Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Immunology

The microbiome: toward preventing allergies and asthma by nutritional intervention

Olaf Perdijk and Benjamin J Marsland

Allergies and asthma have increased in prevalence over recent decades while the development of therapies to treat or prevent them has stagnated. Genetic predisposition and lifestyle changes influence the constituents of the microbiome and these host–environment–microbe interactions represent a key underlying pressure influencing disease susceptibility. Consequently, there has been a surge of interest in shaping the microbiome to a health-promoting state particularly through nutritional intervention strategies. However, mechanistic insights into the nutrition–microbe–host interplay are still needed in order for such approaches to succeed. In addition, little is known about how trans-kingdom interactions might influence disease susceptibility and progression. Future steps toward revealing the underlying mechanisms of host–microbe interactions will be pivotal for the development of effective dietary intervention strategies for the prevention and treatment of allergic diseases.

Address
Department of Immunology and Pathology, Central Clinical School, Monash University, Melbourne, VIC 3004, Australia

Corresponding authors: Perdijk, Olaf (Olaf.perdijk@monash.edu), Marsland, Benjamin J (Benjamin.marsland@monash.edu)

Current Opinion in Immunology 2019, **60**:10–18

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

Edited by **Pamela A Frischmeyer-Guerrerio** and **Joshua D Milner**

For a complete overview see the Issue and the Editorial

Available online 9th May 2019

https://doi.org/10.1016/j.coi.2019.04.001

Introduction

The prevalence of allergies and asthma has increased over the last few decades, now affecting 20–30% of the world's population [1]. Asthma is a multifaceted disease that is influenced by risk factors including genetics, upper respiratory tract infections in early life, atopy, and deficits in lung function [2]. In recent years, a common hypothesis has been that the rise in allergies and allergic asthma is due to lifestyle changes, particularly those that influence the microbiome such as diet, hygiene, and antibiotics [3]. Indeed, antibiotic usage in the first year of life, and thus disruption of the microbiota, is associated with episodes of wheeze, risk of exacerbations, reduced antiviral responses and increased hospital admissions [4]. Animal studies support this notion, as germ-free mice (lacking any microbiome) and young mice treated with antibiotics exhibit exaggerated allergic airway inflammatory responses [5,6]. The ongoing development of sequencing technologies and multi-omics approaches is facilitating the exploration and potential use of the microbiome in new preventive and therapeutic strategies. To date, nutritional interventions (e.g. prebiotics, probiotics or synbiotics) to alleviate or prevent allergic airway inflammation have been largely unsuccessful. In order to develop successful personal medicinal nutrition strategies, we require a thorough understanding of the interplay between nutrition, the intestinal microbiome and host immunity at different stages of life. In this review, we address recent advances in the microbiome field and speculate how manipulation of this complex ecosystem, by means of nutritional intervention, could lead to effective approaches to combat allergies and asthma.

Early life: a 'window of opportunity' for allergy and asthma prevention?

In utero

Viviparity gives the unique possibility to nurture and protect the infant during gestation. The mother's lifestyle is thereby inherently linked to the health of her infant. Mouse models show that colonization of the mother's intestine during gestation increases intestinal innate immune cell numbers and enhances expression of antimicrobial peptides in the intestine that protect the offspring against microbial-induced inflammation [7]. Antibiotic use during pregnancy induces dysbiosis of the maternal microbiome and was shown to increase the risk of allergic airway inflammation in the offspring [8]. Dietary interventions that aim to restore microbial 'harmony' during gestation could be beneficial for both mother and child. Epidemiological and clinical studies show that raw cow's milk exposure, supplementation with vitamin D, or omega-3 during pregnancy, all decrease the risk of developing asthma [9–11]. Although vitamin D levels *in utero* are associated with the presence of specific gut bacteria in the infant's stool in the first months of life [12] and omega-3 fatty acids shape microbiota composition in mice [13], it is to date unknown whether these dietary compounds affect asthma directly via modulation of the microbiota. Future work is required to elucidate the underlying mechanisms by which diet during gestation influences airway responses.

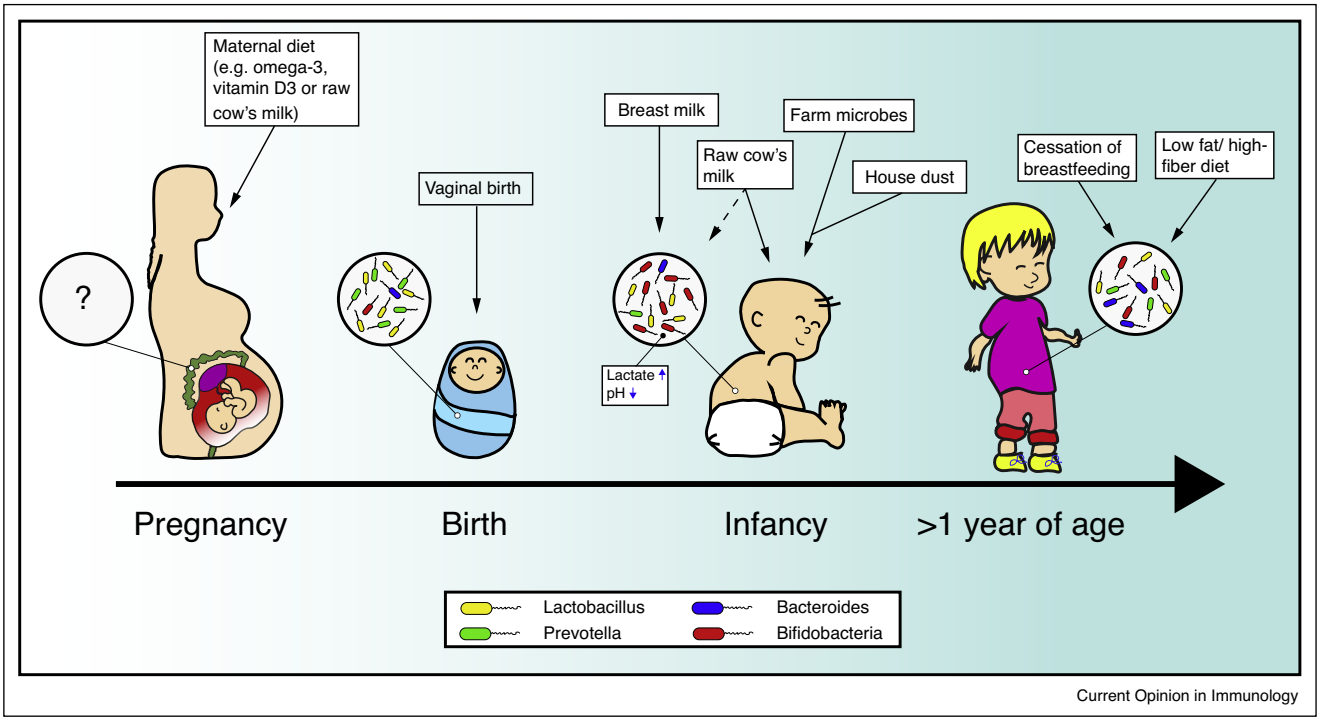
Vertical transmission of the microbiome upon delivery

Upon delivery, the neonate's mucosal surfaces are exposed to the maternal microbiome. Transmission of the microbiome between mother and newborn may have allowed for cospeciation between humans and the microbiome, which is a hallmark of the intimate symbiotic relationship with the bacteria residing in our gut [14]. The gut of vaginally delivered infants are seeded with the maternal vaginal and rectal microbiome and show a community dominated by *Lactobacillus* and *Prevotella* species directly after birth (Figure 1), while infants born by Caesarean section show a community resembling the mothers skin microbiota [15] and show a decreased prevalence of *Bacteroides* in the intestine during the first year of life [16]. It has been debated whether this differential seeding of the gut upon delivery has long-term consequences on the composition of the microbiota. It was recently reported that mode of delivery does not affect microbiota composition and function beyond the first six weeks of life [17]. Nevertheless, birth by caesarean section has been associated with a 20% increased risk of developing childhood asthma [18] and allergic rhinitis in children with a parental history of atopy [19]. Preliminary evidence of a small cohort showed that exposure of caesarean born neonates to maternal vaginal fluid at birth partially aligned their microbiome with vaginally born babies during the first month of life [20]; however, the implications of this microbial seeding are unknown.

Horizontal transmission in early life

The composition and function of the microbiome are highly variable between neonates and converges as it matures during the first years of life [21]. This exposure to microbes in early life plays a central, albeit not exclusive, role in maturing the immune system [22]. Studies indicate that there are discrete timeframes during development where signals from microbes are particularly important for driving immune development. Although it remains challenging to firmly define this timeframe, Olin *et al.* showed that immune cells in peripheral blood of neonates reaches adult-like phenotypes in the first three months of life [22]. A current view is that the neonate's immune system could be educated during this 'window of opportunity' by microbes and their constituents. Nutrition holds promise for the supplementation of microbes and shaping of microbiome composition, for example by prebiotic or probiotic. In early life, exposure

Figure 1



Life style factors and microbiota composition protecting against allergy and asthma.
Maternal diet during gestation, vaginal delivery, consumption of breast milk, early life exposures to raw cow's milk and endotoxin levels in house dust reduce the risk of developing allergy and asthma. The mother's vaginal and rectal microbiome and breast milk promote an ecosystem dominated by *Prevotella* and lactobacilli or bifidobacteria and lactobacilli, respectively. Cessation of breastfeeding and introduction of a diet rich in plant-derived carbohydrates and low in fat leads to a mature microbiome composition, which is characterized by high diversity, clustering by presence of *Prevotella* and elevated short chain fatty acid (e.g. propionate, butyrate) levels. At one year of age this more mature microbial profile is negatively associated with childhood asthma

to a farming environment is negatively associated with the development of allergies and asthma in childhood [23]. The consumption of raw cow's milk is, independently of potential confounding factors (e.g. sibling size), a consistent factor that shows strong negative associations with the prevalence of allergy and asthma [24–27]. It is uncertain whether this association can be attributed to the microbial community and their constituents present in raw cow's milk, its nutritional components (e.g. whey proteins) or both. The potential of raw cow's milk supplementation is highly topical; however, safety concerns rule out its use as a widespread nutritional supplement. A greater understanding of the mechanism of action of raw cow's milk could lead to strategies to minimally process raw milk to ensure it is safe, but efficacious; or alternatively, purification of the active components could open the door to more refined strategies.

The impact of farming environments was also recently illustrated in a study comparing Amish and Hutterite children. Both are agricultural communities in the U.S. with a similar genetic background but distinct farming practices. The Amish practice traditional farming, while the Hutterite live on industrialized communal farms. The Amish children showed a several fold lower allergic sensitization and asthma prevalence than Hutterite children. The fact that Amish were better protected was attributed to higher endotoxin levels in house dust that has been shown in mice to protect against allergic inflammation [28,29••]. Interestingly, infants exposed to higher endotoxin concentrations in house dust also show differences in gut microbiota composition, including a higher abundance of *Bifidobacterium* species [30]. Although the vast majority of studies have focused upon bacteria and their associated products, the microbial diversity, including both bacteria and fungi, is high on a farm and both are negatively associated with the risk of developing asthma [31]. The potential protective effects of fungi are unknown, with their presence typically being associated with infections or allergens; however, fungi could represent important parts of a healthy microbiome, and might hold potential as probiotics as do bacteria. The idea that not only bacteria, but also fungi and helminths play an important role in intestinal homeostasis is supported by mouse studies. Intestinal helminth infection with *Heligmosomoides polygyrus bakeri* (Hpb) was shown to change microbiota composition and function that attenuated allergic airway inflammation [32]. Intestinal dysbiosis of the microbiome after oral supplementation with antifungal drugs resulted in exacerbated airway inflammation [33]. One of the mechanisms through which intestinal fungi impacted airway inflammation was dependent on their sensing by gut-resident CX3CR1+ mononuclear phagocytes and Syk-dependent signalling in the cells [34]. Whether fungal dysbiosis was due to the depletion of keystone 'healthy' fungi, or a bloom of opportunistic fungi is unknown. Fungi can be pathobionts (e.g. *Candida*) that are abundant in children at high risk of developing asthma [35••]. Indeed, supplementation of antifungal drugs to mice also resulted in outgrowth of specific fungal pathobionts in the intestine that were linked with an exacerbation of allergic airway inflammation [33,34]. Thus, environmental microbial exposures and the establishment of a healthy intestinal trans-kingdom microbiome composition may be important factors contributing to protection or susceptibility to disease.

Shaping the microbiome — breast milk

The meconium is suggested to reflect the maternal bacterial community that is transmitted to the infant during gestation (e.g. by swallowing amniotic fluid) [17]. Although actual colonization of the foetus is unproven, early microbial communities rapidly expand and diversify in the intestine during the first weeks of life. Newborns at high risk of developing asthma, which was based on parental history and an array of clinical markers including total serum IgE levels, were found to have a distinct bacterial profile in the meconium as compared to healthy controls [36]. As a potential consequence, the fecal microbiota of neonates at high risk showed a delay in the expansion and diversification of the microbiota and a fecal metabolic profile depleted of specific anti-inflammatory lipids [36]. The authors showed that daily supplementation of the probiotic *Lactobacillus* strain LGG restored this metabolomic profile to one similar to healthy controls. Although the metabolic profile was no longer similar to healthy controls six month after the intervention compared to neonates at high risk of developing asthma, this study shows the potential of future dietary intervention strategies aimed very early in life. A powerful factor for shaping the neonate's gut microbiome is breast milk. Breast milk protects against gastrointestinal and respiratory infections, and prospective cohort studies report that it also reduces the risk of developing childhood asthma [38], although this is controversial [37]. This discrepancy has partly been attributed to variation in breast milk composition between women [39]. Human milk is unique among mammals for its extremely high oligosaccharide concentration compared to other mammals. These human milk oligosaccharides (HMO) are an important carbon source for the microbiota and are fermented in the colon. HMO shape microbiota composition during the first month of life into a bifidobacterial-dominated ecosystem. The utilization of HMO by specific *Bifidobacterium* species is tightly regulated and essential for the development of the ecosystem and function [40••]. Breast milk supports a community dominated by bifidobacteria and lactobacilli and creates a highly acidic environment composed of acetate and lactate [41]. It has been suggested that the acidification of the intestinal environment has decreased (i.e. pH increase) over the last century in breastfed infants [42]. Although its implications remain to be investigated, it could be indicative of changes in microbiome composition over generations that could have adverse consequences for health. Neonates at high

risk of developing multi-sensitized atopy at two years of age and doctors-diagnosed asthma at four years of age show a microbial community profile characteristically depleted of genera including *Bifidobacterium*, *Lactobacillus*, *Akkermansia* and *Faecalibacterium* [35••]. Cessation of breast feeding (i.e. introduction of solid food) induces drastic changes to the microbiota [41]. Moreover, the ecosystem shifts toward an adult-like state, as shown by a reduction in bacteria belonging to the genera *Bifidobacterium* and *Lactobaccilus*, while other genera such as *Bacteroides* increase [41]. Since the age of the neonate largely determines microbiota composition during the first year of life, the faecal microbiota composition of healthy neonates can be modelled to the age of neonates to visualize the maturation process over time [43••]. Interestingly, an 'immature' microbiota composition at one year of age was shown to increase the risk of allergic sensitization at five years of age in children from asthmatic parents [43••]. In line with this finding, higher faecal butyrate or propionate levels, which are indicative of consumption of plant-derived polysaccharides and maturation of the microbiota, at one year of age were associated with lower food and inhaled allergies at six years of age [44]. The immature microbiome signature induced by breast milk may be beneficial only during a special time window (Figure 1). Prebiotic supplementation (often a 9:1 mixture of galactooligosaccharides: fructooligosaccharides) to infant formula also stimulates the outgrowth of bifidobacteria and acidification of the intestinal milieu [45,46]. Although the use of these prebiotics induces a microbial pattern that is more similar to breast milk compared to non-fortified infant nutrition, they poorly reflect the high variety and concentrations of HMO. Therefore, there is an urgent need for fundamental research to decipher the complex interplay between milk glycans, the microbiome, metabolome and its consequences for the host. This will open up new avenues for specific fortification of infant nutrition that could benefit the neonate through to adulthood.

Can lifestyle changes impact microbiome composition and alleviate airway inflammation?

The bacterial community in adults shows long-term steady states based on clustering of the microbiota, so called enterotypes (i.e. three clusters dominated by the genus *Bacteroides*, *Prevotella* or *Firmicutes*) [47]. The *Bacteroides*-dominated enterotype is associated with a diet rich in animal protein and saturated fats, while the *Prevotella*-dominated enterotype is associated with a plant-derived carbohydrate-based diet [48,49,50••,51] (Figure 1). The intestinal microbial community in adults is highly resilient and returns to its original steady state after perturbations. Hence, for nutrition to have an impact on microbiota composition long-term dietary intervention strategies are required [48,52] and may need to be personalized depending on an individual's immunological, microbiological and genetic profile [53]. The notion that long-term dietary changes can induce changes in the microbiota was recently shown by studying hunters-gathers living in Tanzania. The activities of this Hadza tribe are largely based around food acquisition and their faecal microbiota composition and function showed annual cyclic reconfiguration. In the dry-season, the community was dominated by the family *Prevotellaceae* and showed a higher capacity to utilize carbohydrates compared to the wet-season [50••]. Cyclical microbial signatures similarly differentiate individuals from non-industrialized and industrial countries [49,50••]. Individuals from industrialized countries generally show a higher abundance of Verrucomicrobia and higher mucin degrading activity. Smits *et al.* [50••] provided evidence that drastic nutritional and lifestyle changes can induce shifts between two alternative stable states of the microbial ecosystem. It has been shown that shifts between these alternative states are maintained by several bacterial taxa [52]. These taxa may be essential for the functioning of the complete ecosystem. Therefore, targeting these taxa may allow radical shifts in microbiota composition into alternative states. Future research needs to investigate whether therapeutics or nutrition could target these taxa to restore the microbiota into its more favorable state. To date, the challenge is to identify underlying mechanisms that translate such microbial and functional shifts into personalized nutritional strategies that have health benefits.

Potential immunological mechanisms

Early life

The largely immature immune system of the neonate needs to be able to discriminate between harmful and harmless antigens. To prevent allergies it must mount regulatory responses to establish tolerance to allergens. The regulatory compartment of the immune system, particularly regulatory T cells (Treg cells), is essential to maintain tolerance against allergens. Recently, it was shown that allergic patients have Treg cells that are highly antigen-specific; however, they lack Treg cells to specific allergens. These results imply that early-life exposure to allergens is required to mount allergen-specific Treg cell responses [54••]. In early life, it was suggested that the immunoregulatory compounds and allergens in breast milk promote oral tolerance toward inhaled or ingested allergens [55]. Tolerance to food antigens is induced in the small intestine shortly after weaning [56]. Nevertheless, neonatal mice are not susceptible to ovalbumin-induced food allergy, supporting the notion that milk components can suppress allergic responses [56]. For instance, immunoglobulin (Ig) G:allergen complexes in maternal milk were shown to protect mouse pups from developing ovalbumin (OVA)-induced food allergy, which was mediated via uptake by dendritic cells (DC) in a neonatal Fc-receptor-dependent manner [57]. Similarly, the induction of a local regulatory milieu by exposure to homologous regulatory proteins (e.g. tumor growth factor (TGF) β, interleukin (IL) 10 or lactoferrin) present in cow's milk was postulated to be one of the mechanisms by which the consumption of cow's

milk in early life protects against the development of allergies and asthma [58–60]. In contrast to this hypothesis, high levels of the major house dust mite allergen, Der p 1, in breast milk is positively correlated with allergic sensitization in children five years old whose mother's had a family history of asthma [61]. Additionally, a farming environment activates peripheral innate immune responses in children [29••], which may oppose the idea that environmental exposures trigger immune regulatory responses. Indeed, breast milk and cow's milk contain microbes that could activate innate immune responses and shape microbiota composition [62]. Interestingly, *Veillonella*, and *Rothia* are bacterial genera that are transferred in breast milk, and are associated with a reduced risk of asthma [63]. In the context of allergy, it has been postulated that horizontal transfer of bacteria to the neonate shapes a long-lasting protective IgA repertoire that could be cross-reactive to allergens [64,65]. Bacteria (i.e. *Enterobacter cloacae* and *Streptococcus pneumoniae* [64,65]) known to induce protective cross-reactive antibodies in mouse models are also found in raw cow's milk [66]. Microbial exposure and the induction of a regulatory milieu may thereby go hand in hand to prevent allergen-specific T cells from becoming pathogenic T helper (Th) 2 cells.

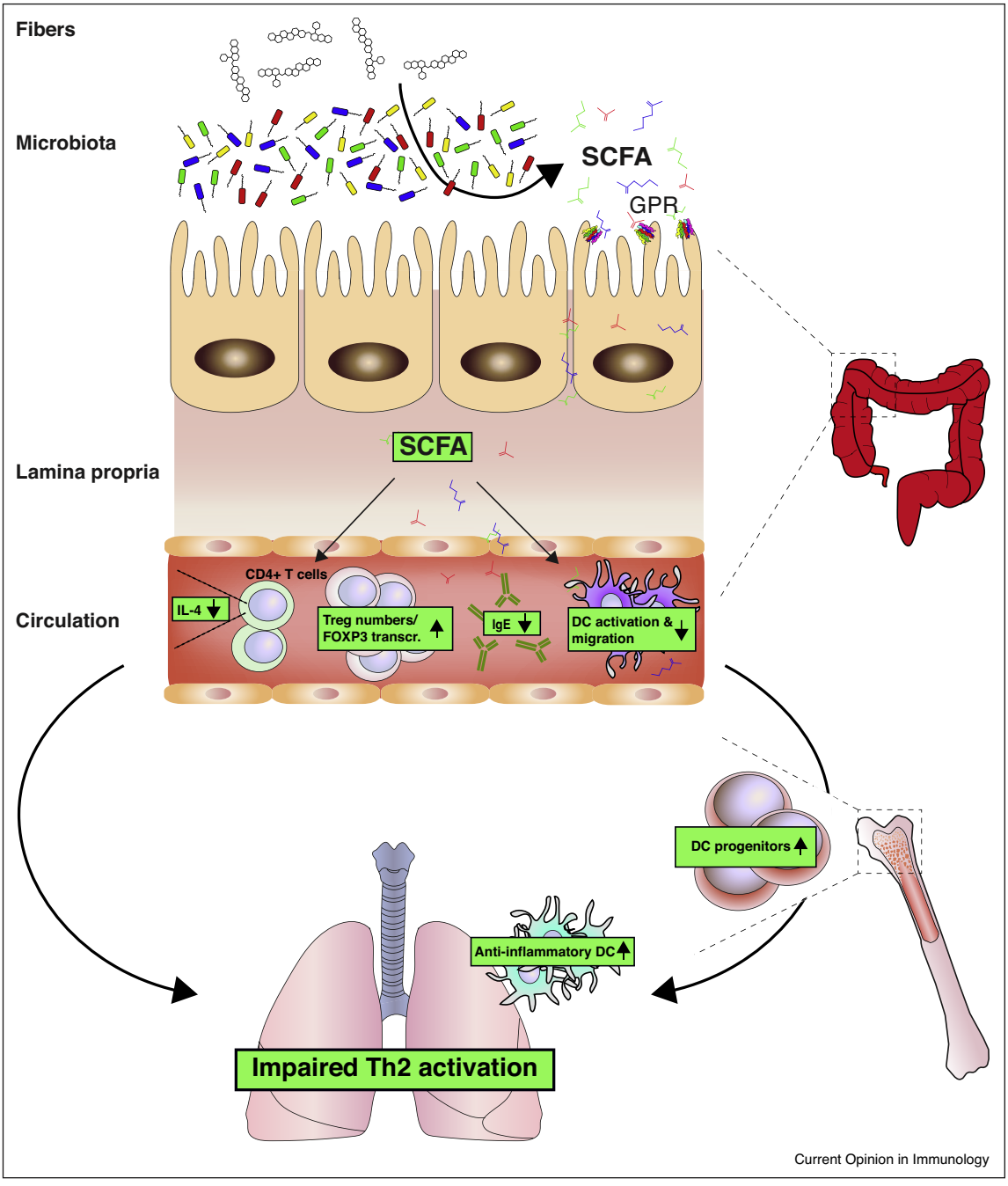
Fibers and allergic airway inflammation

A wealth of information has become available in the last decade showing that long-term dietary interventions with fibers and fat alter microbiota composition and function that affects host physiology [67]. Fibers are plant-derived complex carbohydrate structures that are fermented in the colon by the microbiota into metabolites. To date the best studied metabolites are short-chained fatty acids (SCFA). SCFA are small volatile molecules such as acetate, propionate and butyrate, which are present in high levels (20–100 mM) in the colon [68] and can also reach the circulation, albeit at lower concentrations [69]. The mechanistic evidence of the impact of fibers on allergic diseases comes from mouse models. These models show that a diet low in fibers changes the microbiota drastically — even at a phylum level. In mice fed a low-fiber diet, the Firmicutes/Bacteroidetes ratio was elevated, with a consequently lower production of SCFA [70]. Comparatively, a high fiber diet was shown to increase the abundance of Bacteroidetes and SCFA levels [70]. House dust mite (HDM)-induced asthma exacerbation, as seen in mice fed a low-fiber diet, can be rescued by supplementation with acetate or propionate [70]. Inducing microbial dysbiosis by vancomycin treatment reduces faecal and caecal SCFA levels and leads to exacerbated OVA-induced allergic airway inflammatory responses [71]. Conversely, chronic Hpb infection altered the commensal community and boosted the production of SCFA that protected against allergic asthma [32]. Immunological mechanisms through which fibers/SCFA alleviate airway inflammation are coming to light. A high fiber diet or SCFA supplementation dampens airway inflammation indirectly via modulating haematopoiesis in the bone marrow. In the bone marrow, macrophage-DC progenitors (MDPs) differentiate into monocytes or common DC precursors (CDPs). Propionate increases the number of MDPs and CDPs [70]. In the lung, these newly differentiated and recruited DC were present but expressed lower levels of costimulatory molecules and a reduced capacity to re-activate Th2 cells in a HDM-induced asthma model (Figure 2) [70]. In addition, it was shown that a mixture of acetate, propionate and butyrate directly dampens DC activation and migration, Th2 cell activation and circulating IgE levels in vancomycin-treated mice (Figure 2) [71]. Butyrate and propionate, but not acetate, have been shown to induce *de novo* Treg generation. These SCFA were shown to enhance Treg functioning and enhance the capacity of DC to support Treg differentiation through inhibition of histone deacetylases (HDAC) (Figure 2) [72]. The role of SCFA has been extensively researched in the last decade while the function of the vast majority of other metabolites is poor understood. However, recently the fecal metabolomic profile of neonates at high risk of developing childhood multi-sensitized atopy and asthma was shown to be different to low risk children [35••]. The functional consequences of such metabolic difference were illustrated by *in vitro* cultures of autologous DC:T cell cultures. DC that were exposed to faecal water of children at high risk of developing atopy and asthma showed higher IL-4 production and reduced Treg function, which was attributed to the metabolite 12,13 DiHOME [35••]. This example illustrates the importance of broadening the study of microbiome-derived metabolites in order to tap their potential for modulation of immune pathways underlying respiratory health.

Beyond allergy

This review has focused upon the relationship between intestinal microbiota composition and the development of asthma. Asthma is a multifaceted disease that underlies multiple immune pathways. Upper respiratory tract infections induced by viral (e.g. respiratory syncytial virus (RSV) or rhinovirus) infections can induce structural damage to the airways, result in severe wheezing and are also major risk factors for allergy and asthma [73]. Preventing the negative sequalae of respiratory viral infections with the aid of nutrition may thereby be an important means of preventing asthma exacerbation. For instance, the consumption of raw cow's milk in early life has been associated with reduced respiratory tract infections and fever [74]. Feeding mice a high fiber diet also confers protection against influenza via SCFAs [75]. One of the few mechanisms so far identified involved recruitment of Ly6c-patrolling monocytes from the bone marrow to the lung where they differentiated into alternatively activated macrophages. These macrophages had a limited capacity to recruit neutrophils to the site of inflammation, which

Figure 2



Mechanisms dampening allergic airway inflammation in mouse models.
Fibers escape enzymatic hydrolysis in the gut and are fermented in the colon into SCFA. SCFA are taken up into the circulation where they may reduce DC activation or enhance the generation of Treg and their function, which dampens Th2 pathology. SCFA are also shown to induce haematopoiesis of macrophage-DC progenitors and common DC precursors that seed the lung. These cells have enhanced phagocytic activity and are impaired in their capacity to activate Th2 cells.

dampened immunopathology. In parallel, SCFA boosted CD8+ T cell effector functioning by enhancing cellular metabolism [75]. Future studies should investigate if and how microbial-metabolites also protect against RSV and rhinovirus infections, two of the most common viruses linked with asthma. Modulation of the intestinal microbiome in early life could potentially educate the immune system to induce tolerance against allergens and in addition boost antiviral immunity — a two-pronged approach to confer protection against asthma.

Conclusions

Environmental and intestinal microbes play an important role in the development of our immune system in early life. After the first year of life the microbiome has reached a stable community structure that is more resilient to perturbations. Early life nutrition holds the promise to shape microbiome composition and consequently prevent allergic airway inflammation. Microbial changes are particularly apparent in genetically predisposed neonates, which indicates a crucial role for host genetics and the need for personalized strategies to target the microbiome (e.g. by means of nutrition). We know that specific microbial and nutritional exposures are associated with a reduced risk of developing allergy and asthma. The underlying mechanisms, however, remain poorly understood and *vice versa*, new mechanistic studies from mice need to be translated into clinical settings. The ongoing advances in sequencing technologies, annotation of microbial sequence databases, metabolomics and trans-kingdom interactions will aid in our understanding of how nutrition shapes microbial communities, their function and their impact upon the development of allergies and asthma.

Funding

BJM is a VESKI Innovation Fellow and NHMRC Senior Research Fellow.

Conflict of interest statement

Nothing declared.

References and recommended reading

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

- •• of outstanding interest

1. Pawankar R, Canonica GW, Holgate ST, Lockey RF: *World Allergy Organization (WAO) White Book on Allergy*. 2011.
2. Fuchs O, Bahmer T, Rabe KF, von Mutius E: **Asthma transition from childhood into adulthood**. *Lancet Respir Med* 2017, **5**:224-234.
3. Lambrecht BN, Hammad H: **The immunology of the allergy epidemic and the hygiene hypothesis**. *Nat Immunol* 2017, **18**:1076-1083.
4. Semic-Jusufagic A, Belgrave D, Pickles A, Telcian AG, Bakhsoliani E, Sykes A, Simpson A, Johnston SL, Custovic A: **Assessing the association of early life antibiotic prescription with asthma exacerbations, impaired antiviral immunity, and genetic variants in 17q: A population-based birth cohort study**. *Lancet Respir Med* 2014, **2**:621-630.
5. Herbst T, Sichelstiel A, Schär C, Yadava K, Bürki K, Cahenzli J, McCoy K, Marsland BJ, Harris NL: **Dysregulation of allergic airway inflammation in the absence of microbial colonization**. *Am J Respir Crit Care Med* 2011, **184**:198-205.
6. Russell SL, Gold MJ, Hartmann M, Willing BP, Thorson L, Wlodarska M, Gill N, Blanchet MR, Mohn WW, McNagny KM *et al.*: **Early life antibiotic-driven changes in microbiota enhance susceptibility to allergic asthma**. *EMBO Rep* 2012, **13**:440-447.
7. de Agüero MG, Ganal-Vonarburg SC, Fuhrer T, Rupp S, Uchimura Y, Li H, Steinert A, Heikenwalder M, Hapfelmeier S, Sauer U: **The maternal microbiota drives early postnatal innate immune development**. *Science* 2016, **351**:1296-1302.
8. Stensballe LG, Simonsen J, Jensen SM, Bønnelykke K, Bisgaard H: **Use of antibiotics during pregnancy increases the risk of asthma in early childhood**. *J Pediatr* 2013, **162**:832-838.
9. Lee-Sarwar K, Litonjua AA: **As you eat it: effects of prenatal nutrition on asthma**. *J Allergy Clin Immunol Pract* 2018, **6**:711-718.
10. Ege MJ, Bieli C, Frei R, van Strien RT, Riedler J, Üblagger E, Schram-Bijkerk D, Brunekreef B, van Hage M, Scheynius A *et al.*: **Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children**. *J Allergy Clin Immunol* 2006, **117**:817-823.
11. Riedler J, Braun-Fahrländer C, Eder W, Schreuer M, Waser M, Maisch S, Carr D, Schierl R, Nowak D, von Mutius E: **Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey**. *Lancet* 2001, **358**:1129-1133.
12. Sordillo JE, Zhou Y, McGeachie MJ, Ziniti J, Lange N, Laranjo N, Savage JR, Carey V, O'connor G, Sandel M: **Factors influencing the infant gut microbiome at age 3-6 months: findings from the ethnically diverse Vitamin D Antenatal Asthma Reduction Trial (VDAART)**. *J Allergy Clin Immunol* 2017, **139**:482-491.
13. Kaliannan K, Wang B, Li X, Kim K, Kang JX: **A host-microbiome interaction mediates the opposing effects of omega-6 and omega-3 fatty acids on metabolic endotoxemia**. *Sci Rep* 2015, **5**:11276.
14. Moeller AH, Caro-Quintero A, Mjungu D, Georgiev AV, Lonsdorf EV, Muller MN, Pusey AE: **Cospeciation of gut microbiota with hominids**. *Science* 2016, **353**:380-382.
15. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, Knight R: **Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns**. *Proc Natl Acad Sci U S A* 2010, **107**:11971-11975.
16. Bokulich NA, Chung J, Battaglia T, Henderson N, Jay M, Li H, Lieber AD, Wu F, Perez-Perez GI, Chen Y *et al.*: **Antibiotics, birth mode, and diet shape microbiome maturation during early life**. *Sci Transl Med* 2016, **8**:343ra82.
17. Chu DM, Ma J, Prince AL, Antony KM, Seferovic MD, Aagaard KM: **Maturation of the infant microbiome community structure and function across multiple body sites and in relation to mode of delivery**. *Nat Med* 2017, **23**:314-326.
18. 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.
19. Pistiner M, Gold DR, Abdulkerim H, Hoffman E, Celedón JC: **Birth by cesarean section, allergic rhinitis, and allergic sensitization among children with a parental history of atopy**. *J Allergy Clin Immunol* 2008, **122**:274-279.
20. Dominguez-Bello MG, De Jesus-Laboy KM, Shen N, Cox LM, Amir A, Gonzalez A, Bokulich NA, Song SJ, Hoashi M, Rivera-Vinas JI *et al.*: **Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer**. *Nat Med* 2016, **22**:250.
21. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, Magris M, Hidalgo G, Baldassano RN, Anokhin AP *et al.*: **Human gut microbiome viewed across age and geography**. *Nature* 2012, **486**:222-227.
22. Olin A, Henckel E, Chen Y, Lakshmikanth T, Pou C, Mikes J, Gustafsson A, Bernhardsson AK, Zhang C, Bohlin K *et al.*: **Stereotypic immune system development in newborn children**. *Cell* 2018, **174**:1277-1292.
23. von Mutius E, Vercelli D: **Farm living: effects on childhood asthma and allergy**. *Nat Rev Immunol* 2010, **10**:861-868.
24. Lluis A, Depner M, Gaugler B, Saas P, Casaca VI, Raedler D, Michel S, Tost J, Liu J, Genuneit J *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.

25. Keshav S, Husband AJ, Gleeson M, Onsrud M, Thorsby E, Perry M, Whyte A, Breel M, van der Ende MB, Sminia T *et al.*: **Subpopulations of non-lymphoid cells in bronchus associated lymphoid tissue and lung of the mouse**. *Science* 2012, **6**:220-221.

26. Wickens K, Lane JM, Fitzharris P, Siebers R, Riley G, Douwes J, Smith T, Crane J: **Farm residence and exposures and the risk of allergic diseases in New Zealand children**. *Allergy* 2002, **57**:1171-1179.

27. Wyss AB, House JS, Hoppin JA, Richards M, Hankinson JL, Long S, Henneberger PK, Beane Freeman LE, Sandler DP, O'Connell EL *et al.*: **Raw milk consumption and other early-life farm exposures and adult pulmonary function in the Agricultural Lung Health Study**. *Thorax* 2018, **73**:279-282.

28. Ungar R, Leona M, Helmsley HB, Trust C, Gitlitz J, Foundation A, John L, Schwartz V, Palisades P, Markovitz A *et al.*: **farm dust and endotoxin protect against allergy through A20 induction in lung epithelial cells**. *Science* 2015, **349**:1106-1110.

29. •• Stein MM, Hrusch CL, Gozdz J, Igartua C, Pivniouk V, Murray SE, Ledford JG, Marques dos Santos M, Anderson RL, Metwali N *et al.*: **Innate immunity and asthma risk in Amish and Hutterite farm children**. *N Engl J Med* 2016, **375**:411-421.

This study investigates the underlying differential susceptibility to allergy and asthma between Amish and Hutterite children — two agricultural communities in the U.S. with similar genetic background but distinct farming practices. The Amish were postulated to better protected due to higher exposure of endotoxins in house dust.

30. Sjögren YM, Jenmalm MC, Böttcher MF, Björkstén B, Sverremark-Ekström E: **Altered early infant gut microbiota in children developing allergy up to 5 years of age**. *Clin Exp Allergy* 2009, **39**:518-526.

31. Ege MJ, Mayer M, Normand A-C, Genuneit J, Cookson WO, Braun-Fahrländer C, Heederik D, Piarroux R, von Mutius E: **Exposure to environmental microorganisms and childhood asthma**. *N Engl J Med* 2011, **364**:701-709.

32. Zaiss MM, Rapin A, Lebon L, Dubey LK, Mosconi I, Sarter K, Piersigilli A, Menin L, Walker AW, Rougemont J *et al.*: **The intestinal microbiota contributes to the ability of helminths to modulate allergic inflammation**. *Immunity* 2015, **43**:998-1010.

33. Wheeler ML, Limon JJ, Bar AS, Leal CA, Gargus M, Tang J, Brown J, Funari VA, Wang HL, Crother TR *et al.*: **Immunological consequences of intestinal fungal dysbiosis**. *Cell Host Microbe* 2016, **19**:865-873.

34. Li X, Leonardi I, Semon A, Doron I, Gao IH, Putzel GG, Kim Y, Kabata H, Artis D, Fiers WD *et al.*: **Response to fungal dysbiosis by gut-resident CX3CR1+ mononuclear phagocytes aggravates allergic airway disease**. *Cell Host Microbe* 2018, **6**:847-856.

35. •• Fujimura KE, Sitarik AR, Havstad S, Lin DL, Levan S, Fadrosh D, Panzer AR, Lamere B, Rackaityte E, Lukacs NW *et al.*: **Neonatal gut microbiota associates with childhood multi-sensitized atopy and T-cell differentiation**. *Nat Med* 2016, **22**:1187.

Longitudinal birth cohort study investigating the association between neonatal microbiome composition and risk of childhood multi-sensitized atopy and asthma. The authors show distinct abundances of faecal microbial and fungal genera and metabolome profiles in babies at high risk. *Ex vivo* cocultures of DC:T cells suggested that this microbiome dysbioisis drives CD4+ T-cell dysfunction.

36. Durack J, Kimes NE, Lin DL, Rauch M, McKean M, McCauley K, Panzer AR, Mar JS, Cabana MD, Lynch SV: **Delayed gut microbiota development in high-risk for asthma infants is temporarily modifiable by *Lactobacillus* supplementation**. *Nat Commun* 2018, **9**:707.

37. Victora CG, Bahl R, Barros AJ, França GVA, Horton S, Krasevec J, Murch S, Jeeva Sankar M, Walker N, Rollins NC: **Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect**. *Lancet* 2016, **387**:475-490.

38. Klopp A, Vehling L, Becker AB, Subbarao P, Mandhane PJ, Turvey SE, Lefebvre DL, Sears MR, Daley D, Silverman F: **Modes of infant feeding and the risk of childhood asthma: a prospective birth cohort study**. *J Pediatr* 2017, **190**:192-199.

39. Munblit D, Korsunskiy I, Asmanov A, Hanna H: **The role of breastfeeding and weaning practices in allergic disease development**. *Curr Allergy Clin Immunol* 2017, **30**:76-81.

40. •• Matsuki T, Yahagi K, Mori H, Matsumoto H, Hara T, Tajima S, Ogawa E, Kodama H, Yamamoto K, Yamada T *et al.*: **A key genetic factor for fucosyllactose utilization affects infant gut microbiota development**. *Nat Commun* 2016, **7**:11939.

The authors investigated factors driving microbiota composition during the first months of life. The authors show that the presence of the human milk oligosaccharide fucosyllactose and fucosyllactose-utilizing bacteria are essential to shape microbial community composition and function.

41. Bäckhed F, Roswall J, Peng Y, Feng Q, Jia H, Kovatcheva-Datchary P, Li Y, Xia Y, Xie H, Zhong H *et al.*: **Dynamics and stabilization of the human gut microbiome during the first year of life**. *Cell Host Microbe* 2015, **17**:690-703.

42. Henrick BM, Hutton AA, Palumbo MC, Casaburi G, Mitchell RD, Underwood MA, Smilowitz JT, Frese SA: **Elevated fecal pH indicates a profound change in the breastfed infant gut microbiome due to reduction of *Bifidobacterium* over the past century**. *mSphere* 2018, **3**:e00041-18.

43. •• Stokholm J, Blaser MJ, Thorsen J, Rasmussen MA, Waage J, Vinding RK, Schoos A-MM, Kunøe A, Fink NR, Chawes BL: **Maturation of the gut microbiome and risk of asthma in childhood**. *Nat Commun* 2018, **9**:141.

This study investigates if microbiota composition during the first year of life associates with risk of developing asthma at age 5. The authors showed that a more immature microbial composition at one year of age correlates with increased risk of developing asthma at age 5 in children born to asthmatic mothers.

44. Roduit C, Frei R, Ferstl R, Loeliger S, Westermann P, Rhyner C, Schiavi E, Barcik W, Rodriguez-Perez N, Wawrzyniak M *et al.*: **High levels of butyrate and propionate in early life are associated with protection against atopy**. *Allergy* 2018.

45. Sierra C, Bernal MJ, Blasco J, Martínez R, Dalmau J, Ortuño I, Espín B, Vasallo MI, Gil D, Vidal ML *et al.*: **Prebiotic effect during the first year of life in healthy infants fed formula containing GOS as the only prebiotic: a multicentre, randomised, double-blind and placebo-controlled trial**. *Eur J Nutr* 2015, **54**:89-99.

46. Braegger C, Chmielewska A, Decsi T, Kolacek S, Mihatsch W, Moreno L, Pieścik M, Puntis J, Shamir R, Szajewska H *et al.*: **Supplementation of infant formula with probiotics and/or prebiotics: a systematic review and comment by the ESPGHAN committee on nutrition**. *J Pediatr Gastroenterol Nutr* 2011, **52**:238-250.

47. Costea PI, Hildebrand F, Arumugam M, Blaser MJ, Bushman FD, Vos WM, De, Ehrlich SD, Fraser M, Hattori M, Huttenhower C *et al.*: **Enterotypes in the landscape of gut microbial community composition**. *Nat Microbiol* 2018, **3**:8.

48. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen Y, Keilbaugh SA, Bewtra M, Knights D, Walters WA, Knight R *et al.*: **Linking long-term dietary patterns with gut microbial enterotypes**. *Science* 2011, **334**:105-109.

49. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G, Lionetti P: **Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa**. *Proc Natl Acad Sci U S A* 2010, **107**:14691-14696.

50. •• Smits SA, Leach J, Sonnenburg ED, Gonzalez CG, Lichtman JS, Reid G, Knight R, Manjurano A, Changalucha J, Elias JE *et al.*: **Seasonal cycling in the gut microbiome of the Hadza hunter-gatherers of Tanzania**. *Science* 2017, **55**:9557-9561.

This study longitudinally studied microbiota composition and function of Hadza hunter-gatherers of Tanzania for more than a year. The authors showed cyclic annual reconfiguration that resembled faecal microbial differences observed between individuals living in industrialized and non-industrialized countries. The changes in microbial composition and function suggest that drastic nutritional and lifestyle changes can induce shifts between two alternative stable states of the microbial ecosystem.

51. De Filippis F, Pellegrini N, Vannini L, Jeffery IB, La Storia A, Laghi L, I Serrazanetti D, Di Cagno R, Ferrocino I, Lazzi C *et al.*: **High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome**. *Gut* 2016, **65**:1812-1821.

52. Lahti L, Salojärvi J, Salonen A, Scheffer M, De Vos WM: **Tipping elements in the human intestinal ecosystem**. *Nat Commun* 2014, **5**:4344.

53. Zeevi D, Korem T, Zmora N, Israeli D, Rothschild D, Weinberger A, Ben-Yacov O, Lador D, Avnit-Sagi T, Lotan-Pompan M: **Personalized nutrition by prediction of glycemic responses**. *Cell* 2015, **163**:1079-1094.

54. •• Bacher P, Heinrich F, Stervbo U, Nienen M, Vahldieck M, Iwert C, Vogt K, Kollet J, Babel N, Sawitzki B *et al.*: **Regulatory T cell specificity directs tolerance versus allergy against aeroantigens in humans**. *Cell* 2016, **167**:1067-1078.
The authors-enriched peripheral antigen-specific T cell to investigate the Treg cell repertoire to aeroallergens in allergic donors. Treg cells of allergic patients showed no dysfunctional impairments but lacked allergen-specificity. These findings highlight the importance of inducing allergen-specific tolerance to combat allergy.

55. Verhasselt V: **Oral tolerance in neonates: from basics to potential prevention of allergic disease**. *Mucosal Immunol* 2010, **3**:326-333.

56. Kim KS, Hong SW, Han D, Yi J, Jung J, Yang BG, Lee JY, Lee M, Surh CD: **Dietary antigens limit mucosal immunity by inducing regulatory T cells in the small intestine**. *Science* 2016, **351**:858-863.

57. Ohsaki A, Venturelli N, Buccigrosso TM, Osganian SK, Lee J, Blumberg RS, Oyoshi MK: **Maternal IgG immune complexes induce food allergen-specific tolerance in offspring**. *J Exp Med* 2018, **215**:91-113.

58. Perdijk O, van Splunter M, Savelkoul HFJ, Brugman S, van Neerven RJJ: **Cow's milk and immune function in the respiratory tract: potential mechanisms**. *Front Immunol* 2018, **9**:143.

59. den Hartog G, Savelkoul HFJ, Schoemaker R, Tijhaar E, Westphal AH, de Ruiter T, van de Weg-Schrijver E, van Neerven RJJ: **Modulation of human immune responses by bovine interleukin-10**. *PLoS One* 2011, **6**:e18188.

60. Perdijk O, van Neerven RJJ, van den Brink E, Savelkoul HFJ, Brugman S: **Bovine lactoferrin modulates dendritic cell differentiation and function**. *Nutrients* 2018, **10**:848.

61. Baïz N, Macchiaverni P, Tulic MK, Rekima A, Annesi-Maesano I, Verhasselt V: **Early oral exposure to house dust mite allergen through breast milk: a potential risk factor for allergic sensitization and respiratory allergies in children**. *J Allergy Clin Immunol* 2017, **139**:369-372.

62. Pannaraj PS, Li F, Cerini C, Bender JM, Yang S, Rollie A, Adisetiyo H, Zabih S, Lincez PJ, Bittinger K *et al.*: **Association between breast milk bacterial communities and establishment and development of the infant gut microbiome**. *JAMA Pediatr* 2017, **90095**:647-654.

63. Arrieta M-C, Stiemsma LT, Dimitriu PA, Thorson L, Russell S, Yurist-Doutsch S, Kuzeljevic B, Gold MJ, Britton HM, Lefebvre DL *et al.*: **Early infancy microbial and metabolic alterations affect risk of childhood asthma**. *Sci Transl Med* 2015, **7**:307ra152.

64. Patel PS, King RG, Kearney JF: **Pulmonary α-1,3-glucan-specific IgA-secreting B cells suppress the development of cockroach allergy**. *J Immunol* 2016, **197**:3175-3187.

65. Patel PS, Kearney JF: **Neonatal exposure to pneumococcal phosphorylcholine modulates the development of house dust mite allergy during adult life**. *J Immunol* 2015, **194**:5838-5850.

66. Quigley L, O'Sullivan O, Stanton C, Beresford TP, Ross RP, Fitzgerald GF, Cotter PD: **The complex microbiota of raw milk**. *FEMS Microbiol Rev* 2013, **37**:664-698.

67. Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F: **From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites**. *Cell* 2016, **165**:1332-1345.

68. Tan J, McKenzie C, Potamitis M, Thorburn AN, Mackay CR, Macia L: **The role of short-chain fatty acids in health and disease**. *Adv Immunol* 2014, **121**:91-119.

69. Wolever TMS, Josse RG, Leiter LA, Chiasson JL: **Time of day and glucose tolerance status affect serum short-chain fatty acid concentrations in humans**. *Metabolism* 1997, **46**:805-811.

70. Trompette A, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N, Ngom-Bru C, Blanchard C, Junt T, Nicod LP, Harris NL *et al.*: **Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis**. *Nat Med* 2014, **20**:159-166.

71. Cait A, Hughes MR, Antignano F, Cait J, Dimitriu PA, Maas KR, Reynolds LA, Hacker L, Mohr J, Finlay BB *et al.*: **Microbiome-driven allergic lung inflammation is ameliorated by short-chain fatty acids**. *Mucosal Immunol* 2017.

72. Arpaia N, Campbell C, Fan X, Dikiy S, Van Der J, Rudensky AY: **Metabolites produced by commensal bacteria promote peripheral regulatory T cell generation**. *Nature* 2013, **504**:451-455.

73. Jartti T, Gern JE: **Role of viral infections in the development and exacerbation of asthma in children**. *J Allergy Clin Immunol* 2017, **140**:895-906.

74. Loss G, Depner M, Ulfman LH, Joost van Neerven RJ, Hose AJ, Genuneit J, Karvonen AM, Hyvärinen A, Kaulek V, Roduit C *et al.*: **Consumption of unprocessed cow's milk protects infants from common respiratory infections**. *J Allergy Clin Immunol* 2015, **135**:56-62.

75. Trompette A, Gollwitzer ES, Pattaroni C, Lopez-Mejia IC, Riva E, Pernot J, Ubags N, Fajas L, Nicod LP, Marsland BJ: **Dietary fiber confers protection against Flu by shaping Ly6c — patrolling monocyte hematopoiesis and CD8 + T cell metabolism**. *Immunity* 2018, **48**:992-1005.