

Childhood allergies and asthma: New insights on environmental exposures and local immunity at the lung barrier

Hermelijn H Smits¹, Luciën EPM van der Vlugt^{1,2},
Erika von Mutius^{3,4} and Pieter S Hiemstra²



While certain bacteria and respiratory viruses promote local inflammation and disease onset, a more diverse colonization of the different species in the (gut) microbiome may be linked to more regulatory responses and protection against asthma and allergies. These processes are also influenced in part by food intake, both targeting the composition of the gut microbiome and influencing the immune system via metabolites. Early life environmental microbial exposure also contributes to protection against asthma and allergy and is linked with an early activation of the innate immune system and the development of regulatory immune responses. Although greater mechanistic insight is needed, it is tempting to speculate that part of the environmental effect can be explained by modulation of the microbiome composition at mucosal surfaces, epithelial barrier function and/or local immunity. A review of the latest studies is provided.

Addresses

¹ Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands

² Department of Pulmonology, Leiden University Medical Center, Leiden, The Netherlands

³ Dr von Hauner Children's Hospital, Ludwig Maximilians University of Munich, Munich, Germany

⁴ Comprehensive Pneumology Centre Munich (CPC-M), Member of the German Center for Lung Research, Germany

Corresponding author: Smits, Hermelijn H (h.h.smits@lumc.nl)

Current Opinion in Immunology 2016, 42:41–47

This review comes from a themed issue on **Allergy and hypersensitivity**

Edited by **James McCluskey** and **Robyn E O'Hehir**

<http://dx.doi.org/10.1016/j.coi.2016.05.009>

0952-7915/Published by Elsevier Ltd.

mucus-producing goblet cells and club cells. It not only acts as a selective physical barrier against inhaled components, but is also actively involved in host defense, inflammation and immunity [1]. This is illustrated by the ability of airway epithelial cells to mount robust antimicrobial responses, produce pro-inflammatory mediators and contribute to resolving inflammation. Airway epithelial cells are also actively involved in regulation of adaptive immunity, and may orchestrate Th2 immunity and tolerance in allergy and asthma by for example recruiting and activating dendritic cells (DC) and type 2 innate lymphoid cells (ILC2) [2]. Recent studies have highlighted a role for the epithelial barrier and its interaction with the innate immune system in preventing or reducing allergic and/or asthmatic symptoms [3,4]. An intact barrier layer reduces allergen passage, contributes to host defense against infection and enhances protective innate immune responses. Variations in several genes are associated with asthma and allergies, and a substantial number of these genes are specifically expressed in epithelial cells and contribute to their barrier function. In addition, a variety of environmental (e.g. air pollution, smoke, allergens and respiratory pathogens) and endogenous triggers (e.g. inflammation) may decrease or increase epithelial barrier function suggesting that gene-environment interactions are central to the role of epithelial cells in allergy and asthma.

In the past 50 years childhood asthma and allergies have markedly increased. It is suggested that this partly results from microbial changes in the environment. Here we will discuss recent studies on how these microbial changes may affect the barrier function and local immunity in the lung and the subsequent development of allergic airway diseases. These studies highlight the importance of early-life events by these environmental triggers, suggesting that there is a window of vulnerability for development of allergies and asthma which can be turned into a window of opportunity for early intervention aimed at the prevention of development of these diseases (see also [Figure 1](#)).

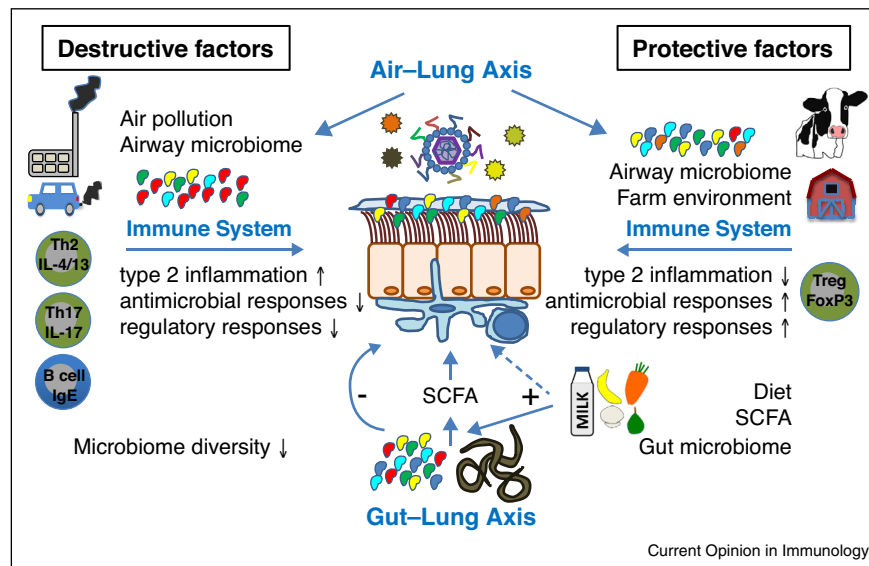
Introduction

Children with asthma and respiratory allergies suffer from symptoms of the upper and lower airways. Here, the airway epithelium is the first site of contact between inhaled allergens and the body, and is composed of various cell types, including basal cells, ciliated cells,

Role of respiratory infections and the airway microbiome

The conventional view that the larger part of the lung is sterile has recently been challenged especially due to the availability of high-throughput sequencing methods for analysis of microbes not captured by conventional culture

Figure 1



Early environmental factors shaping childhood asthma and allergy.

The basis for healthy lungs starts with an intact lung epithelial barrier, keeping pathogenic micro-organisms and allergens at bay and maintaining local immune homeostasis, in which innate antimicrobial responses and regulatory responses are well balanced. Early environmental factors can be distinguished that are of protective or destructive influence on the lung barrier. For example, exposure to air pollution, the presence of certain pathogenic bacteria in the lung microbiome, combined with a relative low abundance and richness of the gut microbiome are all associated with increased risks on early virus-induced wheeze, asthma and allergies. Part of this effect may be explained by an increased type 2 inflammation in the lung at the expense of innate anti-microbial responses and regulatory cells. In contrast, microbial exposure, including infections with worm parasites, during a critical early time window in life is associated with protection against asthma and allergies. Examples are found in the composition of the lung and the gut microbiome, the production of short chain fatty acids (SCFAs) in response to a high fiber diet, the consumption of milk, growing up on farms. These conditions are linked to reduced type 2 inflammation and more pronounced regulatory cells, possibly via the modification of lung DCs and enhanced antimicrobial responses.

techniques [5,6]. Several studies have revealed the presence of an airway microbiome, and showed that the composition of this microbiome is altered in chronic lung diseases such as asthma, chronic obstructive pulmonary disease (COPD) and cystic fibrosis. In both asthma and COPD, the airway microbiome is enriched with members of the Proteobacteria phylum (e.g. *Haemophilus* and *Pseudomonas* spp. [6], suggesting that dysregulation of the host-microbe relationship may be an important determinant for disease development. Interestingly, a study in severe asthma demonstrated that specific members of this airway microbiome are associated with specific asthma phenotypes [7]. To begin to understand their role in these processes, it is important to evaluate microbial presence in (early) childhood and review how these micro-organisms may help to shape (and derange) immune responses contributing to allergy and asthma development [8].

Early life respiratory tract infections with respiratory syncytial virus (RSV) and rhinovirus (RV) are of special interest because of their association with development of asthma [9,10]. Notably, these viruses target the airway epithelial cells directly and studies on RV have shown that airway epithelial cells from asthmatics are more

susceptible to RV infections due to decreased antiviral responses. In addition, RV infection of airway epithelial cells may contribute to Th2 immune responses by increasing epithelial production of innate cytokines such as IL-25 and IL-33 [11*,12**]. These observations help to explain the role of virus infections during and following asthma exacerbations. Interestingly, various studies also suggest a role for early viral infections to increase the risk to develop asthma, which has been further explored in experimental models. For example, when comparing neonatal mice to adult mice, RV infection caused more pronounced IL-13 and IL-25 production, mucus hypersecretion and airway hyperresponsiveness in neonatal mice [13]. These mechanisms were mediated by IL-25 as demonstrated by antibody-mediated blockade, and involved production of IL-13 by ILC2s. Although the authors noted that higher RV titers are needed in mice than in humans, and that RV replication is more pronounced in neonatal mice, this study provides new insight into the role of early-life infection in asthma development. Interestingly, parallel findings were recently reported in a mouse model of RSV infection, showing that neonatal RSV infection causes an IL-33-mediated induction of ILC2s and Th2 immunopathology that is

characteristic of RSV infection [14]. These observations of RV-mediated and RSV-mediated Th2 type inflammation in neonatal models are in line with for example the observation of high titers of IgE antibodies directed against house-dust mite (HDM) allergen in wheezing children infected with RV [15].

The first association between bacterial presence in the (upper) airways and development of recurrent wheeze, asthma and allergy was demonstrated in a birth cohort of children born to mothers with asthma [16]. Here, the presence of *Streptococcus pneumoniae*, *Haemophilus influenzae* or *Moraxella catarrhalis* in nasal samples from neonates was associated with increased risk of asthma diagnosis at the age of 5 years. Whereas this association can also be explained by a shared risk for wheeze, asthma and infection, other studies provided further evidence for such relationships. For example, defective antibody responses to *H. influenzae* and *S. pneumoniae* were reported in children that are sensitized to HDM [17], and characterization of their nasopharyngeal microbiome demonstrated that its composition was associated with infection spread to the lower airways, severity of inflammation, and — especially for *S. pneumoniae* — increased risk for asthma development [18**]. Importantly, early life viral infections may weaken airway host defenses resulting in infection with the above-mentioned opportunistic respiratory pathogens and as a consequence, may contribute to development of asthma. Indeed, interactions between bacterial and viral infections have been suggested, as in children aged 4–12 years the presence of RV in nasal samples increased the likelihood of detecting upper airway bacteria, and the detection of RV together with *M. catarrhalis* and *S. pneumoniae* increased the risk of asthma exacerbations [19].

Collectively these data provide evidence for interactions between respiratory viral and bacterial infections that are associated with or even may contribute to the development of (persistent) airways inflammation, allergy and/or asthma. As a result of such infections or altered colonization and the ensuing inflammatory process in the airways, the barrier function of the airway epithelium may be decreased resulting in increased penetration of allergens. Nevertheless, it is important to consider that the weakened antimicrobial defenses and an altered microbiome, as observed in cross-sectional studies, might also be a consequence of allergy and asthma instead of the cause.

Role of gut microbiome and the influence of diet

New studies, mostly in mouse models, have suggested that not only local interactions between the microbiome and the airway epithelium may affect the balance between health and disease in the lungs, but also microbe-host interactions at other, more distant mucosal areas, such as the gut and the skin. For example, the gut

microbiome maintains a symbiotic relationship with the host, interacts with the host's intestinal mucosal immune system and is critically involved in generating immune tolerance [20] and immune maturation, such as the formation of B cell memory [21]. Indeed, frequent use of antibiotics and subsequent dysbiosis early in life is for example associated with the development of allergic diseases and asthma [22]. Although this relationship may not be causal, it is tempting to speculate that part of the effect is mediated by a defect in the development of regulatory responses at mucosal surfaces. For example, LPS of *Bacteroides* (more abundant in westernized populations with an increased prevalence of inflammatory diseases) was less immunogenic compared to LPS of *Escherichia coli* and even strongly inhibited the innate immune signaling and endotoxin tolerance induced by *E. coli* LPS. Furthermore, it could not reduce type 1 diabetes in NOD mice, suggesting that a predominant colonization of LPS-silencing bacteria can affect certain aspects of immune education by microbiota and enhance the risk to develop inflammatory diseases [23**]. In addition, mucosal Treg cells were shown to be key players in the protection against allergic responses such as in the airways [24]. Furthermore, also IgE production is influenced by the microbiome as germ-free mice or mice with a low diversity microbiome developed elevated levels of serum IgE early in life [25]. Importantly, another explanation for the association between early life antibiotic prescription and childhood asthma may be provided by the early respiratory diseases for which these antibiotics were prescribed [26] and an impaired anti-viral immunity in children with a genetic 'risk' variant at chromosome 17q21 to develop asthma [27].

The microbiome not only consists of bacteria, but it is becoming increasingly clear that also other species like fungi, viruses and worm parasites are, or can be, part of this community in the gut. Changes in the abundance of (specific) gut bacteria will affect the relative abundance of those other species, or vice versa, and may have functional consequences for the final immune outcome. For example, antibiotic treatment with high doses of clindamycin and cefoperazone was shown to enhance type 2 responses and allergic airway inflammation as a consequence of Candida-induced PGE2 [28]. Alternatively, other gut microbiome members, like helminth species, can promote a higher abundance of gut bacteria and a higher degree of phyla variation [29,30]. It was demonstrated that experimental helminth infection can have direct protective effects against secondary challenges by respiratory viruses [31] or against allergic airway inflammation in mice [32]. Part of these effects are accomplished via the formation of regulatory cells [33] or by acting directly on the epithelium of the airways [34*]. In addition, a recent study suggested an additional and/or alternative mechanism via the modulation of the gut microbiome and their capacity to produce short chain fatty acids (SCFAs) [35].

SCFAs, such as propionate, butyrate and acetate, are metabolites produced by the selected species of gut bacteria that can be used as energy sources by host cells and the gut bacteria itself. Their production is influenced by food intake and diet-mediated changes in the gut microbiome. SCFA can also profoundly influence the host immune system, as demonstrated by their ability to directly induce Treg cells [36], influence DC precursors in the bone marrow compartment [37^{••}], or to increase (gut) epithelial barrier function (reviewed in [38]). The importance of these microbiome-derived SCFA for regulation of allergic airway inflammation was recently demonstrated in a mouse model by Trompette *et al.* [37^{••}]. The authors fed a high fiber diet to change the composition of the gut microbiome, resulting in enhanced production of SCFAs such as propionate. This led to accumulation of tolerized DC precursors in the lungs and increased protection against allergic airway inflammation [37^{••}].

Milk is one of the earliest dietary components to which neonates are exposed to. Breastmilk contains indigestible carbohydrate fibers and a wide range of fatty acids, which can impact the composition of the gut microbiome and its capacity to produce metabolites. Interestingly, drinking of unprocessed farm milk during the first year of life is strongly linked to protection against allergies and asthma [39], and the development of more Treg cells [40]. Part of this effect could be explained by higher levels of peptides in the whey fraction and unsaturated omega-3 fatty acids in farm milk compared to shop milk [41]. Although the composition of the microbiome has not been evaluated in these studies, it is tempting to suggest that at least part of the milk effect is due to modifications in gut microbiome.

Altogether, it can be concluded from mouse studies that the gut microbiome is dominant in shaping the maturation of the immature immune system and the development of mucosal tolerance in neonates and that the composition can be influenced by diet. An early habitation of the gut by a wide range of species, leading to a rich composition of the microbiome can positively influence the development of regulatory responses needed to prevent allergic responses at mucosal surfaces, such as in the airways. Studies are needed to investigate whether this concept can be extended to newborn children.

Environmental microbial exposure

Recent studies have highlighted a role for environmental microbial exposure in the education of the immature immune system and the development of sufficient regulatory immune responses in early life, and thus in the protection against allergies and asthma. Environmental microbial exposure may exert these effects through microbial colonization of microbial surfaces, or through other mechanisms. For example, growing up on traditional, family-based farms protects against allergies, asthma and early virus-triggered wheeze [42]. Part of this effect

was explained by the exposure to certain gram negative bacteria and fungi that were found in animal sheds [43]. We found an increased barrier resistance in cultured human primary bronchial epithelial cells (PBEC) in response to farm dust [44], suggesting that environmental microbial exposure — like on farms — can affect the epithelial barrier in the airways. In this context, it has been shown that chronic exposure to microbial compounds in farm dust reduces the innate cytokine production of epithelial cells via the enhanced expression of A20, an ubiquitin-modifying enzyme that inhibits production of pro-inflammatory mediators via attenuation of NF- κ B signaling. A single nucleotide polymorphism (SNP) in the A20-encoding gene of farm children was associated with protection against asthma risk [45[•]]. In addition, children with a SNP at the chromosome 17q21 locus have an increased risk to develop recurrent wheezing and asthma. In farm children with the ‘risk allele’, animal shed exposure was inversely associated with early virus-induced wheeze, suggesting that the 17q21 gene locus modulates susceptibility to environmental influences and virus infections on asthma development [46[•]]. The effect of this modulation is likely an altered maturation of the immune system, a process which has been linked to different expression of anti-inflammatory genes and genes that are associated with a risk for allergy and asthma, and these modifications are probably promoted by a farming environment [47,48].

Similar studies in Karelia at the Finish border with Russia (still a traditional and rural area), also suggested that a traditional lifestyle and more green areas around the house reduce the risk to develop allergic diseases [49,50]. A recent study among Finnish people pointed at the role of the skin microbiome, showing a more abundant presence of *Acinetobacter Iwoffii* in the skin microbiome of healthy individuals compared to allergic ones, which was correlated with a higher expression of anti-inflammatory molecules by their peripheral blood mononuclear cells [51[•]]. This bacterium was also isolated from farm cowsheds and was able to confer transmaternal protection against allergic airway inflammation in mice [52]. Furthermore, the link with development of immune regulatory processes by commensal bacteria in the skin was confirmed in a study where increased Treg cell development was found following commensal colonization of intact neonatal skin [53]. This effect was limited to a critical time window early in life, which might be explained by the fact that during this period the neonatal immune system is still immature and will develop in a more balanced way (both regulatory and inflammatory arms) by appropriate external signals from the environment. Exposure to the commensal flora is currently considered as one of the first and strongest signals in this perspective.

The mode of delivery also has a substantial impact on the first composition of the microbiome: children born by a caesarean (C)-section initially have a gut microbiome that

is more similar to the skin microbiome. A meta-analysis of studies comparing vaginal and caesarean deliveries, showed an increased risk to develop allergies and asthma during childhood for children born by C-sections [54]. Interestingly, the composition of the gut microbiome from children delivered by C-section could partially be restored by immediate exposure to maternal vaginal fluids after birth [55], but the long-term health impact of the restoration of the gut microbiome needs to be established. Questions are raised whether this risk is any different in elective versus post-labor C-sections, as the process of labor may 'stress' the immune system and form the first signal to drive immune activation [56]. A recent study suggested that in addition to the mode of delivery also the gestational age was contributing to immune maturation of circulating cord blood cells [57]. It was hypothesized that neonatal immune maturation is influenced by maternal hormonal changes that normally initiate labor. Immune maturation was less prominent in elective pre-labor deliveries by C-section compared to C-sections or natural births at a higher gestational age [57]. In addition, production of pro-inflammatory innate cytokines in response to exposure to various, but not all, TLR ligands was higher in whole blood cultures from African children in rural and semi-urban areas compared to Europeans [58]. Since previous studies have pointed at an inverse association with allergies and asthma and a high microbial environmental exposure in rural areas of developing countries [59], it would be interesting to study the role of an early development of innate immune responses in this context.

Altogether, these studies suggest that exposure to environmental microbes at early age is a determinant in the risk to develop asthma and allergies. Although part of this effect may be explained by the microbiome composition at mucosal surfaces and a shifted maturation of the (innate) neonatal immune system, several studies now also point at an additional role of maternal hormonal and immune 'stress' during birth in the maturation of the neonatal immune system.

Concluding discussion

Recent developments point to an important role of changes in early life epithelial barrier function and immune maturation in determining the risk of asthma and allergy development. These changes are driven by a variety of factors, including genetic, microbial, diet and environmental factors, potentially influencing the composition of the airway and gut microbiome and the chance to develop acute respiratory tract infections. Variables known to influence asthma and allergy development, including early life exposures, mode of delivery and diet have been shown or are likely to impact on epithelial barrier function and promotion of innate, regulatory and Th2 responses, and involve activation of ILC2s. It will be important to investigate how these findings on complex

gene-environment interactions at the epithelial surface can be translated into better prevention strategies for allergies and asthma. To this end, animal models could provide important information to further develop such strategies, but one of the difficulties in studying the role of a changed microbiome in disease development and progression is that mouse models pose specific problems. These include the fact that the murine airway and gut microbiome is markedly different from that of humans [60], and the notion that mice are relatively resistant to bacteria that readily colonize human airways such as *Staphylococcus aureus* [61]. As discussed in the previous paragraphs, despite these limitations animal models have provided important information on parallel pathways that are operative in mice and humans. These all point to the neonatal period as a decisive time window in which interaction with the microbiome or environmental microbes can modify the risk for asthma and allergy development.

Acknowledgements

HHS is supported by grants from the Lung Foundation Netherlands (#5.1.15.015) and ZonMW-VIDI (#91714352). EM is supported by a grant from Gottfried Wilhelm Leibniz Prize from German Research Foundation (DFG). PSH is supported by grants from the Lung Foundation Netherlands (#3.2.08.032, #3.2.11.009, 5.1.13.033), European Union (Marie Curie Actions Intra-European Fellowship #622815), Boehringer Ingelheim and Galapagos N.V.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Hiemstra PS, McCray PB Jr, Bals R: **The innate immune function of airway epithelial cells in inflammatory lung disease.** *Eur Respir J* 2015, **45**:1150-1162.
2. Hammad H, Lambrecht BN: **Barrier epithelial cells and the control of type 2 immunity.** *Immunity* 2015, **43**:29-40.
3. Georas SN, Rezaee F: **Epithelial barrier function: at the front line of asthma immunology and allergic airway inflammation.** *J Allergy Clin Immunol* 2014, **134**:509-520.
4. Loxham M, Davies DE, Blume C: **Epithelial function and dysfunction in asthma.** *Clin Exp Allergy* 2014, **44**:1299-1313.
5. Marsland BJ, Gollwitzer ES: **Host-microorganism interactions in lung diseases.** *Nat Rev Immunol* 2014, **14**:827-835.
6. Dickson RP, Erb-Downward JR, Martinez FJ, Huffnagle GB: **The microbiome and the respiratory tract.** *Annu Rev Physiol* 2016, **78**:481-504.
7. Huang YJ, Nariya S, Harris JM, Lynch SV, Choy DF, Arron JR *et al.*: **The airway microbiome in patients with severe asthma: associations with disease features and severity.** *J Allergy Clin Immunol* 2015, **136**:874-884.
8. Marsland BJ, Salami O: **Microbiome influences on allergy in mice and humans.** *Curr Opin Immunol* 2015, **36**:94-100.
9. Holt PG: **The mechanism or mechanisms driving atopic asthma initiation: the infant respiratory microbiome moves to center stage.** *J Allergy Clin Immunol* 2015, **136**:15-22.
10. Smits HH, Hiemstra PS, Prazeres da Costa C, Ege M, Edwards M, Garn H *et al.*: **Microbes and asthma: opportunities for intervention.** *J Allergy Clin Immunol* 2016, **137**:690-697.

11. Beale J, Jayaraman A, Jackson DJ, Macintyre JD, Edwards MR, Walton RP *et al.*: **Rhinovirus-induced IL-25 in asthma exacerbation drives type 2 immunity and allergic pulmonary inflammation.** *Sci Transl Med* 2014, **6**:256ra134.

IL-25 is an initiator and regulator of Th2 inflammation and contributes to asthma and allergy. This study demonstrates that rhinovirus infection increases IL-25 in nasal secretions and in cultured bronchial epithelial cells of asthmatics and healthy controls, and that this induction is stronger in asthmatics than in healthy subjects and related to markers of allergy.

12. Jackson DJ, Makrinioti H, Rana BM, Shamji BW, Trujillo-Torralbo MB, Footitt J *et al.*: **IL-33-dependent type 2 inflammation during rhinovirus-induced asthma exacerbations in vivo.** *Am J Respir Crit Care Med* 2014, **190**:1373-1382.

Whereas the ability of IL-33 to induce Th2 responses has been well studied in mouse and *in vitro* human models, this study shows that experimental infection of asthmatics with rhinovirus increases nasal levels of IL-33 and of the Th2 cytokines IL-4, IL-5 and IL-13 *in vivo*, and that this increase is related to symptom severity. Furthermore, the study shows that IL-33 release by rhinovirus-infected cultured bronchial epithelial cells increases production of Th2 cytokines by human T cells and ILC2s *in vitro*.

13. Hong JY, Bentley JK, Chung Y, Lei J, Steenrod JM, Chen Q *et al.*: **Neonatal rhinovirus induces mucous metaplasia and airways hyperresponsiveness through IL-25 and type 2 innate lymphoid cells.** *J Allergy Clin Immunol* 2014, **134**:429-439.
14. Saravia J, You D, Shrestha B, Jaligama S, Siefker D, Lee GI *et al.*: **Respiratory syncytial virus disease is mediated by age-variable IL-33.** *PLoS Pathog* 2015, **11**:e1005217.
15. Soto-Quiros M, Avila L, Platts-Mills TA, Hunt JF, Erdman DD, Carper H *et al.*: **High titers of IgE antibody to dust mite allergen and risk for wheezing among asthmatic children infected with rhinovirus.** *J Allergy Clin Immunol* 2012, **129**:1499-1505 e5.
16. Bisgaard H, Hermansen MN, Buchvald F, Loland L, Halkjaer LB, Bonnellykke K *et al.*: **Childhood asthma after bacterial colonization of the airway in neonates.** *N Engl J Med* 2007, **357**:1487-1495.
17. Hales BJ, Chai LY, Elliot CE, Pearce LJ, Zhang G, Heinrich TK *et al.*: **Antibacterial antibody responses associated with the development of asthma in house dust mite-sensitised and non-sensitised children.** *Thorax* 2012, **67**:321-327.
18. Teo SM, Mok D, Pham K, Kusel M, Serralha M, Troy N *et al.*: **The infant nasopharyngeal microbiome impacts severity of lower respiratory infection and risk of asthma development.** *Cell Host Microbe* 2015, **17**:704-715.

Microbiome analysis of the upper respiratory tract in the first year of life reveals that its composition affects risk for future asthma development, infection severity and pathogen spread to lower airways.

19. Kloepper KM, Lee WM, Pappas TE, Kang TJ, Vrtis RF, Evans MD *et al.*: **Detection of pathogenic bacteria during rhinovirus infection is associated with increased respiratory symptoms and asthma exacerbations.** *J Allergy Clin Immunol* 2014, **133**:1301-1307 1307.e1-3.
20. Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y *et al.*: **Induction of colonic regulatory T cells by indigenous *Clostridium* species.** *Science* 2011, **331**:337-341.
21. Lundell AC, Bjornsson V, Ljung A, Ceder M, Johansen S, Lindhagen G *et al.*: **Infant B cell memory differentiation and early gut bacterial colonization.** *J Immunol* 2012, **188**:4315-4322.
22. Vangay P, Ward T, Gerber JS, Knights D: **Antibiotics, pediatric dysbiosis, and disease.** *Cell Host Microbe* 2015, **17**:553-564.
23. Vatanen T, Kostic AD, d'Hennezel E, Siljander H, Franzosa EA, Yassour M *et al.*: **Variation in microbiome LPS immunogenicity contributes to autoimmunity in humans.** *Cell* 2016, **165**:842-853.

This study shows a molecular mechanism for why a dysbiosis in the gut microbiome may be associated to increased prevalence of inflammatory diseases such as allergy and autoimmunity. *Bacteroides* bacteria were more abundant in Russian Karelian people compared to Finnish Karelian people, who have a more westernized lifestyle. LPS from *Bacteroides* was less immunogenic compared to *E. coli* LPS and can even inhibit innate immune signaling and endotoxin tolerance by *E. coli* LPS. Furthermore, it could not decrease the development of type 1 diabetes in NOD mice,

suggesting that a predominant colonization of LPS-silencing bacteria may affect certain aspects of immune education.

24. Josefowicz SZ, Niec RE, Kim HY, Treuting P, Chinen T, Zheng Y *et al.*: **Extrathymically generated regulatory T cells control mucosal TH2 inflammation.** *Nature* 2012, **482**:395-399.
25. Cahenzli J, Koller Y, Wyss M, Geuking MB, McCoy KD: **Intestinal microbial diversity during early-life colonization shapes long-term IgE levels.** *Cell Host Microbe* 2013, **14**:559-570.
26. Almqvist C, Wettermark B, Hedlin G, Ye W, Lundholm C: **Antibiotics and asthma medication in a large register-based cohort study – confounding, cause and effect.** *Clin Exp Allergy* 2012, **42**:104-111.
27. Semic-Jusufagic A, Belgrave D, Pickles A, Telcian AG, Bakhsholiani E, Sykes A *et al.*: **Assessing the association of early life antibiotic prescription with asthma exacerbations, impaired antiviral immunity, and genetic variants in 17q21: a population-based birth cohort study.** *Lancet Respir Med* 2014, **2**:621-630.
28. Kim YG, Udayanga KG, Totsuka N, Weinberg JB, Nunez G, Shibuya A: **Gut dysbiosis promotes M2 macrophage polarization and allergic airway inflammation via fungi-induced PGE(2).** *Cell Host Microbe* 2014, **15**:95-102.
29. Lee SC, Tang MS, Lim YA, Choy SH, Kurtz ZD, Cox LM *et al.*: **Helminth colonization is associated with increased diversity of the gut microbiota.** *PLoS Negl Trop Dis* 2014, **8**:e2880.
30. Cantacessi C, Giacomini P, Croese J, Zakrzewski M, Sotillo J, McCann L *et al.*: **Impact of experimental hookworm infection on the human gut microbiota.** *J Infect Dis* 2014, **210**:1431-1434.
31. Scheer S, Krempel C, Kalfass C, Frey S, Jakob T, Mouahid G *et al.*: ***S. mansoni* bolsters anti-viral immunity in the murine respiratory tract.** *PLoS One* 2014, **9**:e112469.
32. McSorley HJ, Hewitson JP, Maizels RM: **Immunomodulation by helminth parasites: defining mechanisms and mediators.** *Int J Parasitol* 2013, **43**:301-310.
33. Hussaarts L, van der Vlugt LE, Yazdanbakhsh M, Smits HH: **Regulatory B-cell induction by helminths: implications for allergic disease.** *J Allergy Clin Immunol* 2011, **128**:733-739.
34. McSorley HJ, Blair NF, Smith KA, McKenzie AN, Maizels RM: **Blockade of IL-33 release and suppression of type 2 innate lymphoid cell responses by helminth secreted products in airway allergy.** *Mucosal Immunol* 2014, **7**:1068-1078.

This is the first study to show that helminth-derived molecules can prevent allergic airway inflammation in mice by direct interference with the function of airway epithelial cells and not only by acting on the regulatory arm of the immune system.

35. Zaiss MM, Rapin A, Lebon L, Dubey LK, Mosconi I, Sarter K *et al.*: **The intestinal microbiota contributes to the ability of helminths to modulate allergic inflammation.** *Immunity* 2015, **43**:998-1010.
 36. Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P *et al.*: **Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation.** *Nature* 2013, **504**:451-455.
 37. Trompette A, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N, Ngom-Bru C *et al.*: **Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis.** *Nat Med* 2014, **20**:159-166.
- The gut microbiome and its production of SCFAs is directly influenced by the diet. In this study the application of a high fiber diet resulted in modified and more tolerogenic DCs in the bone marrow compartment and because of that, to less allergic airway inflammation. These processes were dependent on the production of the SCFA propionate and the ligation of the G-protein coupled receptor GPR43.
38. Yamada T, Takahashi D, Hase K: **The diet-microbiota-metabolite axis regulates the host physiology.** *J Biochem* 2016, pii: mvw022.
 39. Loss G, Apprich S, Waser M, Kneifel W, Genuneit J, Buchele G *et al.*: **The protective effect of farm milk consumption on childhood asthma and atopy: the GABRIELA study.** *J Allergy Clin Immunol* 2011, **128**:766-773.

40. Lluís A, Depner M, Gaugler B, Saas P, Casaca VI, Raedler D *et al.*: **Increased regulatory T-cell numbers are associated with farm milk exposure and lower atopic sensitization and asthma in childhood.** *J Allergy Clin Immunol* 2014, **133**:551-559.
41. Brick T, Schober Y, Bocking C, Pekkanen J, Genuneit J, Loss G *et al.*: **omega-3 fatty acids contribute to the asthma-protective effect of unprocessed cow's milk.** *J Allergy Clin Immunol* 2016. pii: S0091-6749(15)01731-5.
42. von Mutius E: **The microbial environment and its influence on asthma prevention in early life.** *J Allergy Clin Immunol* 2016, **137**:680-689.
43. Ege MJ, Mayer M, Normand AC, Genuneit J, Cookson WO, Braun-Fahrlander C *et al.*: **Exposure to environmental microorganisms and childhood asthma.** *N Engl J Med* 2011, **364**:701-709.
44. Van der Vlugt LE, Eger K, Amatngalim GD, Müller C, Bracher F, Von Mutius E *et al.*: **Farm dust extract increases the epithelial barrier function of bronchial epithelial cells.** *Eur Respir J* 2015, **46**(Suppl. 59):PA901.
45. Schuijs MJ, Willart MA, Vergote K, Gras D, Deswarte K, Ege MJ *et al.*: **Farm dust and endotoxin protect against allergy through A20 induction in lung epithelial cells.** *Science* 2015, **349**:1106-1110.
This study provides a mechanistic explanation why a microbe-enriched environment, such as on traditional farms, can protect against allergic airway inflammation. Microbial stimulation, such as by endotoxin from gram-negative bacteria, reduces the capacity of epithelial cells to produce type 2 promoting innate cytokines by the upregulation of A20, an ubiquitin-modifying enzyme. Interestingly, a gene-by-environment interaction was detected for an A20 SNP that showed a protective effect in farm children against asthma development.
46. Loss GJ, Depner M, Hose AJ, Genuneit J, Karvonen AM, Hyvärinen A *et al.*: **The early development of wheeze: environmental determinants and genetic susceptibility at 17q21.** *Am J Respir Crit Care Med* 2015, **193**:889-897.
Genetic variations at the gene 17q21 are linked to a higher risk to develop asthma. In this study, it was shown that the same genetic variation at the gene 17q21 was linked to lower development of virus-induced wheeze in farm children. This suggests that depending on the environmental exposure the genetic susceptibility changes from an increased risk to develop asthma to an increased protection in particular in relationship to early respiratory virus infections.
47. Frei R, Roduit C, Bieli C, Loeliger S, Waser M, Scheynius A *et al.*: **Expression of genes related to anti-inflammatory pathways are modified among farmers' children.** *PLOS ONE* 2014, **9**:e91097.
48. Michel S, Busato F, Genuneit J, Pekkanen J, Dalphin JC, Riedler J *et al.*: **Farm exposure and time trends in early childhood may influence DNA methylation in genes related to asthma and allergy.** *Allergy* 2013, **68**:355-364.
49. Haahtela T, Laatikainen T, Alenius H, Auvinen P, Fyhrquist N, Hanski I *et al.*: **Hunt for the origin of allergy – comparing the Finnish and Russian Karelia.** *Clin Exp Allergy* 2015, **45**:891-901.
50. Ruokolainen L, von Hertzen L, Fyhrquist N, Laatikainen T, Lehtomäki J, Auvinen P *et al.*: **Green areas around homes reduce atopic sensitization in children.** *Allergy* 2015, **70**:195-202.
51. Fyhrquist N, Ruokolainen L, Suomalainen A, Lehtimäki S, Veckman V, Vendelin J *et al.*: **Acinetobacter species in the skin microbiota protect against allergic sensitization and inflammation.** *J Allergy Clin Immunol* 2014, **134**:1301-9.e11.
This study shows that not only the gut or the lung, but also the skin microbiome may influence immune maturation and the risk to develop allergic inflammation. Especially a rich composition and the presence of Acinetobacter species seems to be relevant in this context.
52. Conrad ML, Ferstl R, Teich R, Brand S, Blumer N, Yildirim AO, *et al.*: **Maternal TLR: signaling is required for prenatal asthma protection by the nonpathogenic microbe Acinetobacter lwoffii F78.** *J Exp Med* 2009, **206**:2869-2877.
53. Scharschmidt TC, Vasquez KS, Truong HA, Gearty SV, Pauli ML, Nosbaum A *et al.*: **A wave of regulatory T cells into neonatal skin mediates tolerance to commensal microbes.** *Immunity* 2015, **43**:1011-1021.
54. Thavagnanam S, Fleming J, Bromley A, Shields MD, Cardwell CR: **A meta-analysis of the association between Caesarean section and childhood asthma.** *Clin Exp Allergy* 2008, **38**:629-633.
55. Dominguez-Bello MG, De Jesus-Laboy KM, Shen N, Cox LM, Amir A, Gonzalez A *et al.*: **Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer.** *Nat Med* 2016, **22**:250-253.
56. Romero R, Korzeniewski SJ: **Are infants born by elective cesarean delivery without labor at risk for developing immune disorders later in life?** *Am J Obstet Gynecol* 2013, **208**:243-246.
57. Thyssen AH, Larsen JM, Rasmussen MA, Stokholm J, Bonnelykke K, Bisgaard H *et al.*: **Prelabor cesarean section bypasses natural immune cell maturation.** *J Allergy Clin Immunol* 2015, **136**:1123-5.e6.
58. Labuda LA, de Jong SE, Meurs L, Amoah AS, Mbow M, Ateba-Ngoa U *et al.*: **Differences in innate cytokine responses between European and African children.** *PLoS ONE* 2014, **9**:e95241.
59. Wammes LJ, Mpairwe H, Elliott AM, Yazdanbakhsh M: **Helminth therapy or elimination: epidemiological, immunological, and clinical considerations.** *Lancet Infect Dis* 2014, **14**:1150-1162.
60. Kostic AD, Howitt MR, Garrett WS: **Exploring host-microbiota interactions in animal models and humans.** *Genes Dev* 2013, **27**:701-718.
61. Knop J, Hanses F, Leist T, Archin NM, Buchholz S, Glasner J *et al.*: **Staphylococcus aureus infection in humanized mice: a new model to study pathogenicity associated with human immune response.** *J Infect Dis* 2015, **212**:435-444.