Are infections protecting from atopy?

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The 'Hygiene Hypothesis' proposes that overcrowding and unhygienic contacts early in life may protect from atopic diseases by facilitating exposure to microbes. Longitudinal studies have recently shown that among subjects exposed early in life to other children at home, or at day care, the risk of wheezing steadily declined with age to levels significantly lower than controls. Evidences supporting a protective role of respiratory infections or BCG immunization on the development of allergic asthma are still insufficient. By contrast, the observation of a lower prevalence of atopic sensitization among children raised on a farm has been consistently reproduced. Several new studies have recently investigated the role of changes of human microbial flora, declining exposure to foodborne and orofecal infections, to helminths and to environmental sources of endotoxin as putative contributors to the rise of allergy and asthma cases among populations living with a western lifestyle. Curr Opin Allergy Clin Immunol 1:413–419.

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Current Opinion in Allergy and Clinical Immunology 2001, 1:413–419

1528-4050

Introduction

According to WHO estimates, pathogenic microbes are still responsible for about one-third of deaths worldwide [1], and this is an obvious reason for considering them as enemies to be eliminated. Our understanding of infectious diseases, however, has greatly evolved during the last century: we are now aware that the outcome (disease, colonization, elimination) of the interaction between a host and a given microorganism is only partially determined by the latter, a major role being played by the host, the milieu and the social and physical environment [2]. We have also learnt that our immune system has coevolved with microbes, and it is programmed in such a way that its postnatal development and homeostasis are susceptible to the conditioning influence of the microbes themselves [3]. Thus microorganisms are not just 'enemies' but fundamental components of our ecosystem that can harm, leave unchanged or even improve our health.

Our progressive understanding of such a fascinating scenario is well reflected by the many-sided role attributed to microbes in the natural history of asthma and allergies. The intuitive aggravating role of some airborne pathogenic viruses on asthma and allergies has been investigated since the 1950s and it is now widely accepted [4]; only in the 1990s, however, have elegant studies highlighted the contra-intuitive concept that exposure to some microbes may indeed protect from the same diseases [5]. Here we review articles published in the last year that investigate the putative protective role of certain infections from the development of the atopic phenotype, while details on less recent literature may be found elsewhere (reviewed in [5,6]).

Sociodemographic factors

In a British cohort study, Strachan observed that the risk of having hay fever was directly related to the socioeconomic status (defined by the father's occupation) and inversely related to the overall number of siblings (sibship size 'effect'); he also noted that hay fever was less frequent in the presence of older rather than younger siblings (birth order 'effect') [7]. Assuming that infections were acquired more frequently in large, less affluent families and earlier in the presence of many older siblings, Strachan proposed that exposure to common infections, especially very early in infancy or, through the mother, even *in utero*, may 'protect' from hay fever [7]. As a corollary, he hypothesized that the decline in cross infections within young families due to decreasing family size and improvement in hygienic

standards is, among the set of characteristics of the western lifestyle, the one mostly responsible for the increase in atopy prevalence ('hygiene hypothesis') [6,7].

The direct association between socio-economic status and atopy has been repeatedly observed in developed countries, including the USA [8] and Italy [9]. Interestingly, Bergmann *et al.* [10] recently observed this association among German adults, but not in their children. They reported data on atopic sensitization and atopic diseases in 1314 children born in 1990 and followed up to the age of 6 years as well as in their parents. Among parents, a high socio-economic status was related to skin sensitization (OR 1.76), lifetime prevalence of hay fever (OR 2.36) and asthma (OR 1.74) but not to atopic dermatitis (OR 0.90). Among children, a high socio-economic status was related only to atopic dermatitis starting after 3 years of age (OR 2.42), but not to atopic sensitization, hay fever or asthma. The authors' interpretation was that parents with a high socio-economic status, thanks to their experience, could avoid exposing their children to dangerous factors [10]. A more likely explanation of Bergmann's intriguing data is, in our opinion, the following: factors facilitating atopy may have been typical only of families with a high socio-economic status during the parents' infancy (around the 1960s), but spread across the whole German society (including families with the lowest socio-economic status) during their children's infancy (in the 1990s). Therefore a high socio-economic status would no longer be a risk factor of atopy in German children, not because of a decreased risk among the richest families, but because of an increased risk among the poorest. The rising trend of allergic diseases observed in Western Europe during a period of one generation makes this interpretation more plausible; indeed, in the post-war period, advantaged German families could acquire a 'western lifestyle' decades earlier than the less advantaged families.

The sibship-size 'effect' originally reported by Strachan has been reproduced in more than 20 cross-sectional studies. Only recently, however, was it confirmed for the first time by a prospective study. In a birth-cohort study involving 1035 children, Ball *et al.* [11] observed that among subjects exposed early in life to other children at home, or at day care, the risk of wheezing steadily declined with age, being high (OR 1.4) at the age of 2, low (OR 0.8) at 6 years and very low (OR 0.3) at 13 years. The most plausible explanation for their results was that some of the bacterial or viral infections (facilitated by early contacts with other children) had caused wheezing of infectious etiology early in life, but some others also inhibited atopic sensitization and the subsequent development of wheezing of atopic etiology later in life. Other explanations, including differential exposure to mites, pets and moulds, as well as recall or recruitment biases, were not supported by data and were considered very unlikely by the authors [12].

Airborne viruses

Reports of ecological associations of lower prevalence of atopic diseases with higher prevalence of upper and lower airway infections in Eastern Asia suggested that early acquisition of airborne viral infection in still healthy infants may protect them from the development of asthma later in life [13]. This hypothesis would not be, in principle, in contrast with the well known aggravating role of the same viruses in subjects with already established asthma [4], as they could indeed play different roles according to the health status of the host, the time and the context of infection.

In the above-mentioned birth-cohort of 1314 German children, several kinds of infectious diseases, atopic sensitization and asthma were monitored in the first 7 years of life. In this population, Illi *et al.* [14] used periodic parental questionnaires to record the episodes of respiratory infectious disease in the first 3 years of life; then they separated the episodes limited to the upper airways from those extended also or exclusively to the lower airways and analysed their relation to the development of asthma symptoms or atopic sensitization at the age of 7 years. The main findings were that children with an increasing number of wheezing episodes early in life during airway infectious disease had a higher frequency of asthma at 7 years; children with more than one episode of airways infectious disease limited to the upper airways had a lower risk of having asthma symptoms at 7 years of life. The authors conclude that repeated viral infections early in life other than those affecting the lower respiratory tract may reduce the risk of developing asthma up to school age. An alternative explanation, also mentioned by the authors, is that children already predisposed to asthma are those who wheezed in early infancy in response to viral respiratory infections (reverse causation). This conclusion that respiratory viral infectious diseases had no influence on the development of allergic asthma in Illi's cohort is further supported by the evidence of no relationship between infectious diseases due to airborne viruses and skin sensitization to airborne allergens at the age of 5 years. These kinds of studies are indeed based upon recording host symptoms, so that what is measured is not simply exposure to microbes (infection), but the outcome of the interaction between microbes and the host (infectious disease); the relevance of this problem is much stronger in the study by Illi *et al.*, where the symptoms of the same target organs (the lung and the nose) are utilized to describe both the dependent (asthma) and the independent (infectious disease) variables.

More reliable conclusions may be achieved by studies designed to objectively measure real exposure to airborne viruses (independently from symptoms) and atopic sensitization. In a case–control study among Italian recruits, we found no association between atopic sensitization and serologic evidence of previous exposure to a series of airborne viruses, including measles, mumps, rubella and chickenpox [15]. Although our data were not sufficient to refute the hypothesis [13] that other airborne viruses may protect from atopy, they suggest that the hygiene hypothesis should be examined mainly in the light of exposure to microbes transmitted through other routes.

Mycobacteria

In a paper now considered to be pioneering, Shirakawa *et al.* [16] found that among Japanese children immunized with BCG at 3 months of age, those producing positive skin responses to intradermal tuberculin injection at 12 years of age had lower serum levels of total IgE and TH2 cytokines (IL-4, IL-10 and IL-13), higher serum levels of interferon gamma, and lower prevalences of atopy and atopic diseases [16]. These associations suggested that natural exposure to mycobacteria [16], or immunization with BCG [17] may protect from atopy; a third explanation proposed is that a high skin reactivity to tuberculin and strong immediate reactions to allergens may be mutually excluding phenotypes of the same set of genes regulating predisposition to a TH1 or TH2 predominance in the immune responses [18]. At least four studies have examined these topics in the last year.

Yilmaz *et al.* [19] examined delayed skin reactivity to i.d. PPD in 538 atopic and 198 non-atopic Turkish children, all immunized with BCG since at least 6 months. They found no relationship between atopic diseases or total serum IgE levels and the diameter of PPD induration size.

Omenaas *et al.* [20] examined the relationship of delayed skin reactivity to tuberculin total with serum total and allergen specific IgE in 574 Norwegian adults aged 20–44. All of them had been vaccinated with BCG at the age of 14. They found no relationship between serum total IgE levels or positivity to RAST assays and the reactivity to tuberculin. Their data suggest that a 'protective' effect of BCG against atopy, if any, could not occur if the vaccine is administrated after childhood. In Guinea-Bissau, Aaby *et al.* [21] examined the prevalence of skin sensitization to common airborne allergens among 271 children vaccinated with BCG in infancy in comparison with 53 never vaccinated with BCG. Atopy was observed in 40% of non-vaccinated children and in 21% (adjusted OR 0.19; CI 0.06–0.59) of vaccinated children. The authors concluded that BCG vaccination given early in infancy may prevent the development of atopy in African children. In contrast, Gruber *et al.* [22] found that BCG immunization early in life in German children was not related to a marked decrease in atopic sensitization or allergic disease later in life. While experimental studies continue to demonstrate that BCG immunization may suppress or prevent eosinophilic allergic inflammation in murine models of asthma [23], the extension of such a beneficial role in humans remains to be proven.

Foodborne and orofecal infections

In a retrospective study aimed at testing the hygiene hypothesis, we found that atopy was inversely related to seropositivity for hepatitis-A virus, a marker of high exposure to orofecal microbes, in Italian military cadets [24]. That observation was confirmed in a general population sample [25] and it was suggested that 'fecal contamination of the environment may protect from atopy' [24]. This concept was supported later by studies showing that, among children living in a farming environment, direct exposure to stables where livestock are kept was a strong protective factor from development of atopy and atopic diseases [26]. Evidence of a lower prevalence of atopic sensitization among children raised on a farm was also recently provided from studies in Finland [27], Canada [28], and Austria [29].

A recent study by von Mutius *et al.* [30] has tried to disclose what kind of exposure is mediating this protective effect of a farming environment on the development of atopy. They measured the level of environmental endotoxin exposure in homes of farmers' children, children having regular contact with livestock and control children having no contact with farm animals. They found that endotoxin concentrations were highest in stables of farming families, but were also significantly higher indoors in the dust from kitchen floors (143 versus 39 EU/mg, $P<0.001$) and children's mattresses (49 479 versus 9383 EU/m^2, $P<0.001$) compared with control children from non-farming families. Endotoxin levels were also significantly higher in mattresses and dust from kitchen floors in households where children had regular contact with farm animals (38.6 EU/mg and 23 340 EU/m^2, respectively) compared with control subjects. They concluded that environmental exposure to endotoxin and other bacterial wall components is an important protective determinant for the development of atopic diseases in childhood. Similar conclusions have been reached by Gereda *et al.* [31] who found an association of low levels of endotoxin in the house dust with the proportion of peripheral blood CD4 T lymphocytes producing interferon gamma and with skin sensitization to common allergens. The authors concluded that indoor endotoxin exposure early in life may protect against allergen sensitization by enhancing type 1 immunity.

In principle, exposure to endotoxin should be a proxy of exposure to Gram-negative bacteria. Our studies on Italian recruits seem to support a similar conclusion. By extending our previous work on Italian recruits, we found that atopy was inversely related not only to hepatitis-A virus positive serology but also to other orofecal/foodborne infections (*Toxoplasma gondii* and *Helicobacter pylori*) [15]. These data suggested that food hygiene and declining exposure to orofecal microbes may underlie the allergy and asthma epidemic and focused the attention on the gut mucosa and GALT as the sites where microbes may inhibit the development of the atopic phenotype. Human microflora is by far the most relevant source of Gram-negative bacteria; moreover, it is conceivable that endotoxins stimulating our immune system derive from endogenous, more than from exogenous, bacterial sources.

Microbial flora and its modulation

It is has been known since the 1970s that mice reared under sterile conditions do not develop a fully functional immune system and, more recently, that they are not susceptible to the induction of oral tolerance [32]. The relevance of these observations for the understanding of atopy in humans was underlooked until Bjorksten's group reported on 1-year-old infants living in two countries with a low (Estonia) and high (Sweden) prevalence of atopy, and found that *Lactobacilli* and *Eubacteria* predominated in the intestinal microflora of Estonian infants, while *Clostridia* was more frequent in Swedish infants [33]. It was therefore proposed that a persistent 'pressure' on the human immune system by bacteria colonizing the gastrointestinal tract is a critical factor that may prevent atopic sensitization to airborne allergens [34], a hypothesis in line with the one emerging in parallel from studies among Italian recruits [18] and from studies in developing countries [35,36].

Recently, Bottcher *et al.* [37] found that 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 concluded that the composition of gut microflora in allergic subjects may be causally related to their disease. In 76 infants at high risk of atopic diseases, Kalliomaki *et al.* [38] investigated prospectively the composition of intestinal microflora at 3 weeks and 3 months of age and the appearance of skin sensitization to common food and airborne allergens at 12 months of age. By using fluorescence in-situ hybridization, they found evidence of a reduced ratio of bifidobacteria to clostridia in the stools of children developing atopy, compared with the non-atopic subjects. They concluded that 'differences in the neonatal gut microflora precede the development of atopy, suggesting a crucial role of the balance of indigenous intestinal bacteria for the maturation of human immunity to a non atopic mode'.

If colonization by commensal or pathogenic bacteria is important in conditioning the development of atopy, then it is conceivable that any intervention conditioning both composition and turnover of microflora should also exert effects on the development of the atopic phenotype.

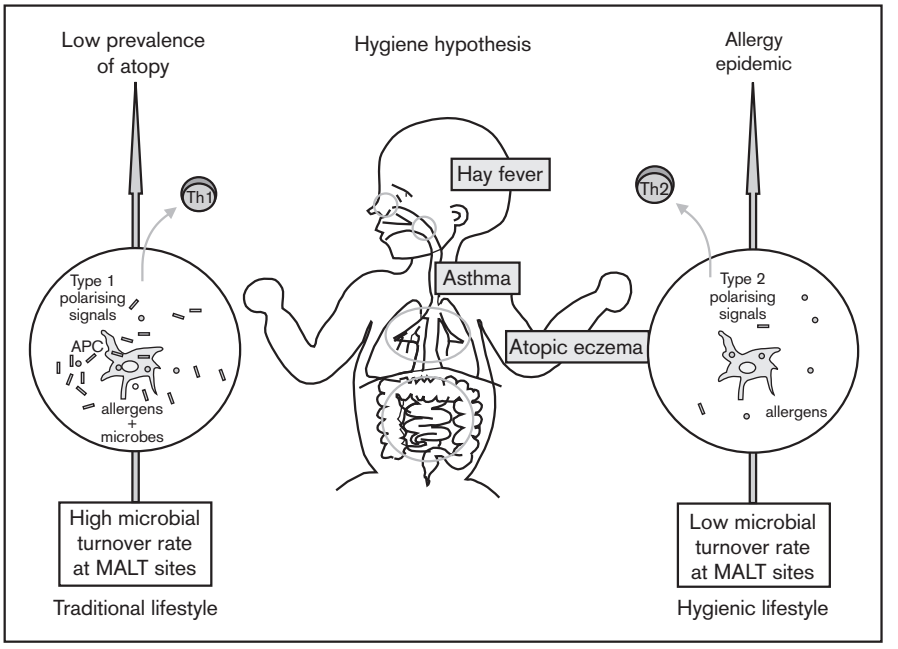
Oyama *et al.* [39] reported that kanamicin given orally for 7 consecutive days to BALB/c mice at 3 and 52 weeks of age increased serum levels of total IgG1 and IgE while decreasing serum IgG2a levels. Spleen cells from treated mice produced more IL-4 and less interferon gamma when stimulated with immobilized anti-CD3 antibody compared with untreated controls. The authors' interpretation was that a Th2 polarized immune deviation occurred in treated mice, possibly through an influence of the antibiotic on the intestinal microflora.

In a population-based sample of Belgian children, Droste *et al.* [40] found that the use of antibiotics during the first year of life was significantly associated with lifetime asthma (OR 1.7), hay fever (OR 2.3) and eczema (OR 1.3) but not with atopic sensitization at 7 or 8 years of age. The authors considered this relationship as ambiguous, since the use of antibiotics may only be the representation of an increased risk on respiratory infectious diseases in children with propensity to asthma and allergies (reverse causation). The observed associations, however, persisted after accounting for known respiratory infectious diseases. Therefore, Droste *et al.* concluded that their data suggest that antibiotics early in life may condition the maturation of the immune system.

Kalliomaki *et al.* [41] recently completed a double-blind placebo controlled trial with 132 Finnish children at high risk of developing atopic eczema. Lactobacillus GG was given daily prenatally to mothers ($n = 64$) in the 2–4 weeks before expected delivery, to bottle-fed newborns or to mothers of breast-fed newborns, respectively, during the first 6 months of life. Controls and their mothers ($n = 68$) assumed placebo. Atopic eczema was diagnosed at 12 months of age in 15/64 (23%) 'treated' children versus 31/68% (46%) of controls (relative risk 0.51; 95% CI 0.32–0.84). The authors concluded that Lactobacillus GG was effective in prevention of early atopic disease in children at high risk. A major limitation of this study is that the authors assumed that giving Lactobacillus GG to mothers during breast-feeding would have resulted in similar amounts of Lactobacillus GG in infant feces, but they based this assumption on previous experience and did not prove this in the cohort

Figure 1. Hygiene hypothesis

A reduced bacterial turnover at mucosal surfaces may be responsible for 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 non-microbial 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 (reproduced from *Clinical and Experimental Allergy* with permission).



participating in the trial. Lactobacillli, however, have been recently shown to stimulate type 1 immune response both *in vitro* [42] and *in vivo* [43].

Helminths

Most of the epidemiologic studies reviewed so far were based on populations living in temperate areas of developed countries, characterized by a very low prevalence of helminth infection. Helminths play a fundamental role in the homeostasis of the so-called IgE network [48], which is considered to have evolved mainly to fight them. Since the 1970s, Gerrard *et al.* had observed that in Saskatchewan metis (living a very traditional lifestyle) had higher total IgE levels but less atopic diseases than white Caucasians (living a hygienic way of life). He anticipated that declining exposure to helminths and the fight against infectious diseases would have caused a rising trend in atopic diseases among populations living with a western lifestyle [44].

Total IgE serum levels were higher and specific IgE antibodies against mites more frequent in rural than in urban areas near Jimma (Ethiopia) [45]. Only in urban Jimma, where total IgE levels were lower, IgE sensitization against mites was associated with allergic asthma (a finding recently confirmed in urban Gondar [46]). These data suggested that helminths infections, very frequent in rural African areas, can potentiate production of IgE against environmental antigens but also induce biological activities that may prevent the inflammatory consequences of exposure to allergens. In the 1970s, it was proposed that polyclonal IgE induced by helminths could compete for IgE binding sites on effector cells with allergen-specific IgE [47].

An inverse association between infection with *Schistosoma mansoni* and skin response to aeroallergens has recently been reported by Araujo *et al.* [48] in Bahia, a tropical area of Brazil. Similarly, van den Biggelaar *et al.* [49] examined 520 Gabonese schoolchildren in a cross-sectional study and found a lower prevalence of a positive skin reaction to house-dust mite among children with urinary schistosomiasis than in those schistosome-free (OR 0.32; 95% CI 0.16–0.63). The production of interlukin-10 obtained by incubating peripheral blood mononuclear cells with schistosoma antigens was inversely associated with skin reactivity to mite allergens. The authors concluded that helminth-induced interleukin-10 can play a part in counteracting inflammation-mediated allergic diseases, even in the presence of specific IgE responses against common allergens. They therefore provided further insights on the mechanisms by which helminths may sustain IgE responses, yet preventing damage from the bystander induction of specific IgE responses against common allergens [50].

Conclusions

Epidemiological studies will by definition never demonstrate if some kind of microbial exposure is able to protect from atopy. A single infection that is able to prevent atopy probably does not exist and it is

evolutionistically unlikely that one single microbial species has the important task of protecting mammals from atopic sensitization. Available data reviewed so far suggest that a high turnover of appropriate bacteria at mucosal level (NALT, BALT, GALT), rather than specific, stable colonization by certain species, can generate the kind of microenvironment necessary to prevent atopy and atopic diseases. This 'turnover' hypothesis not only has evolutionistic plausibility (an atopy-preventing effect being attributed to many different bacterial species), but also has the potential to explain the 'effects' of sibship size and birth order on atopy, that is, the evidence at the origin of the 'hygiene hypothesis' (Fig. 1) [51]. The way ahead to demonstrate what remains a hypothesis, however, is still long.

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