responses are strongly influenced by environmental exposures that stimulate innate immune pathways capable of promoting or protecting from asthma. The dual role played by innate immunity in asthma pathogenesis offers multiple opportunities for both research and clinical interventions and is the subject of this review.

KEYWORDS

asthma, environment, farm effect, hygiene hypothesis, innate immunity, microbiome

1 | INTRODUCTION

Asthma is a complex, chronic disease that affects over 300 million people worldwide and is characterized by inflammation, tissue remodelling and hyperresponsiveness of the airways.¹ The disease is frequently (albeit not invariably) marked by exaggerated allergen-induced type 2 immune responses leading to airway and blood eosinophilia, mucus overproduction and increased levels of allergen-specific serum IgE antibodies (Figure 1). These pathogenetic events, however, are strongly influenced by environmental exposures, as shown by the rapid rise in asthma prevalence observed particularly in Western countries.²⁻⁴ Because the innate immune system has evolved to process environmental stimuli and shape adaptive immune responses accordingly, it should come as no surprise that innate immunity is emerging as a critical regulator of both the induction of and the protection from, the disease. The dual role of innate immunity in asthma pathogenesis is the focus of this review.

2 | ASTHMA-PROMOTING INNATE IMMUNE PATHWAYS

Multiple lines of evidence suggest that innate immunity can play an important role in promoting asthma development. Hypothesis-driven candidate gene studies initially showed that polymorphisms in classical innate immunity genes, such as toll-like receptors (TLRs), nucleotide-binding oligomerization domain containing proteins (NODs) and CD14, are associated with asthma and allergic inflammation (reviewed in ⁵). Hypothesis-generating genome-wide association studies (GWAS) later identified additional innate immunity genes associated with asthma, such as interleukin (IL)33, the IL-33 receptor interleukin-1 receptor like 1 (*IL1RL1*)/*ST2*, thymic stromal lymphopoietin (*TSLP*) and the *ORMDL3* (*ORMDL* sphingolipid biosynthesis regulator 3)/*GSDM* (gasdermin) locus on chromosome 17q.⁶⁻⁸ Together with IL-25, the alarmins IL-33 and TSLP can promote type 2 immune response (reviewed in ⁹). Their identification in asthma GWAS led to a renewed interest in the function of these genes and their protein products, and it rapidly became clear that all three cytokines are implicated in the pathophysiology of asthma. Increased expression of IL-25 and its receptor was found in patients with allergic asthma,¹⁰ while TSLP expression was elevated in asthmatics and correlated with airway obstruction.¹¹ Moreover, treatment with an

Vadim Pivniouk, Joao Antonio Gimenes Junior and Linnea K. Honeker contributed equally to this work.

deficient in the IL-33 receptor subunit ST2 and immunized with two Ova + alum intra-peritoneal (IP) injections²⁸ but was strongly inhibited when a single Ova + alum injection was used.²⁹ Finally, IL-33 was able to induce IL-5 production in *IL4*^{-/-} and *STAT6*^{-/-} mice.²⁹

In combination, these data suggest that IL-33 plays a central role in the local release of IL-5 and IL-13 and the airway inflammation driven by these cytokines but may be dispensable for the induction of systemic Th2 responses. This suggestion is supported by the ability of IL-33 to induce robust airway eosinophilia in Rag2-deficient mice lacking T and B cells,²³ while induction of airway inflammation by *Aspergillus fumigatus*, a strong allergen, was markedly inhibited in these animals.³⁰ Inhalation of papain also induced airway eosinophilia in Rag2-deficient but not in *IL33*^{-/-} or *IL4*^{-/-}*IL13*^{-/-} mice,²³ and influenza virus³¹ and the glycolipid antigen α -GalactosylCeramide³² elicited IL-33-dependent AHR in Rag2-deficient mice.

Notably, the IL-33 axis and lung development appear to be linked in ways that might be relevant to asthma pathogenesis. Shortly after birth, ILC2s, eosinophils, basophils and mast cells were found to spontaneously accumulate in developing lungs in an IL-33-dependent manner. During the phase of postnatal lung alveolarization, HDM exposure further increased IL-33, which boosted cytokine production in ILC2s, activated CD11b⁺ dendritic cells and reprogrammed them to promote Th2 cell skewing. ST2, the soluble decoy IL-33 receptor, had a strong preventive effect on asthma in the neonatal period, less so in adulthood.³³ These data suggest that enhanced neonatal Th2 responses to inhaled allergens result from the postnatal hyperactivity of the IL-33 axis during a period of maximal lung remodelling.

2.2 | Alum, LPS and the regulation of immune responses

The ability of IL-33 to induce airway inflammation in the absence of T and B cells does not rule out the possibility that this cytokine may also influence adaptive Th2 responses relevant to asthma pathogenesis. A case in point is provided by the role of IL-33 in alum-dependent Th2 responses. Alum has a long history in experimental models of allergic asthma. Indeed, one of the earliest observations suggesting a Th2-promoting role of innate immunity was made over 80 years ago when aluminium salts were shown to increase allergic sensitization to pollen extracts in guinea pigs (reviewed in³⁴). Different aluminium salts can be used as adjuvants to increase antibody responses to soluble antigens in research and improve vaccine efficacy in the clinic. A combination of aluminium hydroxide and magnesium hydroxide is widely used in mouse asthma models to induce a robust Th2 response to protein antigens. However, the role of alum was long ignored, probably because its mechanisms of action were unclear.³⁴ After the discovery that Th1 immune responses to Ova mixed with complete Freund's adjuvant require MyD88-mediated TLR signalling, whereas Th2 responses to Ova mixed with alum do not,³⁵ it was suggested that Th2 responses may develop either by default in the absence of TLR activation or through another, yet undefined pathway.³⁶ An influential study by Eisenbarth et al³⁷ addressed this question by immunizing mice

intranasally with Ova mixed with different doses of lipopolysaccharide (LPS), a potent TLR4-dependent immunostimulator present on the cell surface of Gram-negative bacteria. Without LPS, intranasal Ova failed to induce airway inflammation, while Ova with low or high dose of LPS induced Th2 or Th1 type inflammation, respectively. Importantly, both types of inflammation were abrogated in TLR4-deficient mice, suggesting that TLRs may be critical for both Th1 and Th2 immune responses.³⁷

The discovery of TLRs renewed the interest in alum and its powerful Th2-inducing effects (reviewed in³⁴). Interestingly, most of the effects of alum (other than induction of IL-1 β and IL-18³⁸) did not require TLR signalling because they were intact in MyD88-deficient^{35,39} and MyD88/Trif double-deficient mice.^{40,41}

Recently, it was shown that alum induces some cell death and local release of endogenous double-stranded DNA when injected into mice IP or intramuscularly (IM).⁴² Host DNA released from dying cells appeared to be critical for the effects of alum because mouse genomic DNA mixed with Ova was as efficient as alum in inducing Ova-specific IgG1 and IgE, while IP treatment with DNaseI inhibited induction of these antibodies by Ova + alum.⁴² Other studies have shown that among multiple innate immune pathways stimulated by alum, release of IL-33 may play an important role. In the presence of LPS, alum induces IL-33 secretion in THP-1 cells.⁴³ Moreover, alum induces elevated levels of IL-33 after intra-peritoneal injection,⁴⁴ and alum and IL-33 induce comparable cytokine responses.⁴⁴ Finally, alum failed to induce IL-5 and IL-13 secretion in IL-33-deficient mice, while other cytokines, such as IL-6 and G-CSF, were less affected. Surprisingly, antigen-specific antibody responses induced by antigen + alum were comparable in *IL-33*^{-/-} and control mice, suggesting that IL-33 mediates some of the allergy-promoting effects of alum, but its role in this process is somewhat redundant.⁴⁴

3 | ROLE OF INNATE IMMUNITY IN ASTHMA PROTECTION

Recent evidence suggests that the dramatic rise in asthma prevalence seen in westernized countries⁴⁵ may be due not only to a growing number of asthma-promoting factors but also to a dramatic decline in asthma-protective innate stimuli.

3.1 | The farm effect: epidemiology & immunology

Seminal epidemiologic studies in European children raised in farming and non-farming environments have clearly demonstrated the connection between early life exposure to traditional farms and decreased prevalence of allergic diseases and asthma, a pattern referred to as the "farm effect".⁴⁶⁻⁶⁰ Specific exposures that contribute to the farm effect include contact with livestock (cattle, pigs and poultry) and animal feed (hay, grain and straw), consumption of raw milk, high endotoxin levels and environmental microbes.⁶¹⁻⁶³ More recent data have clarified the reach of protective exposures by showing

that physical proximity to a traditional farm within a maximum radius of 100 m is inversely associated with childhood asthma and atopic sensitization.⁶⁴

A remarkable example of the farm effect is offered by children raised in Amish traditional farming communities⁶⁵ and their comparison with Hutterite children.⁴¹ Amish and Hutterites are US farming populations with similar genetic ancestries and lifestyles but distinct farming practices (traditional among the Amish, industrialized and modern among the Hutterites). We found that the incidence of asthma is fourfold lower in Amish children while the level of endotoxin in Amish homes is 6.7-fold higher. There were significant differences in the proportions and phenotypes of peripheral blood innate immune cells between Amish and Hutterites, with lower eosinophils and higher neutrophils in the Amish.⁴¹ These findings suggest that exposure to traditional, microbe-rich farm environments significantly influences innate immune responses, thereby modifying subsequent risk for allergic diseases and asthma.⁴¹

Toll-like receptors play a major role in the interactions between the host's innate immune system and environmental microbiota. These transmembrane pattern recognition receptors are typically (but not exclusively) expressed on a variety of innate immune cells in mucosal linings, both in the lungs and the gut and detect a broad range of microbial-associated molecular patterns (MAMPs). TLR4, TLR2 and CD14 (a co-receptor for LPS detection by TLR4) play a critical role in gene-environment interactions in asthma.⁶⁶ Prenatal exposure to environments rich in microbial diversity, such as farms, appears to prime a child's immune response and protects against the development of atopic sensitization in part by upregulating innate immune receptors such as CD14, TLR4 and TLR2.⁶⁷ Indeed, increased numbers and improved function of regulatory T cells (Tregs) were found in neonates of farming mothers compared with neonates born to non-farm mothers.⁶⁸ Moreover, expression of TLR7 and TLR8 (receptors for single-stranded RNA) and levels of IFN- γ and TNF were higher in cord blood of farm children compared with children not raised on farms.^{69,70} Children of farmers at decreased allergy risk have also increased levels of CD14 and TLR2 mRNA,⁷¹ high expression of interleukin-1 receptor-associated kinase (IRAK)-4 and receptor-interacting serine/threonine kinase (RIPK) 1 and enhanced expression of regulatory molecules such as IL-10, transforming growth factor (TGF)- β , suppressor of cytokine signalling (SOCS) 4 and IRAK-2,⁷² all of which may contribute to protection against asthma. These results suggest that prenatal and/or early life act as a window of opportunity during which exposure to the rich and diverse microbial environment of traditional farms critically upregulates asthma-protective innate immune responses.

3.2 | The farm effect: mechanistic studies

Mouse models have played a crucial role in identifying some of the processes underlying the asthma protection conferred by farm exposure. The initial experimental link between exposure to a traditional farm environment and protection from allergic disease was provided by the Bochum group, which demonstrated that inhalation

of cowshed dust extracts from Alpine dairy farms protected mice from Ova-induced BAL eosinophilia and AHR.⁷³ Because cowshed dust contains a wide variety of microbes and microbial products, the same group next characterized two bacterial species most abundant in cowshed microflora: the Gram-negative *Acinetobacter lwoffii* F78 and the Gram-positive *Lactococcus lactis* G121. Both microbes could reduce allergic inflammation in mice, activate human and mouse cells in vitro and induce a Th1-polarizing program in dendritic cells—activities that might play a role in allergy protection.⁷⁴ Other studies have confirmed a protective role of *A lwoffii*. A decreased rate of asthma was reported in children of mothers exposed to this microbe,⁷⁵ and an inverse association was found between rates of asthma and atopy in school-age children from rural and suburban German communities and the presence of *A lwoffii* in mattress dust.⁷⁶ Other environmental microbes implicated in protection are *Bacillus licheniformis* from animal sheds⁷⁷ and farm-derived Gram-positive *Staphylococcus sciuri* W620.⁷⁸

To gain deeper insights into the immune modulation induced by cowshed dust extracts, again the Bochum group studied the effects of cowshed dust on dendritic cells, showing that intranasal administration of in vitro-generated murine bone marrow-derived dendritic cells stimulated with cowshed dust extract before an Ova pulse could not prime mice for allergic responses.⁷⁹ These cells produced high amounts of IL-10, and in vitro IL-10 blockade partially restored their allergy-inducing capacity, suggesting that an autocrine IL-10-dependent, cowshed dust-induced mechanism might down-regulate dendritic cell function.⁷⁹ In subsequent studies, the Bochum group demonstrated that arabinogalactans, polysaccharides abundant in cowshed dust extracts, also protect mice from Ova-dependent allergic sensitization, lung inflammation and airway hyperresponsiveness.⁸⁰ Studies with allergen-pulsed, arabinogalactan-treated murine BMDCs suggested that the allergy-protective properties of arabinogalactans might be due to attenuation of dendritic cell-mediated Th2 induction.

More recently, the Lambrecht group demonstrated that mice exposed chronically to farm dust or low-dose endotoxin were protected from developing HDM-induced asthma through induction of the negative regulator A20 in epithelial cells.⁸¹ A20 is a ubiquitin-modifying enzyme encoded by the *TNFAIP3* gene that limits the activity of multiple NF- κ B signalling cascades.⁸² Expression of A20 in the airway epithelium was required for the asthma-protective effect of dust extracts from European cowsheds. Additionally, A20 mRNA and protein expression were significantly reduced in air-liquid interface-cultured bronchial epithelial cells from mild and severe asthmatics compared with healthy subjects.⁸¹

Our group also used experimental asthma models to better understand the different levels of asthma prevalence found in Amish and Hutterite children, but our experiments were the first in which farm dust activity was rigorously controlled for: dust collected in Hutterite farm homes served as a control for Amish farm house dust. In this setting, inhalation of Amish house dust was sufficient to strongly protect Ova-treated mice from experimental asthma, as evidenced by decreased AHR, lung eosinophilia and IL-13 and IL-5 secretion in BAL. In contrast, mice that received Hutterite house

dust (either at the same concentration as the Amish house dust, or in amounts that reflected the total dust load in the relevant homes) were not protected from experimental asthma.⁸³ Interestingly, intranasal administration of Hutterite barn dust extracts inhibited HDM- and Ova-induced AHR and BAL eosinophilia as effectively as Amish barn dust extracts.⁸⁴ These results demonstrated that an environment with strong asthma-protective properties exists not only on Amish but also on Hutterite farms. However, unlike Amish children, young Hutterite children typically do not spend time near farm animals or in the barns, which are much larger and located at much greater distances from their homes than are Amish barns, and thus do not benefit from this asthma-protective exposure.

It is noteworthy that the asthma-protective signals delivered by farm living appear to be both microbial and non-microbial in nature. A recent report found that N-glycolylneuraminic acid (Neu5Gc), a sialic acid found in non-human mammalian glycoproteins but absent in bacteria,⁸⁵ is associated with lower rates of asthma and wheezing in farm children. Levels of anti-Neu5Gc IgG antibodies correlated positively with living on a farm, and Neu5Gc exerted anti-inflammatory effects in mouse models of airway inflammation.⁸⁶

4 | MICROBIOME, INNATE IMMUNITY AND ASTHMA PROTECTION

Innate immunity and asthma development are intensely influenced not only by environmental microbes but also by the microbes living on or inside our bodies, collectively called the microbiome. From the moment we are born, and even as early as in utero,^{87,88} our bodies provide niches for a diverse array of microbes to colonize, and a large proportion of microbiota found in the home, where we spend most of our time, comes from the surrounding outdoor environment.⁸⁹ Because microorganisms have existed on this planet well before humans, our evolution was largely affected by these associations: mechanisms were needed to fight off harmful pathogens, while keeping beneficial commensals. This is a delicate balance, deviations from which can result in dysbiosis and increased susceptibility to diseases such as asthma. In fact, asthma development or protection is highly influenced by our microbiome, as evidenced by strong associations between asthma rates and microbial diversity in the gut,^{90,91} lung⁹² and nose.⁸⁹ Innate immunity, the first line of interaction with microbes residing on or in us, largely determines whether a pro-inflammatory or regulatory response will be initiated. Here, we will focus on the host microbiome and how its interactions with the innate immune system protect from asthma.

4.1 | TLRs and MAMPs

Like environmental microbes, mammalian host resident microbes are also detected by TLRs expressed by epithelial and innate immune cells of the lung and gut.⁹³⁻⁹⁵ TLR-detected MAMPs differ in structure between bacterial species and thus lead to different innate immune responses. For example, lipid A, the bioactive component of LPS, can

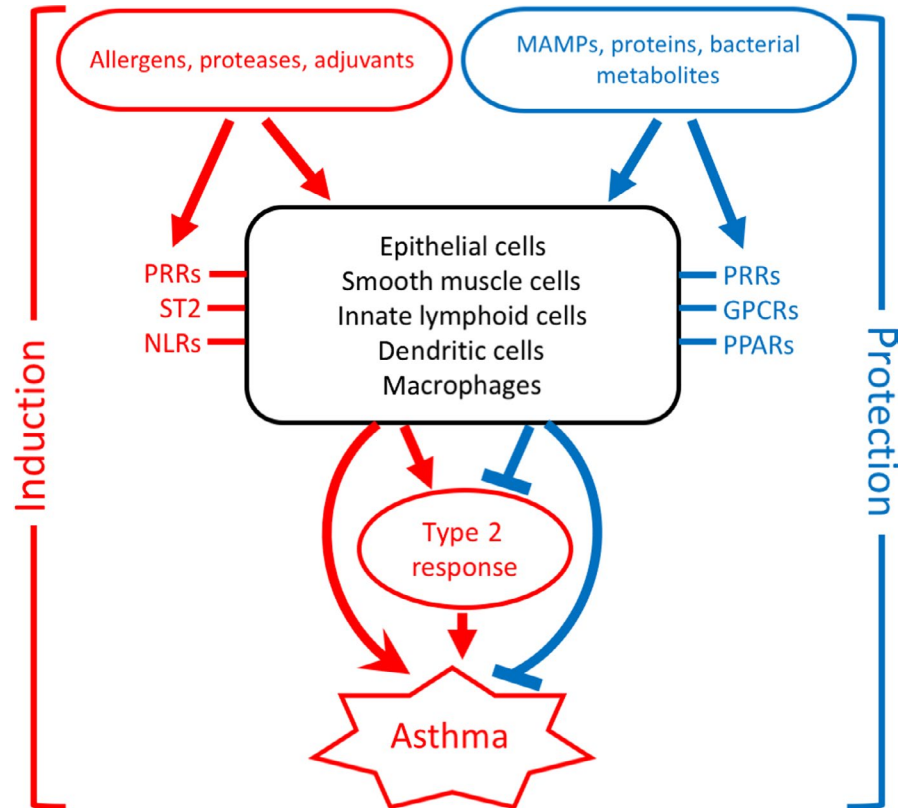
differ in the length and number of acyl chains: hexa-acylated lipid A is the strongest TLR4 agonist^{96,97} while lipid A molecules with shorter or fewer acyl chains are weaker TLR4 agonists.⁹⁷⁻⁹⁹ In fact, gut colonization of infants with *Escherichia coli*, which carries the more potent hexa-acylated lipid A, led to immunostimulation and decreased risk of autoimmunity and allergies later in life, while the less potent LPS of *Bacteroides dorei* inhibited this effect.⁹⁹ Another study found that while all airway-associated bacteria studied in vitro stimulated maturation of adult human dendritic cells, commensals including *Prevotella*, *Veillonella* and *Actinomyces* spp. elicited significantly lower production of pro-inflammatory IL-12 and IL-23 than the pathogens *Haemophilus influenzae* and *Moraxella catarrhalis*, which are associated with asthma and COPD, presumably due to their differences in biologically active lipid A. Moreover, *Prevotella* spp. grown in combination with *Haemophilus influenzae* decreased the overall production of IL-12 and IL-23 in a TLR-dependent manner, suggesting that commensals may be protective in asthma patients.¹⁰⁰ These differences in innate immune responses to MAMPs at different stages of life emphasize the importance of early life microbial exposures in training the immune system. Emerging evidence consistent with this view documents bacterial colonization and presence of bacterial genetic material in the amniotic fluid, cord blood, placenta and placental membranes.¹⁰¹⁻¹⁰³

4.2 | G-protein-coupled receptors and short-chain fatty acids

G-protein-coupled receptors (GPCRs or GPRs), particularly GPR41 (FFAR3), GPR43 (FFAR2) and GPR109A located on innate immune cells, play an important role in immune modulation¹⁰⁴⁻¹⁰⁸ by binding microbial short-chain fatty acids (SCFA) such as acetate, propionate and butyrate. These small molecules are produced by anaerobic bacteria in the gut as metabolic byproducts of dietary fibre fermentation and can either stimulate innate immunity in the gut directly or enter the blood stream, in both cases eliciting systemic effects that extend to the lung—a critical scenario effectively captured by the recently developed notion of a gut-lung axis.¹⁰⁹ Mechanistically, SCFAs bind to GPR41 and GPR43, which induce immune regulation and suppress airway inflammation by promoting the expansion of colonic Tregs^{108,110} or influencing hematopoiesis and the function of DCs recruited to the lung.¹⁰⁵ For instance, despite an absence of SCFAs in the lungs of HDM-sensitized and challenged mice, systemic levels of propionate enhanced the development of DCs impaired in their ability to activate a Th2 immune response. Recruitment of these DCs to the lung led to decreased airway inflammation.¹⁰⁵ Of note, trans-placental transport of microbial products including SCFA and other metabolites¹⁰² might explain why the children of mothers that had higher serum acetate levels during pregnancy had a significantly lower rate of wheeze (both doctor-diagnosed and self-reported) in the first year of life.¹¹¹

SCFA binding to GPRs expressed on leucocytes, including neutrophils, eosinophils and macrophages, has been found to influence myelopoiesis and myelocyte migration.¹¹²⁻¹¹⁴ Intravascular recruitment of neutrophils induced by an LPS challenge was increased in *Gpr43*^{-/-} mice compared with wildtype mice.¹¹⁴ This over-reactive

FIGURE 2 Dual role of innate immunity in asthma pathogenesis. Proposed relationship between signals that induce or inhibit asthma by acting on innate immune cells and their receptors. Depending on the signal, innate immune cells may either promote or block development of type 2 responses and/or asthma. GPCRs, select G-protein-coupled receptors (ie, GPR41, GPR43, and GPR109); MAMPs, microbial-associated molecular patterns; NLRs, NOD-like receptors; PPARs, peroxisome proliferator-activated receptors; PRRs, pattern recognition receptors; ST2/IL1RL1, IL-33 receptor



neutrophil response extended to wildtype mice fed a no-fibre diet compared with mice fed normal chow, further showing how important microbial SCFA is in promoting homeostatic neutrophil responses. These results also demonstrate that the diverging roles of neutrophils (homeostatic or pro-inflammatory)¹¹⁵ may be driven by microbiota—a possibility that is immediately relevant to asthma cases associated with lung neutrophilia.

4.3 | Other metabolites

Besides SCFAs, other microbial metabolites play a key role in host immune modulation and decreased inflammation [reviewed in¹¹⁶]. For instance, D-tryptophan has immunomodulatory properties and was identified as the bioactive substance produced by probiotic *Lactobacillus rhamnosus* GG (LGG) that decreased airway inflammation and Th2 responses in mice.¹¹⁷ Polyunsaturated fatty acids (PUFAs) have also been associated with decreased asthma risk. Compared with 1-month-old infants with an increased relative risk for development of atopy at age 2 and asthma at age 4, infants with a decreased risk had a distinct gut microbiota profile and increased anti-inflammatory faecal metabolites, including docosapentanoic acid, a ω -3 PUFA.⁹⁰ A follow-up study found that this pattern extended to newborns, as indicated by significant differences in microbial communities and enrichment of anti-inflammatory metabolites (including ω -3 PUFA) in the meconium of newborns at decreased risk of asthma.⁸⁸ Interestingly, supplementation of high asthma risk infants with LGG for the first 6 months of life resulted in gut microbiota compositions and metabolic profiles similar

to those with decreased risk of asthma,⁸⁸ further supporting the notion that anti-inflammatory metabolites produced by bacteria can protect from asthma. Mechanistically, PUFAs are potent agonists for peroxisome proliferator-activated receptor- γ (PPAR- γ)¹¹⁸ and PPAR- α ,¹¹⁹ which are expressed on innate immune cells (macrophages and DCs) and inhibit the production of pro-inflammatory cytokines (TNF, IL-6 and IL-1 β).¹²⁰ It is also notable that modelling differences in house dust microbiota composition between farm and non-farm homes of European birth cohorts have recently shown that in children who grow up in non-farm homes, asthma risk decreases as the similarity of their home bacterial microbiota composition to that of farm homes increases. The protective effect was independent of richness and total bacterial load and was associated with reduced pro-inflammatory cytokine responses against bacterial cell wall components *ex vivo*.¹²¹

5 | CONCLUSIONS

A rich literature demonstrates that the innate immune system plays a complex, dual role in both the development of, and the protection from, asthma (Figure 2). Different environmental exposures and host microbiome compositions and products can stimulate the innate immune system to engage asthma-promoting or asthma-protective signalling cascades. The timing of these exposures and host microbiome trajectories also plays a major role in shaping innate immune responses, thus offering multiple opportunities for both research and clinical interventions.

CONFLICT OF INTEREST

All the authors declare no conflict of interest.

ORCID

Vadim Pivniouk  <https://orcid.org/0000-0002-2276-0321>

Linnea K. Honeker  <https://orcid.org/0000-0002-1299-0879>

Donata Vercelli  <https://orcid.org/0000-0001-5276-8976>

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