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Review

A fresh look at the hygiene hypothesis: How intestinal microbial exposure drives immune effector responses in atopic disease

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ABSTRACT

There currently is no consensus on which immunological mechanisms can best explain the rise in atopic disease post industrialization. The hygiene hypothesis lays groundwork for our understanding of how altered microbial exposures can drive atopy; yet since its introduction increasing evidence suggests the exposure of our immune system to the intestinal microbiota plays a key role in development of atopic disease. As societal change shifts our microbial exposure, concordant shifts in the tolerant and effector functions of our immune systems give rise to more hypersensitive responses to external antigens. This is contrasted with the greater immune tolerant capabilities of individuals still living in regions with lifestyles more representative of our evolutionary history. Recent findings, buoyed by technological advances in the field, suggest a direct role for the intestinal microbiota-immune system interplay in the development of atopic disease mechanisms. Overall, harnessing current mechanistic studies for translational research into microbiota composition and function in relation to atopy have potential for the design of therapeutics that could moderate these diseases.

1. Introduction

1.1. The hygiene hypothesis: a history

Our thinking of how environmental exposures can be linked to atopic disease can be traced back to a study in the 1970s, where a low prevalence of allergy in indigenous populations in northern Canada was observed when compared to Caucasian Canadians living in urban environments [1]. This was hypothesized to be the result of fewer infections during childhood. A similar observation was made a decade later, with children with elder siblings being less likely to get hay fever [2]. Henceforth the term "hygiene hypothesis" was used to describe the general phenomenon of the association between hygienic conditions and a higher prevalence of allergic disease. Numerous studies have confirmed this relationship, even extending it to the global epidemiology of other immune-mediated diseases, such as type 1 diabetes and inflammatory bowel disease [3,4]. Recently these observations are being linked to the composition of the microbes that colonize our intestinal tract, collectively referred to as our intestinal microbiota. The microflora hypothesis proposes that shifts in composition of our intestinal microbiota caused by early life antibiotic use and dietary changes can lead to a disruption in immune tolerance [5]. An extension of this idea is found in the "old friends" hypothesis, which gives an evolutionary perspective to these observations. This hypothesis proposes that the microbial exposures vital for immune regulation can be derived from our symbiotic relationship with the microbes we have co-evolved with in our environment [6]. The microbiota has co-evolved with the intestinal immune systems for millions of years, during which most encounters involve commensal and mutualistic bacteria rather than pathogenic ones. With a community of cells expressing one hundred times more genes than its host, the microbiota produces significantly more potential antigens that encounter the mammalian immune system than self or pathogen-derived antigens [7].

The emergence of the jaw in early vertebrates came a series of biological advantages, such as an increase in body size and lifespan. Intriguingly, this evolutionary milestone matches the appearance of the adaptive immune system, which not only left early vertebrates better equipped to fight persistent pathogens, but also required the establishment of immune tolerance toward a vast amount of benign and often beneficial microorganisms. The vertebrates capable of mounting immunological memory have a more diverse microbiota than invertebrates, suggesting that the different features of the immune system and the composition of the microbiota are tightly influenced by one another [8]. Given the

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inevitable exposure of the intestine to microbes, the immune system has also evolved dependency on microbial exposure similar to how our body has become dependent on dietary components that we cannot synthesize.

Following industrialization, microbial exposure from our environment has rapidly changed. The notion of a "disappearing microbiota" links post-industrialization habits to a depletion of our ancestral microbiota that we have become dependent on from our environment [9]. As atopic diseases such as food allergy and asthma have become more prevalent since the industrial revolution, the incidence of these diseases could be linked to lifestyle and dietary changes, altering our exposure to microorganisms that educate our immune system. Much of our microbial exposure throughout life is derived from the intestinal microbiota. Thus, analysis of how our immune system interacts with the intestinal microbiota is critical to understand the link between atopy and microbial exposure.

1.2. The intestinal microbiota: our main exposure to microbial antigens

The human intestine harbors a diverse microbial community comprised of mainly bacteria, but also archaea, viruses, and eukaryotes. The intestine is dominated by over 500 species of bacteria, from 7 to 10 phyla, with species from the phyla Bacteroidetes and Firmicutes being the most abundant [10]. These bacteria colonize to levels reaching 100 trillion microbes along the intestinal tract, and bacteria density increases from the small intestine (10^3–10^7 cells per gram of feces) to the colon (10^{12} cells per gram feces) [11,12]. The colonization of the microbiota starting at birth shapes an intimate relationship where host and microbe have co-evolved together for mutually beneficial outcomes. The intestine provides a protected, nutrient rich environment where the microbiota establishes a remarkably stable and resilient ecosystem [10]. In turn, the host uses the metabolic capacity of the microbiota to its advantage. The intestinal metagenome contains roughly 100 times more microbial genes than human genes [13], supplementing the host cells with a "second genome" [14]. Enzymatic products of these genes enhance our digestive capacity of substances such as polysaccharides and complex carbohydrates [15]. Microbial by-products of digestion provide vitamins and nutrients to host cells and contribute to many aspects of host physiology and development, along with conferring colonization resistance to potentially harmful pathogens [16].

While the gut microbiota is remarkably stable throughout life, pronounced differences in bacterial assemblage and gene repertoires have been observed in humans across the globe [17]. Most notably, there are distinct differences in the composition and diversity of the gut microbiota between adults in industrialized versus developing countries [18]. Differing diets, infection rate and other environmental exposures (rather than purely genetic differences) are thought to be the main drivers of these changes, and we are just beginning to grasp the implications the resulting changes in microbial communities have on immune mechanisms relating to atopy [19].

There remain many outstanding questions. Most importantly, could there be a correlation between population based microbial changes and the epidemiological prevalence of atopic diseases? For example, it is well reported that the incidence of atopy in citizens of hunter-gatherer societies is low to non-existent [20]. The microbial exposure from the surrounding environment is significantly different in these societies, and their lifestyle reflects one closely linked to our evolutionary history. How these lifestyle changes feed into the structure and composition of the intestinal microbiota, and influence T helper 2 (Th2) immune responses after exposure to an allergen is the topic of many current studies. In this review, we highlight how certain components of the intestinal microbiota can direct tolerant versus effector immune responses, and then discuss the relevance of this effect on atopic disease. Particular emphasis is placed on the effect the intestinal microbiota have on T helper cell balance and Toll-like receptor signaling. Finally, we introduce plausible hypotheses and mechanisms as to why atopic diseases are absent in geographical regions with poor sanitation, and a lifestyle more reflective of our evolutionary history.

2. The gut microbiota shapes tolerant and effector immune responses

At homeostasis, the gastrointestinal immune environment is one of controlled inflammation and tolerance. Several immune mechanisms are involved in achieving this state. First, most bacteria in the distal small intestine and the colon are not in physical contact with the intestinal epithelial cells (IECs). Only some microorganisms are capable of inhabiting the mucus layer immediately adjacent to the gut epithelium and interacting with epithelial and immune cells. The mucus layer is morphologically divided into inner and outer portions. Bacteria rarely populate the inner mucus layer, while the outer mucus layer provides mucin glycoproteins that facilitate the colonization by a variety of microbial species [21]. This strategy likely accounts for the ignorance of the systemic immune system toward many of the bacterial antigens inhabiting the colon. The proximal small intestine lacks a continuous mucus layer to anatomically contain and compartmentalize the resident microbes. Here, secretion of antimicrobial proteins by Paneth cells, microbiota-specific IgA secretion by B cells and IL-22 production from innate lymphoid plays an important role in regulating the spatial and compositional arrangement of the microbiota [22,23]. Many microbiota-derived antigens do encounter immune cells and those interactions result, in most cases, in immune tolerance. How are tolerogenic responses favored after these encounters? An overview of these mechanisms highlights the many and sometimes redundant strategies that vertebrate hosts utilize to avoid ongoing intestinal inflammation.

As the first layer of cellular defense, IECs secrete thymic stromal lymphopoeitin (TSLP) and tumor growth factor-beta (TGF-β), both of which induce the secretion of tolerogenic cytokines from intestinal dendritic cells [24]. These antigen-presenting cells in turn induce the development of regulatory T cells (Tregs) by secreting TGF-β and retinoic acid [25,26] and promote the differentiation of IgA-producing plasma cells, downregulating the pro-inflammatory arms of mucosal immunity [27]. Tregs are essential for immune tolerance toward the microbiota and their expansion is favored over effector T cells upon microbial neonatal exposure. Treg-deficient mice spontaneously develop IBD due to the overwhelming intestinal pro-inflammatory effector T cell response [28]. This dependence on Tregs to achieve tolerance to the microbiota forces the question of how these cells are trained to specifically respond to these commensal-derived antigens. These cells are selected in the thymus for their ability to suppress T cells with a high affinity to self-MHC molecules, thus preventing autoimmune responses. A recent study provides evidence that Tregs may receive further education from the intestinal microbiota. The microbiota is essential for the peripheral development of colonic Tregs from naïve T cells. Furthermore, there is a much higher heterogeneity in the repertoires of T cell receptor (TCR) α-chain from FoxP3+ Tregs of the colonic lamina propria compared to Tregs from secondary lymphoid organs [29]. Thus, there are post-thymic mechanisms of T cell education that occur peripherally via interactions with the commensal microbiota, which implies that the immune system may have evolved to rely on the microbiota to complete the training of the population of immune cells.