Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Immunology

The hygiene hypothesis: immunological mechanisms of airway tolerance

Eline Haspeslagh[1,2,3], Ines Heyndrickx[1,3], Hamida Hammad[1,3] and Bart N Lambrecht[1,3,4]

The hygiene hypothesis was initially proposed as an explanation for the alarming rise in allergy prevalence in the last century. The immunological idea behind this hypothesis was a lack of infections associated with a Western lifestyle and a consequential reduction in type 1 immune responses. It is now understood that the development of tolerance to allergens depends on microbial colonization and immunostimulatory environmental signals during early-life or passed on by the mother. These environmental cues are sensed and integrated by barrier epithelial cells of the lungs and possibly skin, which in turn instruct dendritic cells to regulate or impede adaptive T cell responses. Recent reports also implicate immunoregulatory macrophages as powerful suppressors of allergy by the microbiome. We propose that loss of adequate microbial stimulation due to a Western lifestyle may result in hypersensitive barrier tissues and the observed rise in type 2 allergic disease.

Addresses
[1] Laboratory of Immunoregulation and Mucosal Immunology, VIB Center for Inflammation Research, Technologiepark 927, B-9052 Ghent (Zwijnaarde), Belgium
[2] Department of Biomedical Molecular Biology, Ghent University, Technologiepark 927, B-9052 Ghent (Zwijnaarde), Belgium
[3] Department of Internal Medicine, Ghent University, De Pintelaan 185 K12, B-9000 Ghent, Belgium
[4] Department of Pulmonary Medicine, ErasmusMC, 's-Gravendijkwal 230, 3015 CE Rotterdam, The Netherlands

Corresponding authors: Hammad, Hamida (Hamida.Hammad@UGent.be), Lambrecht, Bart N (bart.lambrecht@ugent.be)

Current Opinion in Immunology 2018, **54**:102–108

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.2018.06.007

Introduction

Allergic sensitization is characterized by the presence of allergen-specific immunoglobulin E (IgE) in serum. Exposure to allergens via inhalation, ingestion or contact with the skin can lead to diseases such as asthma, hay fever, eczema and, in some cases, to systemic anaphylaxis. During the last 150 years, allergies have emerged in a very rapid way and their prevalence is still on the rise. Nowadays, more than 30% of children are allergic, up to 10% of children suffer from asthma and allergic rhinitis, and 5–7% of children have developed food allergy. It is still not entirely clear why asthma prevalence is so high, but the rapid time frame of its origination and expansion suggests that environmental or behavioral changes in Western lifestyle are involved.

A modern lifestyle is associated with dysbiosis

An important evolution of the last 150 years is a successful decrease of infectious disease burden, due to the massive introduction of hygiene measures, antibiotics, and vaccines. In 1989, Strachan observed that growing up in large families with more older siblings decreased the chances of developing hay fever or eczema [1]. He postulated that the recent increase in allergy incidence was a result of 'declining family size, improvements in household amenities, and higher standards of personal cleanliness', which had reduced 'the opportunity for cross infection in young families'. The original 'hygiene hypothesis' was thus introduced. Since then, this hypothesis has been supported by numerous studies, especially in murine models, showing that exposure to bacteria, viruses, helminths or microbe-derived products could protect from allergy (reviewed in [2], [3••]). However, it should be kept in mind that not all pathogens are protective; for instance, respiratory syncytial virus (RSV) or rhinovirus are associated with a higher risk to develop wheeze and asthma up to adulthood [4].

Changes in lifestyle can also heavily influence the composition and diversity of the microbiome at several mucosal surfaces. These microbial communities have co-evolved with and within the human body for millions of years, and, consequently, the human immune system has been calibrated and fine-tuned so to maintain and shape symbiotic relationships with them (reviewed in [5]). Two theories, the 'Old friends' and the 'Biodiversity' hypotheses, have been proposed by Rook and by Haahtela as a more accurate, or at least complementary, explanation for the recent allergy pandemic [6,7]. They stipulate that the reason for the increased incidence in allergic disorders is a reduced exposure to such beneficial symbiotic bacteria or parasites. Indeed, several studies have

reported that alterations in the composition of the skin, the nose or the gut microbiome are associated with eczema, asthma and food allergy [8–10]. These changes do not affect a single commensal, but rather reflect a reduced total microbial diversity [11], and they may be caused by several factors, including sibling order in the family [12], exposure to animals [13], and other early-life events [14]. The importance of a healthy microbiome in controlling allergies was further substantiated in mice, with germ-free mice being especially prone to develop overt allergic (airway) disease, a phenotype reverted by microbial recolonization [15,16]. However, other studies showed that germ-free mice are not universally more susceptible to house dust mite driven asthma, and that only selected strains of lung microbiota seem to suppress asthma [17]. During the last 30 years, the body of correlative epidemiological studies has expanded vastly, and is the subject of many excellent reviews. Here, we will zoom in on recent advances in the search for the underlying immunological mechanisms explaining the observed effects.

Microbes induce protective regulatory DCs and T cells

Allergies are generally aberrant immune reactions to innocuous antigens, orchestrated by T helper 2 (Th2) cells and type 2 innate lymphoid cells (ILC2s). In the case of asthma, this type 2 cell activity leads to mucus hypersecretion, goblet cell hyperplasia, smooth muscle cell hyperreactivity, and the infiltration and/or activation of eosinophils, mast cells and basophils, ultimately culminating in breathing difficulties and airway remodeling [18]. Dendritic cells (DCs) are always found at the body's barriers, and because they express a wide range of pattern recognition receptors (PRRs), they can sense the environment for the presence of danger signals [19]. Our group has shown that Th2 responses to house dust mite (HDM) allergens were induced by IRF4-dependent cDC2s in the lungs and in the skin [20,21•] (Figure 1). These cDC2s capture the HDM allergens in the airways and migrate to the draining lymph nodes, requiring ILC2-derived IL-13, where they present the allergens to naïve T cells [22]. It is easy to imagine that environmental changes sensed at the level of the lungs, the skin but also of the gut will modify the context of allergen recognition by DCs, and either protect against or enhance allergic responses.

Chronic *Helicobacter pylori* infection has been inversely linked to asthma in humans and can effectively protect mice from OVA-induced asthma [23,24]. In mice, *H. pylori* infection induced the accumulation of CD103+ cDCs in the lungs, which were required for the protection, as was their IL-10 production [24]. In a recent study, semi-therapeutic *H. pylori* extract treatment also reduced airway allergy, shifted the CD11b+/CD103+ DC ratio in the lungs, and reduced the antigen processing by lung and lymph node DCs [25]. Other studies demonstrated protective modulation of *in vitro* bone-marrow derived DC cultures (BMDCs). A synthetic TLR1/TLR2-agonist induced LPS-tolerance and IL-10 production in BMDCs, whereas the cowshed *Lactococcus lactis* instigated a Th1-polarizing program, both rendering the BMDCs unable to sensitize mice to OVA-allergen upon adoptive transfer [26,27•].

Trompette *et al.* recently found that feeding mice a fiber-rich diet changed the composition of the lung and gut microbiome, the latter metabolizing the fiber into circulating short-chain fatty acids (SCFA's) [28]. The increased SCFA levels protected the mice from allergic lung inflammation. Mechanistically, the SCFA's altered DC precursor generation in the bone marrow, and the DCs subsequently seeding the lungs had a higher phagocytic capacity and were impaired in polarizing Th2 cells. Additional studies have supported the protective effect of dietary fiber supplementation on allergic asthma development in mice [29], and on wheeze in human infants when the fiber was given to the pregnant mother [30].

One mechanism by which the DCs in microbe-exposed animals can confer protection, is by inducing the generation of regulatory T cells (Tregs). Microbial colonization in 2-week old mice was shown to be necessary for the transient upregulation of PD-L1 on lung CD11b + DCs, and the expansion of a specific pulmonary Treg subset [31]. PD-L1 blockade in neonates resulted in exaggerated responsiveness to HDM through adulthood, suggesting a crucial role for this microbial-induced DC–Treg axis for immunological tolerance. In another mouse model of *H. pylori*-mediated asthma protection, the *Helicobacter* infection inhibited TLR-induced DC maturation and reprogrammed the DCs towards a FoxP3+ Treg-polarizing phenotype [32]. The bacterial component flagellin B, given semi-therapeutically together with allergen, could also inhibit murine allergic asthma symptoms in a DC– and CD25+ Treg-dependent manner [33•].

Although helminths are prototypical inducers of type 2 immunity, they have been correlated with reduced allergen skin prick test reactivity, and to some degree with asthma protection (reviewed in [34]). A general explanation for this non-intuitive association is that helminths induce a so-called 'modified Th2′ response, with immunoregulatory cells such as Tregs complementing the Th2-arm of immunity, and regulating the response to bystander antigens such as aeroallergens. Therefore, several groups have tried to find helminth-derived products with immunomodulatory properties that could be used to suppress Th2 immunity. For instance, an anti-inflammatory protein (-2; AIP-2) from the parasitic hookworm was identified to suppress murine airway allergy in a DC-dependent and Treg-dependent manner [35]. In another study, the helminth-derived immunomodulator