

REVIEW

High microbial turnover rate preventing atopy: a solution to inconsistencies impinging on the Hygiene hypothesis?

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The Hygiene hypothesis

The allergy epidemic has been attributed to changes in the interactions between humans and the microbes of their ecosystem consequent to a 'western lifestyle' [1]. This theory, unlike others (pollution, increased allergen exposure), explains the epidemic in evolutionistic terms [2]. Microbes that co-evolved with mammals influenced the evolution of their immune structures and functions along the zootic scale up to primates. Partially deprived of these microbial stimuli, some components of the human immune system (TH2-like activities) are no longer adequately regulated by other components (TH1-like activities) [3,4]. Therefore, in modern societies, allergies spread among new cohorts according to gradients dictated by hygiene and by the individual degree of genetic predisposition to atopy. Collectively, these concepts are known as the 'Hygiene' hypothesis [1,5].

Arguments apparently at variance with the Hygiene hypothesis

The Hygiene hypothesis has been challenged by conflicting data on the alleged atopy-protecting role of measles [6,7] and other airborne viruses [8,9], and by the not infrequent occurrence of severe allergic asthma and atopic sensitization in unhygienic American inner cities [10], in rural Africa [11] and among adults immigrants who in their infancy were exposed to a traditional, unhygienic lifestyle [12]. These inconsistencies, which seemingly confute the Hygiene hypothesis, may, on the contrary, cast further light on the theory.

Measles

An initial report that measles virus may protect from atopy [6], has not been confirmed [7]. Indeed, it seems unlikely that evolution relied on transient infection by a single virus

to down-regulate TH2 responses in mammals. Moreover, measles has only recently become a worldwide disease: natives of South America, for example, were not exposed to measles until the arrival of Europeans in the 15th century [13]. If measles was essential to prevent atopy or asthma, most native Americans would have probably been atopic and asthmatic.

Airborne viruses

The prevalence of atopy and asthma was inversely related to childhood infections by airborne viruses in some studies [reviewed in 8], but not in others [9,14]. From an evolutionistic and epidemiological perspective, these inconsistencies are not surprising. First, airborne viruses that infect humans have not been constantly present throughout mammalian evolution [2]. Second, some of them even induce or exacerbate asthma [15] thereby introducing a potent confounding effect in epidemiological studies addressing the Hygiene hypothesis. Finally, most respiratory viruses are so infectious that hygiene and declining family size could hardly reduce their circulation among humans (as compared, for example, to infections transmitted through other routes) [14]. Therefore, although we have not enough data either to refute or to accept the hypothesis [8] that certain viruses may protect from atopy, both epidemiological background and evolutionistic arguments indicate that the hygiene hypothesis should be examined mainly in the light of exposure to other microbes.

Inner city asthma

Most asthma cases among children living in American inner cities are due to atopic sensitization to indoor allergens, including mites and cockroaches [16]. Hence it was extrapolated that atopic sensitization in inner cities is not less frequent than in more affluent and hygienic urban areas [10], which is in clear contrast to the hygiene hypothesis. It is tempting to speculate, however, that children living in 'unhygienic' inner-city areas may be indeed not so heavily

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exposed to a wide array of bacteria compared with children that have access to natural soil, eat only food not industrially processed, or live in areas endemic for many different orofaecal infections. Thus, it seems that the words 'dirty' or 'hygiene' are too generic to label environments facilitating or protecting from allergy. American inner cities represent very interesting areas for further epidemiological research on the Hygiene hypothesis.

Atopy in developing tropical areas

Inhabitants of rural, unhygienic areas of developing countries, not rarely have serum IgE responses toward airborne allergens [11]. However, severe helminthic infestation, very frequent in such areas, can potentiate TH2 responses through bystander effects [18], which may counteract the downregulating effects exerted by other microbes. These atopic responses are rarely associated with allergic disease [11], suggesting that severe helminthic infestation may stimulate biological activities (e.g. by competing for IgE-binding sites on effector cells [19]) that may prevent the inflammatory consequences of exposure to allergens. Interestingly, eradication of helminths from Venezuelan patients produced a decrease in total serum IgE level, paradoxically associated with increasing skin reactions to airborne allergens [20].

Onset of atopy in adulthood

IgE sensitization to common or occupational allergens not infrequently start in immigrants or adult workers who in their early years were exposed to a traditional, unhygienic lifestyle [12]. Therefore, atopy does not appear to be programmed exclusively in early infancy and infections acquired within a 'window period' early in life may not provide lifelong protection. Downregulation of atopic responses may rather be a dynamic and quantitative process, extending at least to adolescence and probably to adulthood, subjected to genetic factors that dictate how early, intense and prolonged continuous microbial exposure must be to afford protection from atopic sensitization or to delay its onset [12,14].

Bacteria stimulating GALT (and other MALT sites) may prevent atopy: a step forward in the Hygiene hypothesis?

Given these inconsistencies, the simplistic concepts 'hygiene favours allergy' and 'dirt prevents allergy' are not substantiated by evidence-based data. The question is rather: 'which microbes may prevent atopy and where would they exert their preventive effect?'

A preventive role against allergy and asthma was initially attributed to respiratory viruses [4,8], whereas infections of the gastrointestinal tract were thought to protect from food allergies [4]. In contrast, respiratory allergies were less frequent in Italian military cadets with antibodies against hepatitis A virus (HAV) [21] and were inversely related to other orofaecal/foodborne infections, but not to airborne viruses [14]. The inverse association between atopy and HAV antibodies was confirmed in a general population sample [21]. In these studies, exposure to HAV was considered a reliable proxy of being reared in an environment characterized by fecal contamination and high exposure to orofaecal and food-borne microbes may underlie the allergy and asthma epidemic [12,14].

These epidemiological studies suggested that the gut mucosa and GALT had to be included among the sites where microbes may inhibit TH2 responses to inhaled allergens [12,14,21,22]. A link between respiratory diseases due to 'inhalant' allergens and events occurring in the gut is not that paradoxical. Eosinophilic inflammation has been observed in the duodenal mucosa of atopic and asthmatic subjects, thus implicating that the whole mucosal immune system is involved in these conditions [23]. Aerosolized administration to adults of radiolabelled pollen grains of *Poa pratensis* demonstrated that most of these airborne particles, after impact on orofaringeal mucosal surfaces, are ingested, and only a minority is inhaled to bronchi [24]. Moreover, ingestion of allergenic particles should be facilitated in newborns because of their narrower airways. Therefore, in newborns, microbes uptaken by antigen-presenting cells on nasal associated lymphoid tissue (NALT), Waldeyer's ring and GALT may induce dendritic cell maturation [4,25] and a TH1 bystander adjuvant effect on allergen-specific T-cell responses [4]. Lymphocytes from these sites may then home in to other MALT sites (including BALT) and the skin. This scenario is in line with the concept that lymphocytes of nasal, bronchial and enteric mucosae belong to the same recirculating pool.

Bengt Björkstén's group was the first to propose that persistent 'pressure' on the human immune system by bacteria colonizing the gastrointestinal tract may prevent atopic sensitization to airborne allergens [26]. They examined 1-year-old infants living in two countries with a low (Estonia) and high (Sweden) prevalence of atopy, and found that *Lactobacilli* and *Eubacteria* were more frequent in the intestinal microflora of Estonian infants, while *Clostridia* was more frequent in Swedish infants [26]. They then observed that allergic 2-year-old children were colonized less often by *Lactobacilli*, and harboured higher counts of aerobic bacteria (coliforms, *Staphylococcus aureus*) than non allergic children [27].

Böttcher and coworkers evaluate the faecal composition

of short chain fatty acids (an indicator of colon microflora composition) of allergic and nonallergic 13-month-old infants [28]. Allergic subjects had higher levels of i-caproic acid, a marker of colonization by *Clostridium difficile*, and lower levels of propionic, i-butyric, butyric, i-valeric and valeric acid. The authors conclude that the composition of gut microflora in allergic subjects may be causally related to their disease [28]. However, faecal content in short chain fatty acid is affected mainly by the metabolic activity of bacteria growing in the colon lumen; these are only partially representative of the small percentage of bacteria that can adhere to epithelia, translocate and interact with dendritic cells and other cellular components of GALT. Nevertheless, studies from Bjorksten's group suggest therefore that changes in human gut flora due to 'westernization' may be related to the increase in allergy [26,28].

Atopy prevention may require a high turnover of different bacteria genera, species and strains

It may be speculated that the gastrointestinal microflora of westernized children may predispose to atopy because of the stable predominance of certain bacteria (e.g. clostridia) or because of the absence of other bacteria (e.g. *Lactobacilli*). Accordingly, atopy may be prevented simply by substituting one colonizing species with another. Unfortunately, this is probably not the case. In fact, it is evolutionistically unlikely that one single bacterial species has the important task of protecting mammals from atopy. Moreover, translocation through the epithelial barrier and subsequent potent stimulation of the local immune system by a bacterium are only transient and limited to initial colonization phases, and they are soon prevented by a local

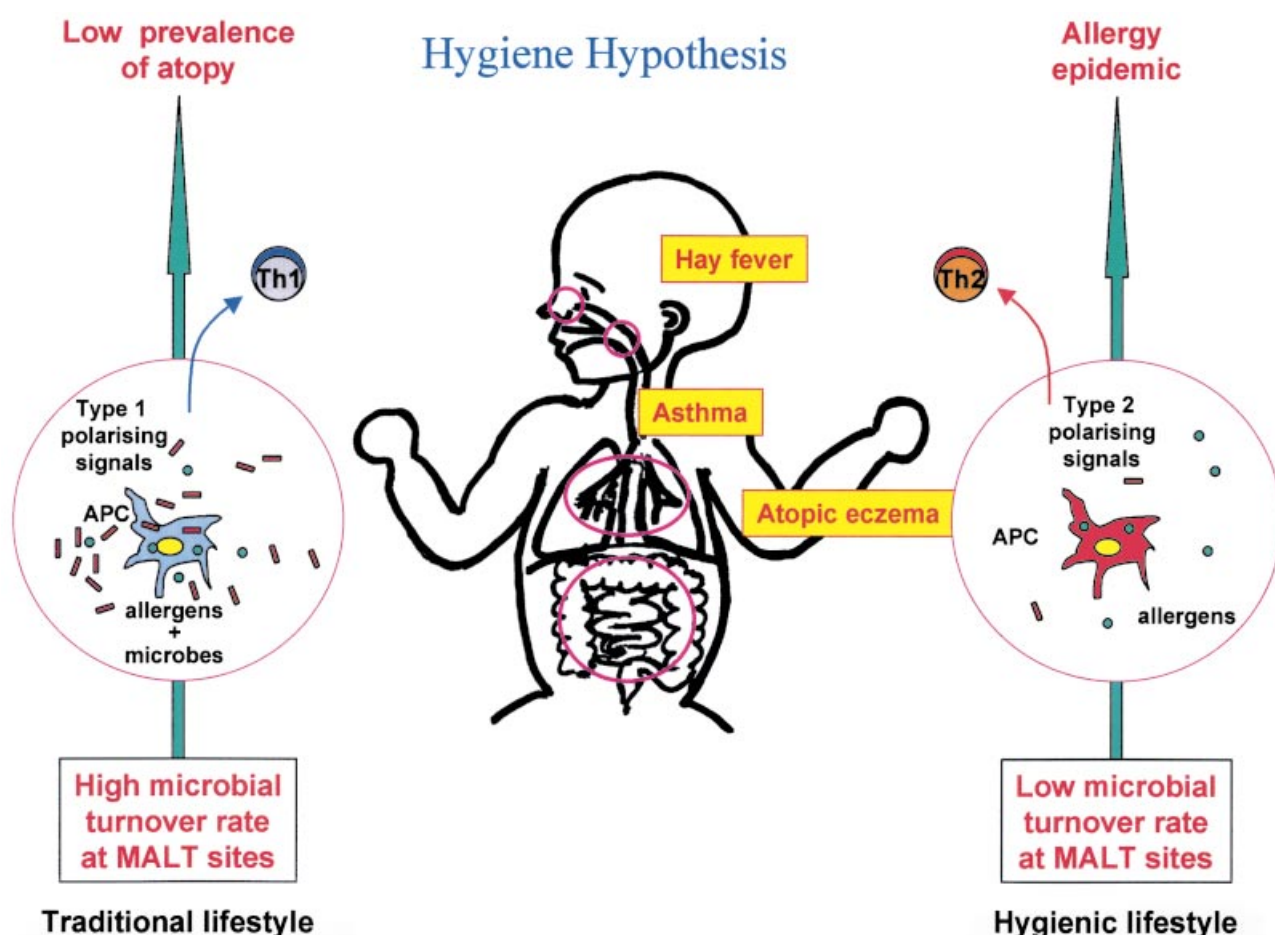


Fig. 1. A reduced bacterial turnover at mucosal surfaces may be responsible of the association between hygiene and atopy or atopic diseases among populations living with a western lifestyle. The sites involved may be those where antigen presenting cells uptake, process and present microbial and nonmicrobial antigens to specific T cells. The quality and intensity of bacterial stimulation would dictate the state of activation and the kind of accessory signals that antigen presenting cells transmit to surrounding cells of the innate and adaptive immune system.

IgA response [30,31], otherwise, we probably could not tolerate our own microflora.

A stronger hypothesis is that a high turnover of appropriate bacteria at mucosal level (NALT, BALT, GALT), rather than specific, stable colonization by certain species, provides the potent, continuous immune stimulation necessary to prevent atopy and atopic diseases. Interestingly, longitudinal studies of the gut microflora composition of infants living in Pakistan (a country with low prevalence of allergies) [31] and Sweden (higher prevalence of allergies) [32], showed that most infants in both countries harboured *Escherichia coli*. However, Pakistani infants were colonized earlier and more frequently by new *E. coli* strains [31–33], while Swedish infants carried the same strain for months and years [32]. As only new colonization by a different strain would exert, although transiently, new immune stimulation, Agnes Wold hypothesized that only a high turnover of bacterial species and strains at the gut level [30] would guarantee that ‘continuous pressure’ [26] on the immune system considered essential to prevent atopy. This hypothesis not only has evolutionistic plausibility (an atopy-preventing effect being attributed to many different bacterial species), but can also explain the ‘effects’ of sibship size and birth order on atopy [1,34]. Indeed, cross-infections with new bacterial strains, rather than a specific and stable colonization, are facilitated in large families living a traditional lifestyle. We should consider, however, that not only GALT, but also other MALT sites (e.g. NALT, Waldeyer’s ring, BALT and related lymph nodes) may be the target of such an atopy protective effect [14].

Should a high bacterial turnover be essential to prevent atopy, different bacterial species must share common immune-modulating effects. There are indeed many examples of molecules that stimulate type-1 immunity and that are shared by many bacteria, including cell wall components of Gram-negative bacteria (lipopolysaccharide) [35], mycobacteria [2], and DNA containing immune-stimulating sequences (ISS, CpG motifs) [36]. Indeed, the concentration of exogenous lipopolysaccharide in house dust was inversely related to the risk of atopy among infants at risk for asthma [37]; higher exposure to saprophytic mycobacteria was proposed as a cause of lower risk of atopy in Japanese children [38]; and bacterial DNA-ISS inhibited *in vivo* airway allergic inflammation in animal models of asthma [39]. Different bacterial factors may therefore lead, through different molecular mechanisms, to the same outcome: inhibition of TH2 responses and allergic inflammation.

‘Immuno-education’ in early life: a novel strategy or wishful thinking?

A diversified, persistent and continuously renewed microbial stimulation of human mucosal lymphoid components

and related lymph nodes may be necessary to sustain physiological bystander effects on the microenvironments at the site(s) of priming, immune deviation and clonal expansion of T cells against allergens in order to afford protection against atopy (Fig. 1). These concepts may at least partially solve the inconsistencies impinging on the Hygiene hypothesis. If we knew how high microbial turnover ‘educates’ our immune system, perhaps we could learn to mimic its action without giving up our hygienic lifestyle. This novel concept of safe ‘immuno-education’ [40], aimed at facilitating harmonious development and maintenance of the immune system, may one day become normal practice not only for protection against infectious diseases, but also for prevention of immune-mediated diseases, including allergies [12].

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