

Microbiome influences on allergy in mice and humans

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The microbiota plays a pivotal role in the development and calibration of host immunity. Over many millennia, finely balanced interactions between the microbiota and host tissue compartments have evolved, imparting metabolic advantages and protection against pathogens, while restricting deleterious immune responses against innocuous antigens. Perturbations in host–microbiota crosstalk at critical developmental windows in early life may underlie allergy and chronic inflammation. Although the microbiota's of the gut and skin have been extensively characterized, the lung microbiota has also, in recent years, received considerable attention. This ever-expanding field is pushing the boundaries of pulmonary research, with potential implications for novel strategies in the treatment and prevention of chronic lung diseases. In this article, we provide a summary of the development of the microbiota in early life, and describe the evidence from human and murine studies of how microbial dysbiosis in early life can alter the trajectory of immune development and provide the setting for allergic disorders in later life.

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Introduction

In recent decades, there has been an upsurge in the prevalence of allergic diseases, now reported to affect up to 400 million people across the globe [1]. While allergy encompasses a wide spectrum of diseases [2], it is notable that the majority of clinical manifestations occur in the respiratory, gastrointestinal and cutaneous epithelial surfaces: sites which not only harbour vast microbial communities but are constantly exposed to waves of environmental microbes and allergens throughout life.

The microbiota in early life and development of allergy

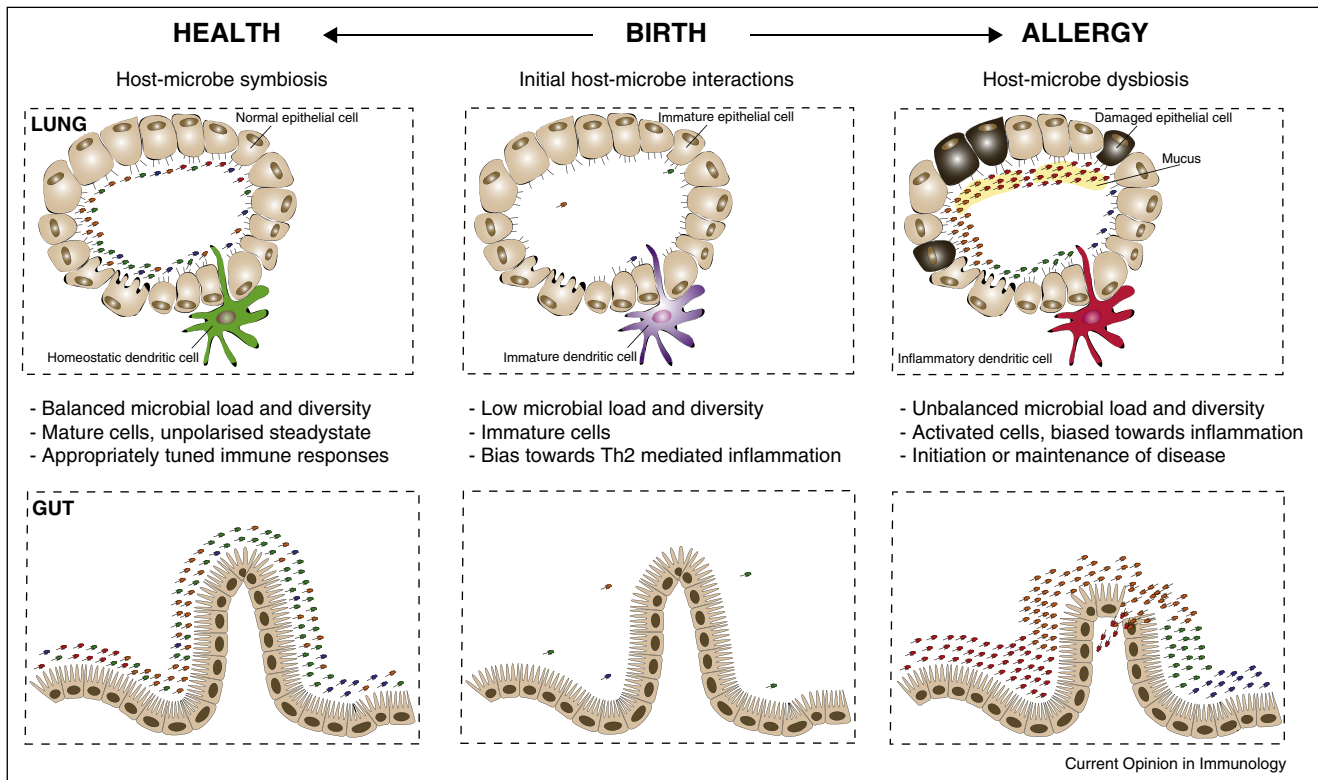
Following birth, the mammalian newborn makes a transition from an intrauterine milieu to an external, microbe-laden environment. The age-long paradigm of a sterile, intrauterine habitat has been called into question by recent studies that reveal the presence of a low-abundance microbiota in the placenta [3,4] and meconium [5,6], suggesting that the earliest encounters between the developing neonatal immune system and bacteria occurs *in utero*. Little is known about how initial host–microbial interactions *in utero* could determine future programming of immune responses; however, one could speculate that the types and nature of bacterial exposure in the amniotic fluid-bathed environment will be vastly different to those that occur postnatally. Moreover, the magnitude of post-natal microbial exposure is far greater (Fig. 1).

Postnatal microbial colonization of the gut occurs in the early neonatal period [7]. Whereas differences in initial bacterial communities are shaped by mode of delivery [7], subsequent temporal changes occurring within the first months of life are influenced by mode of feeding [8,9], gestational age [10] and use of antibiotics [11,12], with a gradual convergence towards an adult-like profile by the age of 1 year [13].

Mounting evidence points to low diversity of the gut microbiota in early infancy as an important risk factor for subsequent development of allergy [14–16]. Furthermore, data from large cross-sectional [17,18,19] and longitudinal studies [20–22] suggest a particularly strong association between growing up in a farm environment, with early life exposure to barn-derived microbes and unpasteurized milk, and decreased risk of allergy.

Similarly, evidence from experiments in mice alludes to the crucial role of the microbiota in early life in dampening allergic airway inflammation. For example, experiments in mice have demonstrated that tolerance to aeroallergens begins in early life and is dependent on the presence of the microbiota [23]. Furthermore, microbial colonization of germ free mice within the first days or weeks of life was shown to be protective against elevated IgE levels [24] and iNKT [25] cell responses in adulthood. The kinetics of early life microbial colonization in the gut has been well characterized; however, little is known about similar trends in the skin and lungs and whether there is an interplay between the microbiota within the three tissue compartments in early life. Overall, these findings suggest a key role for the early life microbiota in modulating allergic inflammation in later life.

Figure 1



Early life impacts of microbial colonization upon immune development in the lung and gut. Before birth there is a negligible load of bacteria present in mucosal tissues. Immune cells present at these sites can broadly be considered 'immature' and have a functionality that is representative of the stimuli present in the intrauterine environment. Immediately following birth, mucosal tissues are exposed to, and colonized by, bacteria. These early host-microbe interactions could be critical in shaping the trajectory of an individual's immune development, and consequently risk of disease. Formation of a balanced and diverse microbiota is associated with 'healthy' immune development and low risk of allergies, whilst a lack of microbial diversity and an over-representation of bacteria, typically considered as pathogens, is associated with the development of allergy.

The tissue microenvironment and the microbiota

The total surface area of the lungs in humans measures approximately 75 m² and is constantly exposed to the external environment. Distally, within the alveoli, a single layer of epithelial cells sets the boundary between the external environment and the underlying capillaries [26]. Multiple studies are now in support of the existence of a lung microbiota [27–31], challenging the age-long paradigm that the lungs are sterile. Furthermore, technical difficulties associated with collection of 'pure' lower airway samples, free of bronchoscopic carryover from the upper respiratory tract, have been overcome by improved sampling techniques [27,29]. Sampling of the lower airways via a broncho-alveolar lavage (BAL) [32] remains an established diagnostic and experimental procedure and the obtained BAL fluid with its cellular (leukocytes, bacteria, fungi, epithelial cells) and acellular (proteins, nucleic acids, viral particles) components provides a snapshot of the airway composition in a variety of

disease states [32]. However, the microenvironment of the lower airways is heterogeneous, with topographical differences in ventilation [33], perfusion [33], oxygen tension [34], and temperature [35]. Thus, akin to findings in the skin [36] and gastrointestinal tract [37,38], the microenvironment may induce regional differences in the lung microbiota in an individual with important implications for disease development. Indeed there are indications in the literature that support the case for discrete niches in the airways of individuals [28], however, a direct causal relationship of local host-microbiome interactions leading to local tissue damage is yet to be forthcoming. This is a key concept that needs to be challenged and tested.

Compared with other tissue habitats like the gastrointestinal tract and skin, the lung microenvironment is relatively nutrient-poor. Therefore, colonization and long-term survival of the microbiota will depend on its inherent ability to harness environment-derived nutrients vital

for cellular biosynthesis. Currently, little is known about such airway-derived nutrient sources required for optimal bacterial growth. However, biochemical profiling of low-molecular-weight metabolites in BAL, sputum and exhaled breath [39,40,41] revealed that patients with cystic fibrosis had higher levels of lactate, valine, taurine and alanine, in comparison to healthy controls [41]. Furthermore, glycolipids and glycol-proteins found in bronchial mucus [42], and lung surfactant [42] are also potential energy sources available in the lung microenvironment. Currently, little is known about the regulation and topographic distribution of these putative endogenous energy sources in the airways, and how this might shape the microbiota in the context of allergic airway inflammation. Another important consideration is that the microbiota could be in constant flux and many of its constituents are short-term residents; however, evidence from a variety of studies describing the lung microbiota in health and disease show striking similarities, thus arguing that even if there is a constant flux of the microbial constituents, the lung environment is selecting for certain microbes.

The mammalian skin is composed of diverse habitats, comprising of appendages and invaginations, hairy and hairless regions; all of which possess endogenous physico-chemical properties which select for unique, well adapted microbial communities [43]. The composition of the skin microbiota is largely dependent on the micro-environment sampled [44], with specific bacteria associated with dry, moist or sebaceous skin sites [44]. Microbial diversity is lowest in sebaceous skin sites of the forehead, back, retro-auricular crease and alar folds, demonstrating a strict, site-specific selection for lipophilic bacteria [44]. *Propionibacterium* spp., with a variety of lipases which metabolize lipid-rich sebum, has evolved to dominate sebaceous skin regions [45], while *Staphylococci* and *Corynebacteria*, which are halotolerant, that is capable of thriving in high salt concentrations, are well adapted to moist regions of the skin like the axillae, submammary and inguinal regions [44]. Clinically, the symptomatology of atopic dermatitis displays a proclivity for certain skin sites, for example, in infants, the scalp, face, neck, trunk and extensor surfaces of the extremities are generally affected, while typical involvement of flexural surfaces of the extremities, hands and feet is observed in adolescents and adults [46]. The determinants of the predilection for inflammation in specific skin sites observed in AD remain unknown. However, we speculate that topographical differences in the microbial communities present in skin sites and appendages may underlie the localization of skin inflammation.

In the gastrointestinal tract, local factors also, to a large extent, regulate regional differences in the microbiota. The highly acidic environment of the stomach and duodenum is prohibitive to bacterial colonization and the result is a low diversity of the gastric microbiota [47]. The

upper small intestine is populated by aerobic species, while the low oxygen tension in the colon is highly permissive to anaerobic species. Furthermore, the differential availability of dietary components like vitamin A, flavonoids, and fibre not only determine local microbiota, but also influence immune functions [48].

Microbiota-immune interactions in allergic inflammation

Our knowledge of the critical role of the microbiota in regulating allergic inflammation, and functional consequences of its perturbation, has improved over the past decades. Evidence from studies in germ-free mice suggests that the microbiota can regulate both local and systemic allergic responses. Germ-free and antibiotic-treated mice were shown to display higher levels of allergen-induced airway inflammation [49,50], which was reversed by re-colonization with microbiota from mice raised under SPF conditions [49]. In addition, direct administration of innocuous bacteria or bacterial products into the airways in mice induces protection from airway inflammation [51,52]. Microbiota in early life can also promote tolerance to aeroallergens via the induction of T-regulatory cells [23]. Specific bacterial species can also mediate protective effects. Mice infected with *Helicobacter pylori* were protected from allergen-induced pulmonary and bronchoalveolar infiltration with eosinophils, Th2 cells, and Th17 cells [53], and oral administration of a mix of *Clostridium* strains to BALB/c mice stimulated allergen-induced expansion of Treg cells in the colonic mucosa and reduced systemic IgE production [54].

In the gastrointestinal tract, the role of the microbiota in immune maturation and development is well established [55–57] and dysbiosis is not only linked with chronic inflammatory diseases of the gut such as Crohn's disease and ulcerative colitis [58], but also plays a key role in maintaining intestinal tolerance to food-derived antigens [59].

Currently, there is limited evidence supporting a direct link between the microbiota and food allergy in humans. One study compared faecal microbiota from healthy infants and those allergic to cow-milk proteins [60]. Allergy was associated with higher anaerobic and lactobacilli counts, a higher proportion of lactobacilli, a lower count and proportion of bifidobacteria, and lower proportions of enterobacteria [60]. Studies in IL4raF709 mice, which have a gain-of-function mutation in the IL-4 receptor α chain, and are particularly susceptible to allergic sensitization, have demonstrated the presence of a specific gut microbiota signature which, when transferred to wild type mice, was capable of transmitting susceptibility to food allergy [61]. In another study, Clostridia enriched microbiota were protective against allergen sensitization by regulating innate lymphoid cell function and intestinal epithelial permeability [62].

Taken together, these findings support a protective role for the microbiota in allergic intestinal inflammation. Another important consideration in the context of allergy in the gut is the timing of allergen exposure. Tolerance to food-derived antigens occurs in early life [63], coincident with the development of the gut microbiota [13], and delayed exposure to food antigens has been shown to be associated with increased risks of allergy [64,65]. Currently, epidemiological evidence supports the role of oral, early-life antigen exposure in the protection against food allergy [66,67] and recently, a randomised clinical trial examined the effect of early life peanut consumption on allergy development in infants at high risk of allergy [68**]. The authors of this study concluded that the early introduction of peanuts in infancy led to a significant decrease in the frequency of peanut allergy, and the induction of peanut-specific protective immune responses [68**]. Currently however we know very little about the role of the microbiota in food allergy and one might speculate that early life interactions between developing immune cells, the microbiota and food-derived antigens could be critical in regulating allergic responses in the gut.

Bacterial dysbiosis is associated with chronic inflammatory disorders of the skin, such as atopic dermatitis (AD) and psoriasis [69]. However, it remains unknown whether dysbiosis is a cause or consequence of skin inflammation. Studies in skin samples of patients with AD have shown that outgrowths of *Staphylococcus* species correlate with disease flares, with associated reduction in the overall diversity of microbial communities in the skin [70]. Following antimicrobial therapy, a reduction in severity of symptoms was linked with a return of *Streptococcus*, *Propionibacterium*, and *Corynebacterium* species to the skin [70], suggestive of a role for bacterial diversity in regulating skin inflammation.

Recent studies have demonstrated strong similarities within the skin microbiota of mice and humans [71,72**], making them useful animal models for exploring dysbiosis in the skin. In one study, topical mono-association of germ-free mouse skin with *S. epidermidis* induced the production of IL-17A by CD8+ T-cells that home to the epidermis [73,74]. This effect, mediated by microbe specific non-inflammatory dendritic cells [73], was downstream of the activation of the IL-1 signalling pathway [74], which has been shown to play a key role in other chronic inflammatory skin disorders, like psoriasis [75,76].

The interplay of diet, microbiota and allergy

Diet is known to affect the gut microbiota in mice and humans and dietary habits have the potential to impact overall intestinal health, and in particular, protection from intestinal inflammation. For instance, in a study comparing faecal samples from children in a rural village in

Burkina Faso with European children, the Burkinabe children, whose diets comprised essentially of plant polysaccharides had low levels of Firmicutes and increased levels of Bacteroidetes in their faecal microbiota as compared to the European children, who had high levels of Enterobacteriaceae [77]. In addition, the Burkinabe children had higher levels of faecal microbiota richness and diversity, as well as increased short chain fatty acid (SCFA) levels, supporting the role of dietary fibre intake in shaping gut microbiota. SCFAs are produced by fermentation of dietary fibre by intestinal microbiota, and they exert both local and systemic immunomodulatory effects [78]. Mechanistic insights into the systemic immunomodulatory effects of gut-derived SCFAs were recently provided in mice, where intake of high fibre diet induced protection from allergic airway inflammation, and treatment with the SCFA, propionate, induced the production of bone-marrow derived DCs with impaired Th2 stimulatory abilities [79**].

Conclusions

There is a substantial body of evidence now showing symbiotic host–microbe relationships at all our body surfaces, particularly the gut, skin and lung. Dysregulation of this relationship is a fundamental cause of inflammation and disease development. An alteration in the nature of microbes, whether that be the representation of certain species within the microbiota or a general change in diversity and load, could be a key factor underlying the global increase in allergies seen in recent decades. With this possible causality comes opportunity. A better understanding of how microbes impart beneficial effects on the host needs to be reached; an accurate definition of when windows of ‘opportunity’ or ‘disease susceptibility’ occur at different tissue sites needs to be established. Indeed, the origin of allergies could well be a convergence of certain exposures (e.g. allergens or infections) during stages of immune and microbiome development. Armed with this information we might be able to move from symptomatic management of allergies to cures and prevention strategies effective on a population level.

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