



Microbiota and Type 2 immune responses

Kathy D McCoy¹, Aline Ignacio^{1,2} and Markus B Geuking³

The trillions of microbes that colonize mucosal surfaces are critical for educating the immune system and microbial-derived signals continually shape and set the tone of immune responses. Although Type 2 immune responses are important for mediating protection from helminth infection they also underlie atopy and allergy. Microbes modulate Type 2 immune responses through effects on Type 2 cytokines, dendritic cells and regulatory T cells. Microbial colonization in the gut, the lung and the skin during an early and critical time period in immune development appears to be of particular importance for tolerance induction and regulation of aberrant Type 2 immune responses. This is illustrated by studies showing microbial alterations in early life that are associated with allergies later in life.

Addresses

¹ Department of Physiology and Pharmacology, Snyder Institute for Chronic Diseases, Cumming School of Medicine, University of Calgary, Calgary, Alberta T2N 4N1, Canada

² Laboratory of Transplantation Immunobiology, Institute of Biomedical Sciences, Department of Immunology, University of São Paulo, São Paulo, SP, Brazil

³ Department of Microbiology, Immunology and Infectious Diseases, Snyder Institute for Chronic Diseases, Cumming School of Medicine, University of Calgary, Calgary, Alberta T2N 4N1, Canada

Corresponding author: McCoy, Kathy D (kathy.mccoy@ucalgary.ca)

Current Opinion in Immunology 2018, 54:20–27

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

Edited by **Onur Boyman, Alexander Eggel and Mario Noti**

<https://doi.org/10.1016/j.coi.2018.05.009>

0952-7915/© 2018 Elsevier Ltd. All rights reserved.

Introduction

The human body is home to trillions of microbes that play a critical role in educating the immune system. This microbiota is a complex community of microorganisms that includes bacteria, archaea, fungi, viruses (including bacteriophages), and protozoa species [1]. All mucosal surfaces, including the gastrointestinal (GI) tract, skin, oral mucosa and lung, are colonized by microbes, although the colon of the GI tract exhibits the highest density and complexity. Microbial colonization is a dynamic process throughout the first years of life that

is heavily influenced by external factors, such as mode of birth, diet, breast vs formula feeding, and antibiotic use, and ultimately leads to relatively stable microbial community in adults. The microbiome plays a dominant role in shaping development of the immune system and helps to regulate and fine-tune immune responses. Disturbances in microbial composition, termed dysbiosis, are associated with many chronic diseases including allergic disease, Inflammatory Bowel Disease (IBD), cancer, and autoimmunity (reviewed in [2]). Although microbial dysbiosis could be a consequence of disease, it is also possible that alterations in the microbiota could occur prior to disease onset and may even be a primary driver of disease pathogenesis. In this short review, we discuss recent murine and human studies that provide insight into the role of the microbiota in inducing, regulating, or preventing Type 2 immune responses. We highlight key studies from the past two years and discuss how they have advanced our view of the pervasive interactions of the microbiota and Type 2 immune responses, particularly during the early life ‘window of opportunity’.

Microbes and regulation of Type 2 immune responses

Type 2 immune responses are often mounted at mucosal surfaces, in keeping with their protective role in defense from helminth infection and their involvement in wound healing. Many innate and adaptive immune cells are involved in orchestrating Type 2 immune responses, including epithelial cells, Type 2 innate lymphoid cells (ILC2), dendritic cells, macrophages, eosinophils, mast cells, basophils and CD4⁺ T helper (Th2) cells. In addition, a variety of cytokines, such as Interleukin-4 (IL-4), IL-5, IL-13, IL-9, IL-33, IL-25 (IL17E), and thymic stromal lymphopoietin (TSLP), are involved in promoting or mediating Type 2 immune responses. During the last few years it has become increasingly clear that the commensal microbes that colonize the gut, lung, and skin mediate strong regulatory effects on Type 2 immune responses. A clear example of this is the observation that in the absence of microbes, germ-free or antibiotic-treated mice have increased Type 2 immune responses with elevated levels of IL-4, circulating basophils, serum IgE and susceptibility to allergy [3–6]. This indicates that microbes are required to control Type 2 immunity although the precise cellular and molecular pathways involved in such regulation have not yet been fully elucidated. However, one pathway is likely via microbial control of induced regulatory T cells (iTreg), which then dampen the induction of Type 2 immunity. Microbial colonization induces intestinal regulatory T cells [7–9], predominantly through production of the short chain fatty

acids butyrate and propionate [10–12], and these iTreg appear to be critical for control of Type 2 immune responses. In support of this, mice deficient in the intronic *Foxp3* enhancer Conserved Noncoding Sequence 1 (CNS1) do not generate microbially induced iTreg and consequently develop increased Type 2 responses and mucosal inflammation in both the GI tract and the lung [13]. Targeted deletion of the autophagy gene *Atg16l1* in T cells led to a decrease in intestinal Treg and an expansion in Th2 cells leading to aberrant intestinal Type 2 immune responses to both dietary and microbial antigens, illustrating a role for autophagy in regulating Treg and Th2 responses [14]. In the lung, the microbiota is critical to promote tolerance to allergens through the induction of Programmed Death Ligand 1 (PD-L1)-dependent iTreg [15]. More in-depth analysis revealed that microbial-induced iTreg can also express the transcription factor retinoic acid receptor-related orphan nuclear receptor γ (ROR γ t) and iTreg co-expressing FoxP3 and ROR γ t are required to control Type 2 immune responses [16]. However, there is also evidence that microbially induced Treg differ from those induced by food. In a landmark study, Kim *et al.* demonstrated that dietary antigens induce Treg in the small intestine and it is these Treg that are responsible to control immune responsiveness to food antigens [17].

Microbial control of Type 2 immune responses is not only mediated through the induction of Treg and likely involves multiple cell types, including epithelial cells, dendritic cells, innate lymphoid cells (ILC) and other innate cell types. The systemic increase in propionate that occurs as a result of microbial fermentation of fiber inhibits lung dendritic cells from promoting Type 2 immune responses and protects from allergic airway inflammation [18]. Despite their critical role in promoting Type 2 immune skewing (reviewed in [19]), a role for microbes in the control of Type 2 innate lymphoid cells (ILC2) is still scarce. Equal numbers of ILC2 in the lungs of germ-free and colonized mice during homeostasis suggest their recruitment may not require microbial signals [20]. However, microbial control of intestinal macrophages and ILC3 is involved in Treg induction and is required to induce tolerance and limit reactivity to food antigens [21]. Further studies are likely to reveal microbial regulation of IL-33, IL-25 and TSLP, which will impact on Type 2 immune responses at mucosal sites.

Microbial colonization in early life and regulation of Type 2 immunity

Early life development of the gut microbiota and maturation of the immune system occur in parallel, cross regulate each other, and are highly susceptible to modulation by external factors. The current opinion suggests that alterations in the gut microbiome during this dynamic period in early life results in profound consequences on health later in life. For example, microbial

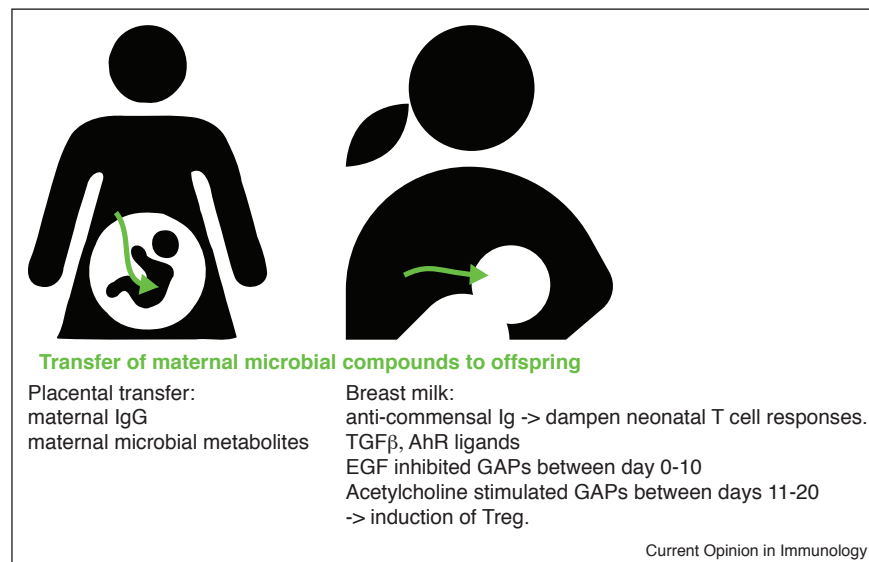
dysbiosis with reductions in four bacterial genera at 3 months of age was associated with an increased risk to develop asthma at one year of age [22] and in a small twin study intestinal dysbiosis with overgrowth of *Ruminococcus gnavus* was predictive of allergy development [23]. Similar results were found in a study of children in rural Ecuador where microbial dysbiosis at 3 months of age was associated with later development of atopic wheeze, although in this cohort fungal dysbiosis was more prominent than bacterial dysbiosis [24]. Fujimura *et al.* found that out of three compositionally distinct neonatal gut microbiotas identified in a US birth cohort, those children that harbored a gut microbiota with lower relative abundance of key bacteria, identified as *Bifidobacterium*, *Akkermansia*, and *Faecalibacterium*, and increased abundance of some fungi, had the highest risk for developing atopy against multiple food and aeroallergens at 2 years of age [25]. This neonatal gut microbiota was also found to be enriched for pro-inflammatory metabolites that decreased the proportion of Treg cells and increased the proportion of CD4⁺IL-4⁺ T (Th2) cells and secreted IL-4 in an *in vitro* T cell stimulation assay, suggesting that the functional effect of early life microbial dysbiosis may be mediated by alterations in T cell populations [25]. Alterations in invariant natural killer T (iNKT) cells producing IL-13 with potential cytotoxic effects on intestinal epithelial cells (IECs) have been identified in ulcerative colitis (UC) patients [26]. Although this observation does not necessarily suggest a causal role for these cells, germ-free mice have increased iNKT cells numbers in both the colon and lung compared to specific pathogen-free (SPF) mice and accumulation of these cells was only suppressed if the mice were colonized prior to weaning [27], suggesting the importance of early microbiota exposure. Similar to UC patients, the enhanced iNKT cells numbers in germ-free mice conferred increased susceptibility to colitis and allergic asthma [27,28]. In mice, neonatal iNKT cell numbers could be controlled by microbiota-induced epigenetic modification of the gene encoding for the chemokine ligand CXCL16 [27] or through providing inhibitory bacterial glycosphingolipids that inhibit iNKT cell activation and proliferation independent of CXCL16 [28]. A similar requirement for microbial colonization within this critical window in early life has also been illustrated for control of IgE. Preventing the high IgE observed in germ-free mice required neonatal colonization with a diverse microbial consortia [3]. Furthermore, vancomycin treatment of neonatal mice induces significant alterations in gut microbiota composition and increases eosinophil infiltration, antigen-specific IgE levels and airway hyper-responsiveness in OVA-challenged mice, effects that are not observed in adult mice also treated with vancomycin [29]. In agreement with these findings, postnatal exposure to antibiotics increases the risk for asthma in children [30]. A recent study has also illustrated that early life exposure to a more diverse microbial environment led to

increased microbial diversity in adulthood and decreased lung inflammation in a mouse model of OVA-induced asthma [31]. Similarly, the neonatal microbiome is also likely to be important to protect from early sensitization to food allergens and tolerance to skin commensals (discussed below).

No discussion of early life would be complete without reference to the role of the mother and breast milk (Figure 1). In a recent study, we found that metabolites derived from the maternal microbiota can be transferred to the offspring via the milk and across the placenta where they stimulate the early development of the innate immune system [32^{••}]. Breast milk is also an important source of anti-commensal immunoglobulins that function to dampen neonatal T cell responses during early life [33^{••}]. Breast milk also provides transforming growth factor (TGF)- β and transfer of TGF- β 1 from mother to child through breast milk has a significant beneficial impact on allergy prevention during infancy [34]. Mice that are lactated by OVA-exposed dams developed oral tolerance mediated by induction of interferon-gamma (IFN γ)-producing CD4⁺ T cells via a mechanism dependent on transfer of TGF- β to offspring [35]. Interestingly however, in this model oral tolerance could not be induced during the first week of life [36]. During this early time, activation of neonatal T cells is normally

suppressed by the presence of maternal antibodies and regulatory T cells [33^{••},37]. Surprisingly however, oral tolerance could be rescued by supplementation with Vitamin A, which led to increased DC expression of RALDH with subsequent Th1 differentiation and IFN γ production [36]. Although these data would suggest a beneficial role for Vitamin A supplementation in early life, others have shown that neonatal supplementation with Vitamin A is associated with increased atopy in Guinean girls [38,39]. The induction of tolerance to gut commensals may also be limited to a time-limited period early in life. Knoop and colleagues found that exposure of the immune system to antigens from the gut lumen was very limited between days 0 and 10 after birth because the amount of bacteria during this time was low and the presence of high levels of epidermal growth factor (EGF) in the breast milk inhibited development of goblet cell-associated antigen passages (GAPs) [40^{••}]. GAPs then developed from days 11 to 20 in response to acetylcholine, leading to induction of antigen-specific regulatory T cells. Finally, the high microbial load post weaning again inhibited GAPs, limiting further exposure to luminal antigens. Although this study specifically investigated induction of an anti-commensal tolerance, it will be interesting to investigate whether tolerance to other luminal antigens could also be affected by the temporal expression of GAPs.

Figure 1



Maternal transfer of microbial products/metabolites. Metabolites derived from the maternal microbiota can be transferred to the offspring across the placenta and via the milk (green arrows) to stimulate early development of the innate immune system. Breast milk is also an important source of anti-commensal immunoglobulins that function to dampen neonatal T cell activation. Breast milk also provides transforming growth factor β (TGF β), which has a beneficial impact on allergy prevention during infancy as well as aryl hydrocarbon receptor (AhR) ligands. Tolerance induction towards commensals may also be limited to a period early in life. Exposure of the immune system to antigens from the gut lumen is very limited between days 0 and 10 after birth because the load of bacteria during this time is low and the presence of high levels of epidermal growth factor (EGF) in the breast milk inhibits development of goblet cell-associated antigen passages (GAPs). GAPs then develop from days 11 to 20 in response to acetylcholine, leading to induction of antigen-specific regulatory T cells (Treg).

Impact of microbiota on food allergy

Food allergy has been increasing dramatically over the past several years and is considered to occur due to a breakdown in oral tolerance pathways. Altered exposure to microbes, especially early in life, is likely to be an important modifier of food allergy susceptibility (for a recent review see [41]). Recent reports in both human and animal models continue to support this view. For example, the early life gut microbiome in children sensitized or allergic to egg was recently reported to differ significantly from unaffected children, with predicted differential purine metabolism [42^{*}]. An early gut microbiome may even predict resolution of food allergy as the gut microbiota composition at 3–6 months of age, specifically the enrichment of Clostridia and Firmicutes, was found to associate with the resolution of milk allergy at the age of 8 years [43^{*}]. Furthermore, a recent case control study of the fecal microbiome of food allergic children, their siblings and healthy children revealed differences in microbial compositions, with alterations in Clostridia driving the separation of food allergic children from the control groups [44^{*}]. Animal models support these associations in humans and provide further insight into the putative mechanisms involved. Earlier studies in mice illustrated the importance of Toll-like receptor 4 (TLR4) signaling in susceptibility to food allergy [45] and that reconstitution of germ-free mice with a donor food allergic microbiota increased disease susceptibility when challenged with the allergen [46]. Germ-free mice or mice treated with antibiotics prior to weaning developed higher levels of peanut-specific IgE and IgG1 antibodies in a food allergy sensitization model but were protected when supplemented with a mixture of Clostridia species [47]. In this model, a microbiota containing Clostridia was able to induce Treg, IgA and IL-22, which reinforced the intestinal barrier and decreased allergen access to the bloodstream [47]. A high fiber diet, which increased acetate and butyrate levels, enhanced retinal dehydrogenase activity in CD103⁺ DC, and boosted IgA, T follicular helper (Tfh) cells and germinal center reactions, was able to improve induction of oral tolerance and prevent food allergy [48]. In this model, protection for food allergy was dependent on Vitamin A, Treg, and the G-protein coupled receptors GPR43 and GPR109a. These recent studies illustrate how specific bacterial species in early life can provide metabolites that change the immune landscape resulting in changes in tolerance induction and susceptibility to develop food allergy. An area of intense interest is the link between microbes, food allergy and atopic dermatitis (AD).

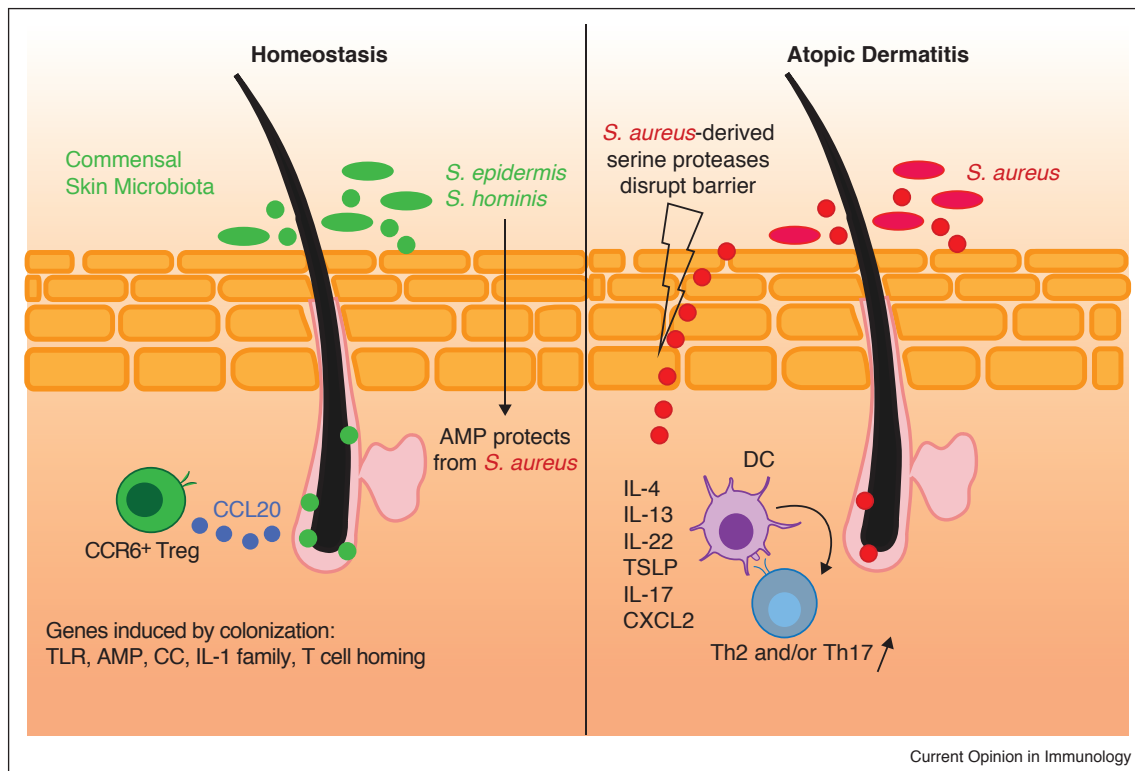
Commensal bacteria, skin immunity, and the atopic march

Atopy is associated with increased immune responses to environmental allergens alongside high concentrations of IgE that can lead to pathologies at a variety of anatomical sites. The first manifestation of atopy is often the

development of AD early in life and then subsequently followed by allergic rhinitis, allergic asthma, and food allergy in a developmental trajectory referred to as atopic march. The observation that AD is positively associated with atopic disease at other sites of the body points to the skin as an important site for allergic sensitization. A recent study identified a fecal microbiome signature in children with AD that could discriminate between the presence or absence of food allergy [49] but a role for the skin microbiota at the first stages of the atopic march is also likely. The skin is the natural habitat for a diverse population of bacteria whose colonization begins at birth and continues postnatally driven by environmental exposure (reviewed in [50]). Composition of the skin microbiome differs greatly from that found in the gut but also differs between skin sites dependent on the local environment, such as temperature, humidity, pH, and the presence of lipids (reviewed in [51]). As in the gut and lung, the skin microbiota is involved in maintaining barrier function and educating the immune system (Figure 2). A recent study compared the cutaneous transcriptome of germ-free mice with that of mice raised under specific pathogen free conditions and found a large number of genes differentially expressed following microbial colonization, with many genes involved in innate immunity and antimicrobial proteins upregulated [52^{*}]. The skin commensal microbiota enhances keratinocyte production of antimicrobial peptides, such as human β -defensin (hBD2) and hBD3, thus protecting from infection with pathogens [53]. Furthermore, the functional potential of effector T cells in the skin is dependent on signals from the skin commensal bacteria through a pathway involving the interleukin-1 receptor (IL-1R) [54] and commensal bacteria interact with skin-resident dendritic cells to drive homeostatic commensal-specific T cell responses [55]. Of particular importance to Type 2 immune responsiveness, colonization of the skin is required during a critical developmental window in order to induce immune tolerance to commensal bacteria [56]. Furthermore, commensal microbial colonization of hair follicles during their development by stimulates production of the chemokine CCL20, which specifically recruits CCR6-expressing Tregs to the follicles during the neonatal period [57^{**}].

Microbial dysbiosis in the skin is associated with a number of skin disorders, including AD, suggesting that such microbial alterations may drive or exacerbate disease. *Staphylococcus aureus* is a pathobiont that can be found in healthy individuals but is highly prevalent in AD. Specifically, *S. aureus* has been shown to disrupt the skin barrier by increasing serine protease production by keratinocytes [58]. Disruptions in the skin barrier may then allow penetration of *S. aureus* through the epidermis, where it stimulates production of IL-4, IL-13, IL-22, TSLP, IL-17, and CXCL2 [59]. In healthy individuals the resident skin commensal bacteria, including *S. epidermidis* and *S. hominis*, produce AMP that protect from *S. aureus* infection but these protective bacterial species

Figure 2



Skin microbiota in homeostasis and atopic dermatitis. Schematic illustration of the mechanisms involved in skin homeostasis and deregulation in atopic dermatitis. Antimicrobial peptide (AMP), Toll-like receptor (TLR), complement cascade (CC), interleukin (IL), dendritic cell (DC), regulatory T cell (Treg), Chemokine (C-C motif) ligand 20 (CCL20), chemokine receptor 6 (CCR6), chemokine (C-X-C motif) ligand 2 (CXCL2), T helper type (Th), thymic stromal lymphopoietin (TSLP).

appear to be rare in AD patients [60]. Despite the evidence that *S. aureus* is highly prevalent in AD patients, a role for *S. aureus* in driving disease onset as opposed to blooming as a consequence of disease has been more difficult to determine. Meylen *et al.* performed multiple skin sampling from the time of birth through to 2 years of age and found that skin colonization with *S. aureus* was positively associated with later development of AD [61]. In contrast, another smaller study from a different birth cohort found no association between early *S. aureus* colonization in early life and subsequent AD development [62]. However, this study did find that infants with AD at 1 year were colonized with less commensal *Staphylococcus* spp., specifically *S. epidermidis* and *S. cohnii*, at 2 months of age [62]. This points to early alterations in the commensal bacteria as potential drivers of disease. This is supported by the observation that *S. aureus* induces AD-like lesions in a mouse model with altered barrier integrity [63]. Of importance, the ability of *S. aureus* to drive inflammation has been shown to be strain dependent with isolates from AD patients with severe flares stimulating greater Th2 and Th17 cell expansion in a mouse model of cutaneous colonization [64^{*}]. Further studies are required

to gain a better understanding of how specific strains of commensal bacteria may modulate Type 2 responses or tolerance during homeostasis and inflammation.

Conclusions

In this review we have attempted to highlight recent studies that have increased our understanding of the mechanisms by which the microbiota can modulate Type 2 immune responses. We have also described the importance of microbial exposure during the neonatal period in promoting the induction of tolerance and the prevention of aberrant Type 2 immunity. Finally, we highlighted recent evidence supporting a role for the skin microbiota in allergen sensitization and food allergy development. With increasing understanding of the role of the microbiota in regulating the immune system comes increased opportunity to apply microbial-based therapies to treat or prevent Type 2 driven immunopathology. While there has been an explosion of studies providing information on the microbial composition based on 16S rDNA sequencing, this does not provide in-depth information at the species or strain level and does not identify other microbial components, such as the virome or mycobiome.

Future studies using shotgun metagenomics will help to provide greater depth, although the current cost for performing this on large sample sizes has been prohibitive. Furthermore, increased integration of metabolomics and other omic platforms with microbiome information will help to define the precise molecular pathways utilized by the microbiota to modulate immune responses. This was elegantly illustrated by a recent study utilizing an integrated transcriptomic analysis to reveal key drivers of acute peanut allergic reactions [65]. The recent expansion of single cell approaches is likely to provide greater clarity of the precise cellular targets of the microbiota and animal models colonized with defined microbes will reveal the underlying mechanisms.

Conflict of interest statement

Nothing declared.

Acknowledgements

This work was supported by a Fellowship from São Paulo Research Foundation (2016/15882-7) to AI, and a Canadian Institutes for Health Research operating grant (PJT-3901060) to MBG.

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. Thursby E, Juge N: **Introduction to the human gut microbiota.** *Biochem J* 2017, **474**:1823-1836.
 2. Belkaid Y, Hand TW: **Role of the microbiota in immunity and inflammation.** *Cell* 2014, **157**:121-141.
 3. 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.
 4. Herbst T, Sichelstiel A, Schar C, Yadava K, Burki 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.
 5. Hill DA, Siracusa MC, Abt MC, Kim BS, Kobuley D, Kubo M, Kambayashi T, Larosa DF, Renner ED, Orange JS *et al.*: **Commensal bacteria-derived signals regulate basophil hematopoiesis and allergic inflammation.** *Nat Med* 2012, **18**:538-546.
 6. McCoy KD, Harris NL, Diener P, Hatak S, Odermatt B, Hangartner L, Senn BM, Marsland BJ, Geuking MB, Hangartner H *et al.*: **Natural IgE production in the absence of MHC Class II cognate help.** *Immunity* 2006, **24**:329-339.
 7. Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, Fukuda S, Saito T, Narushima S, Hase K *et al.*: **Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota.** *Nature* 2013, **500**:232-236.
 8. Geuking MB, Cahenzli J, Lawson MA, Ng DC, Slack E, Hapfelmeier S, McCoy KD, Macpherson AJ: **Intestinal bacterial colonization induces mutualistic regulatory T cell responses.** *Immunity* 2011, **34**:794-806.
 9. Lathrop SK, Bloom SM, Rao SM, Nutsch K, Lio CW, Santacruz N, Peterson DA, Stappenbeck TS, Hsieh CS: **Peripheral education of the immune system by colonic commensal microbiota.** *Nature* 2011, **478**:250-254.
 10. Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, Liu H, Cross JR, Pfeffer K, Coffey PJ *et al.*: **Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation.** *Nature* 2013, **504**:451-455.
 11. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, Nakanishi Y, Uetake C, Kato K, Kato T *et al.*: **Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells.** *Nature* 2013, **504**:446-450.
 12. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly YM, Glickman JN, Garrett WS: **The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis.** *Science* 2013, **341**:569-573.
 13. Josefowicz SZ, Niec RE, Kim HY, Treuting P, Chinen T, Zheng Y, Umetsu DT, Rudensky AY: **Extrathymically generated regulatory T cells control mucosal TH2 inflammation.** *Nature* 2012, **482**:395-399.
 14. Kabat AM, Harrison OJ, Riffelmacher T, Moghaddam AE, Pearson CF, Laing A, Abeler-Dorner L, Forman SP, Grecnis RK, Sattentau Q *et al.*: **The autophagy gene Atg16l1 differentially regulates Treg and TH2 cells to control intestinal inflammation.** *Elife* 2016, **5**:e12444.
- First paper illustrating a role for autophagy in the control of Treg and Th2 cells.
15. Gollwitzer ES, Saglani S, Trompette A, Yadava K, Sherburn R, McCoy KD, Nicod LP, Lloyd CM, Marsland BJ: **Lung microbiota promotes tolerance to allergens in neonates via PD-L1.** *Nat Med* 2014, **20**:642-647.
 16. Ohnmacht C, Park JH, Cording S, Wing JB, Atarashi K, Obata Y, Gaboriau-Routhiau V, Marques R, Dulauroy S, Fedoseeva M *et al.*: **Mucosal immunology. The microbiota regulates type 2 immunity through RORgammat(+) T cells.** *Science* 2015, **349**:989-993.
 17. 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.
- Seminal discovery of food antigen-mediated induction of small intestinal Treg.
18. 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.
 19. Stier MT, Peebles RS Jr: **Innate lymphoid cells and allergic disease.** *Ann Allergy Asthma Immunol* 2017, **119**:480-488.
 20. Monticelli LA, Sonnenberg GF, Abt MC, Alenghat T, Ziegler CG, Doering TA, Angelosanto JM, Laidlaw BJ, Yang CY, Sathaliyawala T *et al.*: **Innate lymphoid cells promote lung-tissue homeostasis after infection with influenza virus.** *Nat Immunol* 2011, **12**:1045-1054.
 21. Mortha A, Chudnovskiy A, Hashimoto D, Bogunovic M, Spencer SP, Belkaid Y, Merad M: **Microbiota-dependent crosstalk between macrophages and ILC3 promotes intestinal homeostasis.** *Science* 2014, **343**:1249288.
 22. Arrieta MC, 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**:307ra.
 23. Chua HH, Chou HC, Tung YL, Chiang BL, Liao CC, Liu HH, Ni YH: **Intestinal dysbiosis featuring abundance of *Ruminococcus gnavus* associates with allergic diseases in infants.** *Gastroenterology* 2018, **154**:154-167.
 24. Arrieta MC, Arevalo A, Stiemsma L, Dimitriu P, Chico ME, Looz S, Vaca M, Boutin RCT, Morien E, Jin M *et al.*: **Associations between infant fungal and bacterial dysbiosis and childhood atopic wheeze in a nonindustrialized setting.** *J Allergy Clin Immunol* 2017. pii: S0091-6749(17)31649-4.
 25. Fujimura KE, Sitarik AR, Havstad S, Lin DL, Levan S, Fadrosch D, Panzer AR, LaMere B, Rackaityte E, Lukacs NW *et al.*: **Neonatal gut microbiota associates with childhood multisensitized atopy and T cell differentiation.** *Nat Med* 2016, **22**:1187-1191.
- This study illustrated that the microbiota of neonates could be divided into three compositional types that were differentially associated with childhood atopy and asthma.

26. Fuss IJ, Heller F, Boirivant M, Leon F, Yoshida M, Fichtner-Feigl S, Yang Z, Exley M, Kitani A, Blumberg RS *et al.*: **Nonclassical CD1d-restricted NK T cells that produce IL-13 characterize an atypical Th2 response in ulcerative colitis.** *J Clin Invest* 2004, **113**:1490-1497.
27. Olszak T, An D, Zeissig S, Vera MP, Richter J, Franke A, Glickman JN, Siebert R, Baron RM, Kasper DL *et al.*: **Microbial exposure during early life has persistent effects on natural killer T cell function.** *Science* 2012, **336**:489-493.
28. An D, Oh SF, Olszak T, Neves JF, Avci FY, Erturk-Hasdemir D, Lu X, Zeissig S, Blumberg RS, Kasper DL: **Sphingolipids from a symbiotic microbe regulate homeostasis of host intestinal natural killer T cells.** *Cell* 2014, **156**:123-133.
29. 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.
30. Metsala J, Lundqvist A, Virta LJ, Kaila M, Gissler M, Virtanen SM: **Prenatal and post-natal exposure to antibiotics and risk of asthma in childhood.** *Clin Exp Allergy* 2015, **45**:137-145.
31. Qian LJ, Kang SM, Xie JL, Huang L, Wen Q, Fan YY, Lu LJ, Jiang L: **Early-life gut microbial colonization shapes Th1/Th2 balance in asthma model in BALB/c mice.** *BMC Microbiol* 2017, **17**:135.
32. Gomez de Agüero M, Ganai-Vonarburg SC, Fuhrer T, Rupp S, Uchimura Y, Li H, Steinert A, Heikenwalder M, Hapfelmeier S, Sauer U *et al.*: **The maternal microbiota drives early postnatal innate immune development.** *Science* 2016, **351**:1296-1302.
- This is a key study demonstrating that bacterial metabolites derived exclusively from the maternal microbiota can impact the development of the neonatal immune system.
33. Koch MA, Reiner GL, Lugo KA, Kreuk LS, Stanbery AG, Ansaldo E, Seher TD, Ludington WB, Barton GM: **Maternal IgG and IgA antibodies dampen mucosal T helper cell responses in early life.** *Cell* 2016, **165**:827-841.
- This is a key study demonstrating that bacterial metabolites derived exclusively from the maternal microbiota can impact the development of the neonatal immune system.
34. Oddy WH, Halonen M, Martinez FD, Lohman IC, Stern DA, Kurzius-Spencer M, Guerra S, Wright AL: **TGF-beta in human milk is associated with wheeze in infancy.** *J Allergy Clin Immunol* 2003, **112**:723-728.
35. Verhasselt V, Milcent V, Cazareth J, Kanda A, Fleury S, Dombrowicz D, Glaichenhaus N, Julia V: **Breast milk-mediated transfer of an antigen induces tolerance and protection from allergic asthma.** *Nat Med* 2008, **14**:170-175.
36. Turfkruyer M, Rekima A, Macchiaverni P, Le Bourhis L, Muncan V, van den Brink GR, Tulic MK, Verhasselt V: **Oral tolerance is inefficient in neonatal mice due to a physiological vitamin A deficiency.** *Mucosal Immunol* 2016, **9**:479-491.
37. Torow N, Yu K, Hassani K, Freitag J, Schulz O, Basic M, Brennecke A, Sparwasser T, Wagner N, Bleich A *et al.*: **Active suppression of intestinal CD4(+)TCRalpha-beta(+) T-lymphocyte maturation during the postnatal period.** *Nat Commun* 2015, **6**:7725.
38. Aage S, Kiraly N, Da Costa K, Byberg S, Bjerregaard-Andersen M, Fisker AB, Aaby P, Benn CS: **Neonatal vitamin A supplementation associated with increased atopy in girls.** *Allergy* 2015, **70**:985-994.
39. Kiraly N, Aage S, Benn CS: **Disparity between vitamin A-induced Th1-dependent oral tolerance in newborn mice and vitamin A-induced atopic sensitization in Guinean girls.** *Mucosal Immunol* 2016, **9**:564.
40. Knoop KA, Gustafsson JK, McDonald KG, Kulkarni DH, Coughlin PE, McCrate S, Kim D, Hsieh CS, Hogan SP, Elson CO *et al.*: **Microbial antigen encounter during a preweaning interval is critical for tolerance to gut bacteria.** *Sci Immunol* 2017:2.
- This study illustrated that exposure to commensal antigens in early life was limited by high levels of growth factors in breast milk that inhibit formation of goblet cell associated passages.
41. Ho HE, Bunyavanich S: **Role of the microbiome in food allergy.** *Curr Allergy Asthma Rep* 2018, **18**:27.
42. Fazlollahi M, Chun Y, Grishin A, Wood RA, Burks AW, Dawson P, Jones SM, Leung DYM, Sampson HA, Sicherer SH *et al.*: **Early-life gut microbiome and egg allergy.** *Allergy* 2018 <http://dx.doi.org/10.1111/all.13389>.
- This study on a subset of CoFAR subjects illustrates specific microbial Genera that were differentially abundant in children with egg allergy. They also found distinct Genera associated with egg sensitization.
43. Bunyavanich S, Shen N, Grishin A, Wood R, Burks W, Dawson P, Jones SM, Leung DYM, Sampson H, Sicherer S *et al.*: **Early-life gut microbiome composition and milk allergy resolution.** *J Allergy Clin Immunol* 2016, **138**:1122-1130.
- This study shows that alterations in early life microbiome may also be associated with later resolution of milk allergy.
44. Kourosh A, Luna RA, Balderas M, Nance C, Anagnostou A, Devaraj S, Davis CM: **Fecal microbiome signatures are different in food allergic children compared to siblings and healthy children.** *Pediatr Allergy Immunol* 2018 <http://dx.doi.org/10.1111/pai.12904>.
- This cohort study included food allergic and non-allergic siblings as well as unrelated healthy controls and revealed that differences in Clostridia explained the observed microbial differences.
45. Bashir ME, Louie S, Shi HN, Nagler-Anderson C: **Toll-like receptor 4 signaling by intestinal microbes influences susceptibility to food allergy.** *J Immunol* 2004, **172**:6978-6987.
46. Noval Rivas M, Burton OT, Wise P, Zhang YQ, Hobson SA, Garcia Lloret M, Chehoud C, Kuczynski J, DeSantis T, Warrington J *et al.*: **A microbiota signature associated with experimental food allergy promotes allergic sensitization and anaphylaxis.** *J Allergy Clin Immunol* 2013, **131**:201-212.
47. Stefká AT, Feehley T, Tripathi P, Qiu J, McCoy K, Mazmanian SK, Tjota MY, Seo GY, Cao S, Theriault BR *et al.*: **Commensal bacteria protect against food allergen sensitization.** *Proc Natl Acad Sci U S A* 2014, **111**:13145-13150.
48. Tan J, McKenzie C, Vuillermin PJ, Goverse G, Vinuesa CG, Mebius RE, Macia L, Mackay CR: **Dietary fiber and bacterial SCFA enhance oral tolerance and protect against food allergy through diverse cellular pathways.** *Cell Rep* 2016, **15**:2809-2824.
49. Fieten KB, Totte JEE, Levin E, Reyman M, Meijer Y, Knulst A, Schuren F, Pasmans S: **Fecal microbiome and food allergy in pediatric atopic dermatitis: a cross-sectional pilot study.** *Int Arch Allergy Immunol* 2018, **175**:77-84.
50. Grice EA, Segre JA: **The skin microbiome.** *Nat Rev Microbiol* 2011, **9**:244-253.
51. Byrd AL, Belkaid Y, Segre JA: **The human skin microbiome.** *Nat Rev Microbiol* 2018, **16**:143-155.
52. Meisel JS, Sfyroera G, Bartow-McKenney C, Gimblet C, Bugayev J, Horwinski J, Kim B, Brestoff JR, Tyldsley AS, Zheng Q *et al.*: **Commensal microbiota modulate gene expression in the skin.** *Microbiome* 2018, **6**:20.
- This study characterized the gene expression profile in the skin before and after colonization, which revealed 2820 differentially expressed genes involved in host immunity.
53. Lai Y, Cogen AL, Radek KA, Park HJ, Macleod DT, Leichtle A, Ryan AF, Di Nardo A, Gallo RL: **Activation of TLR2 by a small molecule produced by *Staphylococcus epidermidis* increases antimicrobial defense against bacterial skin infections.** *J Invest Dermatol* 2010, **130**:2211-2221.
54. Naik S, Bouladoux N, Wilhelm C, Molloy MJ, Salcedo R, Kastnermuller W, Deming C, Quinones M, Koo L, Conlan S *et al.*: **Compartmentalized control of skin immunity by resident commensals.** *Science* 2012, **337**:1115-1119.
55. Naik S, Bouladoux N, Linehan JL, Han SJ, Harrison OJ, Wilhelm C, Conlan S, Himmelfarb S, Byrd AL, Deming C *et al.*: **Commensal-dendritic-cell interaction specifies a unique protective skin immune signature.** *Nature* 2015, **520**:104-108.
56. Scharschmidt TC, Vasquez KS, Truong HA, Gearty SV, Pauli ML, Nosbaum A, Gratz IK, Otto M, Moon JJ, Liese J *et al.*: **A wave of**

regulatory T cells into neonatal skin mediates tolerance to commensal microbes. *Immunity* 2015, **43**:1011-1021.

- 57. Scharschmidt TC, Vasquez KS, Pauli ML, Leitner EG, Chu K, Truong HA, Lowe MM, Sanchez Rodriguez R, Ali N, Laszik ZG **et al.**: **Commensal microbes and hair follicle morphogenesis coordinately drive treg migration into neonatal skin.** *Cell Host Microbe* 2017, **21** 467-477.e465.

This study provided underlying mechanisms explaining the influx of regulatory T cells in the neonatal skin.

- 58. Williams MR, Nakatsuji T, Sanford JA, Vrbanc AF, Gallo RL: **Staphylococcus aureus induces increased serine protease activity in keratinocytes.** *J Invest Dermatol* 2017, **137**:377-384.
- 59. Nakatsuji T, Chen TH, Two AM, Chun KA, Narala S, Geha RS, Hata TR, Gallo RL: **Staphylococcus aureus exploits epidermal barrier defects in atopic dermatitis to trigger cytokine expression.** *J Invest Dermatol* 2016, **136**:2192-2200.
- 60. Nakatsuji T, Chen TH, Narala S, Chun KA, Two AM, Yun T, Shafiq F, Kotol PF, Bouslimani A, Melnik AV **et al.**: **Antimicrobials from human skin commensal bacteria protect against Staphylococcus aureus and are deficient in atopic dermatitis.** *Sci Transl Med* 2017, **9**.
- 61. Meylan P, Lang C, Mermoud S, Johannsen A, Norrenberg S, Hohl D, Vial Y, Prod'homme G, Greub G, Kypriotou M **et al.**: **Skin**

colonization by Staphylococcus aureus precedes the clinical diagnosis of atopic dermatitis in infancy. *J Invest Dermatol* 2017, **137**:2497-2504.

- 62. Kennedy EA, Connolly J, Hourihane JO, Fallon PG, McLean WHI, Murray D, Jo JH, Segre JA, Kong HH, Irvine AD: **Skin microbiome before development of atopic dermatitis: early colonization with commensal staphylococci at 2 months is associated with a lower risk of atopic dermatitis at 1 year.** *J Allergy Clin Immunol* 2017, **139**:166-172.
- 63. Kobayashi T, Glatz M, Horiuchi K, Kawasaki H, Akiyama H, Kaplan DH, Kong HH, Amagai M, Nagao K: **Dysbiosis and Staphylococcus aureus colonization drives inflammation in atopic dermatitis.** *Immunity* 2015, **42**:756-766.
- 64. Byrd AL, Deming C, Cassidy SKB, Harrison OJ, Ng WI, Conlan S, Program NCS, Belkaid Y, Segre JA, Kong HH: **Staphylococcus aureus and Staphylococcus epidermidis strain diversity underlying pediatric atopic dermatitis.** *Sci Transl Med* 2017:9.

This study illustrates the importance of investigating microbial differences at strain level.

- 65. Watson CT, Cohain AT, Griffin RS, Chun Y, Grishin A, Hacyzniska H, Hoffman GE, Beckmann ND, Shah H, Dawson P **et al.**: **Integrative transcriptomic analysis reveals key drivers of acute peanut allergic reactions.** *Nat Commun* 2017, **8**:1943.