The Role of LPS and CpG in the farm effect against allergies, and beyond

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Abstract
The prevalence of allergic disease has increased significantly over the past decades. Although allergies are inherently multifactorial and heterogenous; environmental, maternal, and early life microbial exposures could strongly modify disease risk. The effects of environmental microbiota are illustrated by the ‘farm effect’, showing protection against asthma when growing up on traditional farms. Recent studies have further revealed an important role for early-life exposure to a microbe-rich environment imposing lung and gut microbiome maturation and immune education, preventing allergic disease in childhood. Advances are made in the field of immunology and microbiome research, which identified entire microbial taxa, as well as specific microbial metabolites and bacterial products associated with reducing disease risk. Here we discuss the cross-talk between the microbiota and allergic disease pathogenesis, including bacterial products as lipopolysaccharide and CpG in the farm effect.

Introduction
Over the last decades the prevalence of allergic disease, like: asthma, food allergy, and atopic dermatitis has increased dramatically, especially in areas with Western life styles and environmental exposures. Although asthma is heritable and polygenic, with many genetic variants contributing to disease risk, the interaction of these genetic factors with
environmental factors such as, diet, allergens and microbes appear important for disease pathogenesis\textsuperscript{1}. It is increasingly well recognized that early life exposure to environmental factors play a pivotal role in the development of asthma and other allergies. This is underlined by the fact that prevalence of childhood asthma is significantly greater in urban areas compared to rural environments, with the traditional farming environment showing the strongest protective effect, also referred to as the ‘farm effect’\textsuperscript{2}. This protective effect can, at least in part, be explained by the high-level exposure to lipopolysaccharide (LPS), a cell wall component of Gram-negative bacteria, and bacterial DNA (CpG) on farms. Central to the effect of these exposures are maternal and early-life microbial and immune networks, which start during pregnancy when the mother’s microbiome shapes the immune system and microbiota of the developing fetus, and continues to do so during the first year of life, e.g. via breastfeeding. This ultimately regulates the microbiome and immune maturation, and influences the risk for developing allergic disease\textsuperscript{2,3}. Although our understanding of gene-microbial interactions in asthma has expanded, we still lack specific knowledge on the biological mechanisms as to how the ‘farm effect’ and microbiota interact with host genetics and immune system to modify disease risk. In this review we will discuss the effects of environmental microbial exposure on allergic pathogenesis, focusing on LPS and CpG-derived immunotolerance.

The farm effect: environmental microbiota diversity
The hygiene hypothesis, as proposed by Strachan in 1989, postulates that decreased exposure to environmental or commensal microbes, as is the case in modern day urban lifestyles, is responsible for the increased prevalence of allergic disease in Western societies\textsuperscript{4}. Over time it has become clear that respiratory viral infections, like: RSV, and rhinovirus (RV)\textsuperscript{5}, as well as lifestyle changes and dietary habits could also affect the incidence of asthma later in life\textsuperscript{6}. Moreover, genetic predisposition, especially the chromosome 17q21 risk alleles, also play an important part in the outcome of host interactions with its microenvironment (reviewed in \textsuperscript{7}). Supporting the hygiene hypothesis is the observation of the “farm effect”, which is hypothesized to provide immunomodulatory stimuli sufficient to promote childhood immune maturation. As introduced above, studies have associated maternal and early-life exposure to farming environments or environments with high endotoxins loads with reduced incidence of allergic disease in children\textsuperscript{8}. 

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The best example of the farm effect is observed in the Amish and the Hutterite, two populations with similar genetic background but distinct farming patterns. Researchers have shown a sharply different asthma and allergy prevalence between the Amish children that live near animal barns and whose families use more traditional farming practices and the more industrialized farming techniques seen near the homes of Hutterite children. With Amish showing 4-5 times lower asthma and allergy prevalence9. This difference was reflected in different bacterial composition and levels of house dust endotoxin, which were found to be seven times higher among the Amish. In addition, the proportions, phenotypes and functions of innate and adaptive immune cells were different between both farming practices10. Interestingly, dust from Hutterite barns is similar in microbial content to that of Amish barn dust, and not surprisingly showed similar protection when tested in animal models9. A recent study in Finnish birth cohorts has revealed that in children growing up in non-farming homes, the risk of developing asthma decreased as the similarity of their home microbiota composition increased towards that of farming homes11. These data were replicated in German children living in rural non-farming homes, whose asthma risk was observed to be reduced when the microbiota composition was more similar to the Finnish farm homes11. This illustrates that it may not only be the bacterial load, but rather the microbial diversity that determines protection against asthma.

The importance of environmental microbial exposure was further found in studies comparing Finnish and Russian children. Here, higher microbial load in the Russian drinking water was associated with allergy protection in Russia12,13. Similarly, in a recent study with Croatian school children receiving drinking water from individual wells, a higher cumulative bacterial load in drinking water was associated with lower life-time prevalence of allergic disease14. It is important to note that the interaction between environmental microbes and allergic disease is not limited to children. In a recent large-scale house dust microbiome study, a US adult-farming population was sampled. Bacterial community profiles were less diverse in homes of atopic individuals, compared to controls. Additionally, specific bacterial taxa were abundantly present in house-dust of atopic patients: Cyanobacteria, Bacteroidetes, and Fusobacteria. In contrast several taxa of Firmicutes were more abundant in homes of individuals without atopy15. In all, these data illustrate that the bacterial composition in environmental exposure greatly affects the risk of developing atopic disease or asthma. However, other nonbacterial microbes, like fungi, may also play a role in farm effect and
protect against allergic disease, however, these mechanisms are still unclear and likely involve complex microbial ecologies\textsuperscript{16}.

**The farm effect: instructing human immunity and microbiome**

Farm animals provide a large microbial diversity, a relevant aspect in the farm effect. An import marker of bacterial exposure is the level of endotoxin containing LPS, an immunomodulatory molecule that binds to TLR4 and thereby induces the release of pro-inflammatory cytokines\textsuperscript{17}. However, a dichotomy exists where LPS can either promote or prevent Th2-driven allergic responses. This dual role is illustrated in a study by Kaur et al. showing that local LPS drives protection from allergic inflammation in the presence of GM-CSF, whereas a Th2-favourable effect was observed in the absence of GM-CSF\textsuperscript{18}. In addition to LPS, another microbial product with marked immunomodulatory properties is bacterial DNA. Bacterial DNA contains unmethylated cytosine-guanine (CpG) dinucleotide motifs, which binds to TLR9. Hereby, CpG potently activates innate immune responses and induces the production of IL-10, type I IFN, IL-12, and suppress Th2 cytokines, having the potential to reverse type 2 responses\textsuperscript{19}. These data suggest that exposure to bacterial products, such as LPS and CpG DNA, present in the environmental microbial cocktail seen on farms may favor protection to Th2-mediated allergic disorders. Several strategies using synthetic CpG oligonucleotides (ODNs) for the treatment or prevention of allergic disease, have been studied in both animal models and human trials. Despite the positive results of preclinical studies, trials in patients evaluating the effect of CpG ODNs on allergic disease have returned mixed results\textsuperscript{19}.

Besides the mere release of cell wall components, environmental microbes can also colonize and change the host microbiome and influence the etiology of asthma\textsuperscript{20}. In urban settings, the indoor microbial environment is largely composed by the human skin microbiota, resulting in people being exposed to the relative low-diversity of their own microbes\textsuperscript{21}. Interestingly, exposure to urban green space was sufficient to ensure transfer of environmental microbes, allowing the microbial composition of the skin to become more similar to soil microbiota and nasal samples to become more closely related to the air microbiota\textsuperscript{22}. Other studies showed that farm living increased microbial diversity in mattress dust, but also in microbes colonizing nose and throat\textsuperscript{23}. These data support the idea that a combination of microbes or a diverse composition of the microbial environment offers the
increased protection against atopic disease. Experimental evidence in mice has also revealed that intranasal administration of different bacterial strains (*Lactococcus lactis*, *Acinetobacter lwoffii*, or *Staphylococcus sciuri*), present in cowsheds, could protect from allergic airway inflammation in a TLR-dependent manner. In-depth analysis of the Karelia study revealed that when comparing skin and nasal microbial networks, these were richer and far more diverse with high abundance of *Acinetobacter* in the Russian subjects and correlated with suppression of innate allergic immune responses. The latter hints towards a role for microbiota sharing between hosts, which has also been observed with household pets that have been associated with increased bacterial composition affecting the human microbiome. This close contact transfer of microbes may also explain why the ‘farm effect’ can be passed on from mother to child, when shedding of the maternal microbiome occurs upon delivery and early childhood.

Moreover, a recent birth cohort study showed that the maturation of the gut microbiome during the first year of life may contribute to the protective farm effect. This study found inverse correlations between asthma, levels of fecal short-chain fatty acids (SCFA) butyrate, and bacteria that could produce butyrate. Similarly, Gio-Bata et al. found that children living on farms have higher levels of fecal SCFA acid compared to non-farm living children, which was associated with lower incidence of eczema. This study thereby touches upon the importance of the gut-lung axis and asthma-protection through microbiome produced metabolites (reviewed in ). The importance of the lung-gut axis was further highlighted by studies in humans associating the pro-inflammatory metabolite 12,13-dihydroxy-9Z-octadecenoic acid (12,13-diHOME), with microbial dysbiosis and increased risk for childhood atopy. These observations where further strengthened when introduction of the bacterial epoxide hydrolase genes, responsible for 12,13-diHOME production into the mouse gut microbiome, was sufficient to reduce Treg cells and exacerbate allergic airway inflammation. Moreover, using 16S shotgun sequencing, it was observed that the microbiome of infants who went on to develop allergic sensitization during childhood lacked genes encoding key enzymes for carbohydrate breakdown and butyrate production. Finally, studies in a Finnish/Estonian birth cohort have revealed profound associations between regulatory T cell frequencies and colonization with butyrate-producing bacteria, which occurred earlier in life in Estonian children at low risk of developing allergic disease. In murine studies it was further shown that dietary fermentable fiber content changed the
composition of the lung and gut microbiota and dampened allergic disease. Metabolized fiber increased the levels of circulating SCFA, altering bone marrow hematopoiesis and seeded the lungs with DCs impaired in their ability to promote Type-2 inflammation\textsuperscript{29,34}.

These results show that it is not solely direct microbial exposure (e.g. LPS and CpG) but also the microbial company we keep that affects our disease risk and should be considered an important factor in the ongoing effort to reduce the prevalence of allergic disease.

**The farm effect: when timing is critical**

Data from epidemiological studies provide ample evidence that early-life environmental exposure has a stronger effect on asthma pathogenesis. Human studies have suggested that \textit{in utero} exposure to the farming environment may protect against asthma, hay fever, and eczema\textsuperscript{35}. At birth the adaptive immune system is still immature and neonates have to rely on the innate immune system together with maternal antibodies for protection. The neonatal immune system is still skewed towards type-2 responses, as imposed \textit{in utero}, necessary to condition the maternal immune system and prevent rejection of the fetus. Furthermore, at the first gasp of breath an early wave of IL-33 has been reported in mice and intubated infants\textsuperscript{36,37}. Enhanced type 2 immune responses before the age of 2.5 years further form a strong risk factor for persistent childhood asthma\textsuperscript{38}. Interestingly, polymorphisms in the \texttt{IL33} gene or in the IL-33 receptor (\texttt{IL1RL1}, coding for ST2), are associated with childhood asthma and blood eosinophil count\textsuperscript{39}. Exposure to HDM during this period exacerbates the type-2 environment and accumulation of type-2 innate lymphoid cells (ILC2s), while suppressing the cytokine IL-12p35 in neonatal DCs, which could be responsible for allergic sensitization in early life\textsuperscript{36,40}. On the other hand, neonatal exposure to LPS abrogated the development of OVA-induced allergic airway inflammation\textsuperscript{41}.

It is becoming clear that time-dependent effects of endotoxin or farm exposure are strongest in at least two critical windows: 1.) prenatal and 2.) during early childhood. In most studies, this early childhood window refers to the first three weeks and first year of life, in mice and humans, respectively\textsuperscript{42}. A landmark study profiled blood from 100 newborn babies over the first three months of life. This longitudinal sample set enabled investigators to follow immune maturation during the neonatal period. They reported that the important factors shaping immune maturation in early life are microbial exposures both from the infant's
microbiota as well as from the direct living environment\textsuperscript{43}. These newly developed ideas have also been instrumental for a diametrical shift in guidelines recommending the timing of introduction of potential allergens. Indeed, children for whom allergenic foods were introduced early in life had significantly lower rates of both peanut and egg allergies at three years of age, compared to children from whom introduction was delayed\textsuperscript{44}. Where food exposure at a young age prevents from food allergy, a birth cohort study in the United States showed that exposure to high levels of bacterial content in household dust throughout the first year of life was associated with protection against atopy and wheeze\textsuperscript{45}. Along these lines Roduit \textit{et al.} have reported that high levels of the bacterial metabolites’ butyrate and propionate in the feces of one-year-old children is associated with protection against atopy \textsuperscript{46}. Together, these studies add to the notion that delaying exposure to potential allergens may be detrimental and increase the prevalence of allergies. This notion can be further extended to exposures such as farming environments and animal barns, unpasteurized milk, and daycare attendance, which seem more active when exposures occur in the first two years of life\textsuperscript{5}.

The protective effects of LPS exposure during childhood have been modelled in murine studies, however given the different asthma models used, the timing of the LPS treatment, and concentration of LPS used, the results are somewhat contradictory (reviewed in \textsuperscript{47}). We have shown that in mice the protective effects of LPS exposure could last until adulthood\textsuperscript{48,49}. Something that was also observed in human studies, where the protective effects of early farm exposure could persist into adulthood\textsuperscript{50}. Although it is difficult to exactly model the distinctions between early- and late-onset disease in humans, it should be noted that many patients present with their first onset symptoms of asthma or allergy in adult life.

\textbf{Concluding remarks and future perspectives}

The protective effect of microbial exposure is not a single factor-driven, but rather a complex interaction of microbiome, immunomodulatory effects of microbial components and timing, coming together in an individual with its own genetic make-up and lifestyle. Herein, prenatal and early-life exposure have significant implications on the risk of developing disease in childhood or later in life. LPS and CpG DNA can modulate the human immune system affecting Th2-like responses, however as a single-molecule they haven’t been able to fully recapitulate the farm effect found in human setting. It is likely the diverse composition of bacteria and
other microbes that make up the gut and lung microbiome, and the metabolites they produce that are necessary to optimally reduce disease risk. With this in mind Kirjavainen et al. distilled a protective ‘farm like’ cocktail of bacteria, including: low Streptococcaceae, and high Sphingobacetria, Clostridia, and Alphaproteobacteria\textsuperscript{11}.

Moving forward one can question whether there is going to be a ‘standardized’ microbial cocktail, to be used in all individuals at risk. As the ‘farm effect’ encompasses more than just microbial exposure, it is important to obtain a better mechanistic understanding on the role of the host genetic background in the host-microbiome cross-talk, to combat the asthma epidemic.

**List of abbreviations**

CpG: cytosine-guanine dinucleotide motifs, DC: Dendritic cell, GM-CSF: granulocyte-macrophage colony stimulating factor, LPS: lipopolysaccharide, ODNs: oligonucleotides, RSV: Respiratory Syncytial Virus, RV: Rhinovirus, SCFA: short-chain fatty acid, TLR: Toll-like receptor.

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**Conflicts of interest**

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1. Tang HHF, Teo SM, Sly PD, Holt PG, Inouye M. The intersect of genetics, environment, and microbiota in asthma-perspectives and challenges. J Allergy Clin Immunol 2021;147:781-93.
2. Lynch SV, Vercelli D. Microbiota, Epigenetics, and Trained Immunity. Convergent Drivers and Mediators of the Asthma Trajectory from Pregnancy to Childhood. Am J Respir Crit Care Med 2021;203:802-8.
3. Gilbert JA, Lynch SV. Community ecology as a framework for human microbiome research. Nat Med 2019;25:884-9.
4. Strachan DP. Hay fever, hygiene, and household size. Bmj 1989;299:1259-60.
5. von Mutius E, Smits HH. Primary prevention of asthma: from risk and protective factors to targeted strategies for prevention. The Lancet 2020;396:854-66.
6. Frew JW. The Hygiene Hypothesis, Old Friends, and New Genes. Front Immunol 2019;10:388.
7. Schoettler N, Rodríguez E, Weidinger S, Ober C. Advances in asthma and allergic disease genetics: Is bigger always better? J Allergy Clin Immunol 2019;144:1495-506.
8. Vercelli D. Microbiota and human allergic diseases: the company we keep. Curr Opin Immunol 2019;72:215-20.
9. Ober C, Sperling AI, von Mutius E, Vercelli D. Immune development and environment: lessons from Amish and Hutterite children. Curr Opin Immunol 2017;48:51-60.
10. Hrusch CL, Stein MM, Gozdz J, et al. T-cell phenotypes are associated with serum IgE levels in Amish and Hutterite children. J Allergy Clin Immunol 2019;144:1391-401.e10.
11. Kirjavainen PV, Karvonen AM, Adams RI, et al. Farm-like indoor microbiota in non-farm homes protects children from asthma development. Nature medicine 2019;25:1089-95.
12. Haahtela T, Laatikainen T, Alenius H, et al. Hunt for the origin of allergy - comparing the Finnish and Russian Karelia. Clin Exp Allergy 2015;45:891-901.
13. Ruokolainen L, Fyhrquist N, Laatikainen T, et al. Immune-microbiota interaction in Finnish and Russian Karelia young people with high and low allergy prevalence. Clin Exp Allergy 2020;50:1148-58.
14. Turkalj M, Drkulec V, Haider S, et al. Association of bacterial load in drinking water and allergic diseases in childhood. Clin Exp Allergy 2020;50:733-40.
15. Lee MK, Wyss AB, Carnes MU, et al. House dust microbiota in relation to adult asthma and atopy in a US farming population. J Allergy Clin Immunol 2021;147:910-20.
16. van Tilburg Bernardes E, Gutierrez MW, Arrieta MC. The Fungal Microbiome and Asthma. Front Cell Infect Microbiol 2020;10:583418.
17. Hammad H, Chieppa M, Perros F, Willart MA, Germain RN, Lambrecht BN. House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells. Nat Med 2009;15:410-6.
18. Kaur K, Bachus H, Lewis C, et al. GM-CSF production by non-classical monocytes controls antagonistic LPS-driven functions in allergic inflammation. Cell Rep 2021;37:110178.
19. Montamat G, Leonard C, Poli A, Klimek L, Ollert M. CpG Adjuvant in Allergen-Specific Immunotherapy: Finding the Sweet Spot for the Induction of Immune Tolerance. Front Immunol 2021;12:590054.
20. Patrick DM, Sbihi H, Dai DLY, et al. Decreasing antibiotic use, the gut microbiota, and asthma incidence in children: evidence from population-based and prospective cohort studies. Lancet Respir Med 2020;8:1094-105.
21. Adams RI, Bateman AC, Bik HM, Meadow JF. Microbiota of the indoor environment: a meta-analysis. Microbiome 2015;3:49.
22. Selway CA, Mills JG, Weinstein P, et al. Transfer of environmental microbes to the skin and respiratory tract of humans after urban green space exposure. Environ Int 2020;145:106084.
23. Depner M, Ege MJ, Cox MJ, et al. Bacterial microbiota of the upper respiratory tract and childhood asthma. Journal of Allergy and Clinical Immunology 2017;139:826-34. e13.
24. Wypych TP, Wickramasinghe LC, Marsland BJ. The influence of the microbiome on respiratory health. Nat Immunol 2019;20:1279-90.
25. Barberán A, Dunn RR, Reich BJ, et al. The ecology of microscopic life in household dust. Proc Biol Sci 2015;282.
26. Gomez de Agüero M, Ganal-Vonarburg SC, Fuhrer T, et al. The maternal microbiota drives early postnatal innate immune development. Science 2016;351:1296-302.
27. Depner M, Taft DH, Kirjavainen PV, et al. Maturation of the gut microbiome during the first year of life contributes to the protective farm effect on childhood asthma. Nat Med 2020;26:1766-75.
28. Gio-Batta M, Sjöberg F, Jonsson K, et al. Fecal short chain fatty acids in children living on farms and a link between valeric acid and protection from eczema. Scientific Reports 2020;10:22449.
29. Dang AT, Marsland BJ. Microbes, metabolites, and the gut-lung axis. Mucosal Immunol 2019;12:843-50.
30. Fujimura KE, Sitarik AR, Havstad S, et al. Neonatal gut microbiota associates with childhood multisensitized atopy and T cell differentiation. Nat Med 2016;22:1187-91.
31. Levan SR, Stamnes KA, Lin DL, et al. Elevated faecal 12,13-diHOME concentration in neonates at high risk for asthma is produced by gut bacteria and impedes immune tolerance. Nat Microbiol 2019;4:1851-61.
32. Cait A, Cardenas E, Dimitriu PA, et al. Reduced genetic potential for butyrate fermentation in the gut microbiome of infants who develop allergic sensitization. J Allergy Clin Immunol 2019;144:1638-47.e3.
33. Ruohutla T, de Goffau MC, Nieminen JK, et al. Maturation of Gut Microbiota and Circulating Regulatory T Cells and Development of IgE Sensitization in Early Life. Front Immunol 2019;10:2494.
34. Maslowski KM. Metabolism at the centre of the host-microbe relationship. Clin Exp Immunol 2019;197:193-204.
35. Douwes J, Cheng S, Travier N, et al. Farm exposure <em>in utero</em> may protect against asthma, hay fever and eczema. European Respiratory Journal 2008;32:603-11.
36. de Kleer IM, Kool M, de Bruijn MJW, et al. Perinatal Activation of the Interleukin-33 Pathway Promotes Type 2 Immunity in the Developing Lung. Immunity 2016;45:1285-98.
37. Pattaroni C, Watzenboeck ML, Schneidegger S, et al. Early-Life Formation of the Microbial and Immunological Environment of the Human Airways. Cell Host Microbe 2018;24:857-65.e4.
38. Thysen AH, Waage J, Larsen JM, et al. Distinct immune phenotypes in infants developing asthma during childhood. Sci Transl Med 2020;12.
39. Shrine N, Portelli MA, John C, et al. Moderate-to-severe asthma in individuals of European ancestry: a genome-wide association study. Lancet Respir Med 2019;7:20-34.
40. Steer CA, Martinez-Gonzalez I, Ghaedi M, Allinger P, Mathä L, Takei F. Group 2 innate lymphoid cell activation in the neonatal lung drives type 2 immunity and allergen sensitization. Journal of Allergy and Clinical Immunology 2017;140:593-5.e3.
41. Gao L, Wu M, Liu H, et al. Neonatal LPS Administered Before Sensitization Reduced the Number of Inflammatory Monocytes and Abrogated the Development of OVA-Induced Th2 Allergic Airway Inflammation. Front Immunol 2021;12:725906.

42. Lloyd CM, Saglani S. Opening the Window of Immune Opportunity: Treating Childhood Asthma. Trends Immunol 2019;40:786-98.

43. Olin A, Henckel E, Chen Y, et al. Stereotypic immune system development in newborn children. Cell 2018;174:1277-92. e14.

44. Perkin MR, Logan K, Tseng A, et al. Randomized Trial of Introduction of Allergenic Foods in Breast-Fed Infants. N Engl J Med 2016;374:1733-43.

45. Lynch SV, Wood RA, Boushey H, et al. Effects of early-life exposure to allergens and bacteria on recurrent wheeze and atopy in urban children. J Allergy Clin Immunol 2014;134:593-601.e12.

46. Roduit C, Frei R, Ferstl R, et al. High levels of butyrate and propionate in early life are associated with protection against atopy. Allergy 2019;74:799-809.

47. Zhu Z, Oh SY, Zheng T, Kim YK. Immunomodulating effects of endotoxin in mouse models of allergic asthma. Clin Exp Allergy 2010;40:536-46.

48. Schuijs MJ, Willart MA, Vergote K, et al. Farm dust and endotoxin protect against allergy through A20 induction in lung epithelial cells. Science 2015;349:1106-10.

49. Ding F, Liu B, Niu C, et al. Low-Dose LPS Induces Tolerogenic Treg Skewing in Asthma. Front Immunol 2020;11:2150.

50. Wlasiuk G, Vercelli D. The farm effect, or: when, what and how a farming environment protects from asthma and allergic disease. Curr Opin Allergy Clin Immunol 2012;12:461-6.