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Review article

Asthma and atopy – the price of affluence?

Irrespective of improved knowledge of many aspects of atopic diseases, the unfavorable trends in their prevalence particularly among children could not have been reversed. A growing body of evidence suggests that something may lack from our societal affluence that has the capacity to provide protection against the development of atopic diseases. Much attention during the last years has been devoted to the hygiene hypothesis. This review outlines the impact of environment and lifestyle, particularly from the perspective of the East–West gradient, on the development of atopic diseases, with a special emphasis on the hygiene hypothesis in its broadest sense.

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Numerous epidemiological studies from different parts of the world have consistently revealed two main patterns in the occurrence of atopic diseases; (i) the substantial increase over the past 30–40 years particularly in affluent countries (temporal changes) and (ii) the enormous variation between countries or areas, even between areas that are geographically adjacent (spatial variation). These patterns, which were already discernible 20 years ago (1), point to environmental determinants that are associated with increasing affluence and associated societal changes (2).

Much of the current asthma/atopy research is focused on attempts to clarify the causes of these phenomena. Atopic asthma is the most common chronic disease of children, and the still rising trends in asthma and atopy prevalence worldwide could not have been reversed irrespective of improved knowledge of many aspects of the diseases. This review briefly outlines the epidemiological data on the impact of environment and lifestyle, particularly from the perspective of the East–West gradient, on the development of atopic diseases. A special emphasis will be laid on the hygiene hypothesis. The data were identified from Medline searches and references from relevant articles. Search terms were 'allergy', 'asthma', 'atopy', 'epidemiology', 'international', 'infections', and 'hygiene hypothesis'. Papers in English language were reviewed.

The East–West gradient in the occurrence of atopic diseases

In this review, the term 'West' refers generally to developed, affluent countries with modern lifestyle and a market economy, whereas the term 'East' refers broadly to developing or formerly socialist, less-affluent countries with more traditional lifestyle.

Several international comparisons from the late 1980s lent support for the observations by Gregg (1) that considerable variation in asthma prevalence between countries existed, and that asthma was markedly more common in developed than in developing countries (3–5). However, these studies included only a limited number of countries, and are not comparable because of different and mostly nonstandardized methodology.

To date, two comprehensive international surveys using standardized methods, one in children [the International Study of Asthma and Allergy in Childhood (ISAAC)] and the other in adults [the European Community Respiratory Health Survey (ECRHS)], have been completed. These studies have finally confirmed the earlier observations and provided a reliable global map of the prevalence of atopic diseases (6). The worldwide ISAAC study, which was undertaken to identify factors that may explain the rise in atopic diseases, comprised over a half a million children aged 6–7 and 13–14 years from 155 centers in 56 countries. By using standardized written and videotaped questionnaires, the highest prevalence of asthma symptoms were mainly found in affluent English-speaking countries, and lowest in Eastern Europe, Russia, China, India and Ethiopia. For allergic rhinoconjunctivitis and eczema, the areas of the lowest prevalence were similar to those for asthma symptoms (7, 8). The ECRHS, which measured the occurrence of asthma, atopy and bronchial hyperresponsiveness by standardized methods in adults from 22 countries,

including countries not only from Europe but also from Australia, Africa and India, showed that, similarly to children, asthma symptoms occurred most frequently in English-speaking affluent countries (the UK, Australia, New Zealand, the Republic of Ireland and the USA). Lower prevalence was measured in other European countries, North Africa and India. The highest prevalence of atopy, defined as one or more positive allergen-specific-immunoglobulin E (IgE) result, showed a pattern similar to asthma symptoms (9).

Another line of compelling evidence of the East–West gradient in the occurrence of atopic diseases comes from studies performed in Germany after the reunification in 1989. von Mutius et al. (10) showed that in genetically similar individuals, the prevalence of atopic diseases [current asthma, bronchial hyperresponsiveness, atopic sensitization assessed by skin prick tests (SPT)] were significantly higher among school children ($n = 7445$) living in the former West (Munich) as compared with the children ($n = 4534$) in former East Germany (Leipzig and Halle). This study confirmed the preliminary findings published 2 years earlier by the same group (11). Nicolai et al. (12) showed in a study with 5313 adults from the Western and 2617 adults from the Eastern part of the country that a similar West–East gradient in the prevalence of atopy (defined by specific IgE levels) could be found also among adults, albeit only in those aged <40 years. Later on, these results have been replicated in several studies among children and adults performed in Sweden and Poland (13), Sweden and Estonia (14), Finland and Russia (15), in the Baltic area (16) and in a comparison between Sweden, the Baltic countries and Uzbekistan (17).

The impact of environment and lifestyle on the development of atopic diseases have been clearly demonstrated also in several migrant studies. In the early 1970s, Morrison Smith (18) reported that the prevalence of asthma among Asian and West Indian children born in the UK was very similar to those of native English children, whereas asthma prevalence among immigrant children born in their native countries was much lower as compared with children born in the UK. Further, Waite et al. (19) compared children who or whose parents had migrated from the Tokelau Island to New Zealand for a decade earlier because of a hurricane, with children who still lived in the Tokelau Island. The prevalence of atopic disorders, asthma, rhinitis and eczema, showed all to be higher among the immigrants living in New Zealand than among the native children on the Tokelau Island. There is also evidence to suggest that not only among children but also even among adult immigrants, a new environment and lifestyle may affect the expression of atopic diseases. Kalyoncu et al. (20) found that the spectrum of allergy in 134 adult immigrants to Sweden changed with time in the new environment, and gradually became more similar to the native inhabitants. In addition, the elevated total serum IgE levels of immigrants showed a decline with time and reached the same levels as for the native inhabitants in 10.5 years at the group level, indicating that environmental rather than hereditary factors are involved in the IgE state. These data also show that immigrants may become sensitized to common allergens still during adulthood. Removal of 'protection' during adulthood, e.g. reduction of exposure to infections in the new environment, may release atopic responses in genetically predisposed individuals (21).

In addition, several studies, most performed in the Tropics, have provided further evidence that atopic disorders occur more commonly among individuals living in urban, more affluent and westernized areas as compared with those living in rural areas with traditional lifestyle (22–26). As to sub-Saharan Africa, the situation appears to be more complex. The ISAAC study revealed high prevalence of hay fever and asthma even in some West-African low-income countries, the reasons for which remain to be elucidated. It is also noteworthy that the assessment of asthma and atopy prevalence in Africa is complicated by concomitant infestations by helminths that are endemic in some African countries and may confound the expression of atopic disease (27).

Which factors may explain the East–West differences in the prevalence of atopic diseases?

Lifestyle and standard of living

It has become clear that traditional risk factors do not account to any substantial extent for the observed geographical differences, neither the time trends (6). As noted earlier (28), the measures which have been established as the norm in affluent Western countries over the past 50 years, including effective public health and hygiene programs targeted to household water and food stuffs, vaccination programs, widespread use of antibiotics in early life, reduction in family size, appear to be involved. These measures, by dramatically reducing the exposure to infections in early life, may have crucially impaired the maturation of the immune system, the development of Th1 memory immunity and tolerance to inhalant allergens (28), particularly in individuals genetically susceptible to atopic diseases. The German studies have shown that, contrary to preliminary expectations, the prevalence of atopic conditions were not associated with car traffic and exposure to particulate mater, such as NO_2 and SO_2 (29). They also showed that old-fashioned and less urban living conditions conferred protection against atopic diseases (30, 31). The differences in prevalence of atopic diseases between Eastern and Western Europe have been found to be largely limited to populations born after the late 1950s, at a time when the lifestyle between the two parts of the continent began to drift apart (12, 14, 17, 32, 33).

Family size and early attendance to day care

There is abundant evidence to show that sibship size contributes to the development of atopic diseases. Number of older, and, to a lesser extent, also number of younger siblings is inversely related to the occurrence of hay fever, atopic eczema, skin test reactivity and the presence of specific IgE antibodies in children, adolescents and adults [reviewed by von Mutius (34)]. For asthma, however, the relationship appears to be more complex, as several studies have not been able to confirm such inverse relationship for asthma (34). In a recent review, Karmaus et al. (35) came to a similar conclusion: the incidence and prevalence of atopic eczema, hay fever, and allergic sensitization are inversely related to the number of siblings, and most studies indicated a dose-response relationship between the number of siblings atopy. For asthma again, this pattern could not be consistently found. The reasons for this inconsistency have been suggested to include the ambiguities over the definition of asthma, especially during infancy and early childhood that may easily lead to misclassifications whereas this is not expected for hay fever and atopic eczema, and also by the fact that the strong determinant of the sibling effect, atopy, is not always associated with asthma (35). It has also been proposed that the 'window' period of life, during which environmental stimuli are required for the maturation of the immune system, particularly in genetically predisposed individuals, for asthma may be narrower and restricted to very early in life, whereas that for atopy and hay fever may be broader. It is also possible that several such 'windows' may exist (36) or they may not be linked to chronological age, but to the timing of exposure to specific allergens (37). The mechanisms behind the sibling effect are not wholly understood. Increased exposure to childhood infections is undoubtedly an important factor, but most probably other factors, such as those associated with fetal life and the maternal endocrine system during pregnancy are also involved (38, 39). Whether exposure to lead *in utero*, which has been shown to be associated with elevated levels of total IgE in cord blood (40), plays a role in the development of atopy, remains to be seen.

Analogously to increased family size, early attendance to day care is considered to reduce the risk for atopic conditions, largely as a result of increased exposure to infections (41, 42). A recent review (43), however, showed that the results from the eight studies included in the analysis were highly discrepant, and any conclusions whether there is an (inverse) relationship between day care attendance and asthma and atopy could not be drawn. The causes of such inconsistency were proposed to be largely methodological. The exposure variable 'day care attendance' included different types of day care, not clearly defined in the studies. The type of the reference group in some studies also remained unclear, and whether the reference group occasionally used some type of day care outside home was not reported. Further, none of these studies had characterized the indoor air quality that may be of importance in this context (43).

Maternal stress

One factor characteristic of our modern life in Western societies is work-related stress that also affects women to a growing extent. Sustained maternal stress during pregnancy may influence the developing immune system and this could be one additional factor in the regional differences (and in temporal changes) observed in asthma and atopy prevalence. It is well recognized that the prenatal intrauterine environment plays a significant role in the individual's subsequent health (44). Prolonged maternal stress associated with sustained excessive cortisol secretion could affect the developing immune system, particularly the Th1/Th2 cell differentiation, and further increase the susceptibility to atopic diseases in genetically predisposed individuals. However, although there is compelling evidence obtained from animal models to support this concept, data obtained from humans are still very scanty [reviewed by von Hertzen (39)].

The hygiene hypothesis

Much attention during the last decade has been devoted to the hygiene hypothesis (45–47) – the apparent inverse relationship between infections in early life and the subsequent development of asthma and atopy – which was once based on epidemiological associations, but has gained strong support also on immunologic grounds (48), and is widely considered as the most plausible working hypothesis to explain both the temporal changes and the regional differences in asthma ad atopy prevalence. The hygiene hypothesis in its broadest sense can be considered to include, not only infections caused by bacterial and viral pathogens, but also helminth infections and exposure to bacterial components, such as lipopolysaccharide (LPS), exposure to commensal bacteria in the gastrointestinal tract, as well as exposure to farm environment (i.e. exposure to a wide spectrum of microorganisms from diverse sources). The immunological basis and the mechanisms that are thought to operate in this immunomodulation, have been discussed in detail in several recent reviews and will not be reiterated here (49–54). In addition, a number of animal experiments have provided support for the hygiene hypothesis (55–58), but these data are also beyond the scope of this review.

In the following, the abundant epidemiological literature on the hygiene hypothesis available to date will be considered in the five above mentioned categories, (1) exposure to microbial pathogens (single agent, multiple agents, unspecified), (2) exposure to LPS, (3) exposure to gastrointestinal commensals, (4) exposure to farm/country environment and pets, and (5) exposure to helminths. These studies are summarized in Table 1.

Table 1. Studies of an inverse association between exposure to a potential immunomodulatory agent(s) and the occurrence of atopic diseases

First author, year	Type of study	Immunomodulatory agent(s)	Ref. no.
Exposure to bacterial, viral and protozoan pathogens			
Shaheen, 1996	Longitudinal (retrospect)	Measles	57
Matricardi, 1997	Cross-sectional	Hepatitis A	58
Shirakawa, 1997	Cross-sectional	*M. tuberculosis*	59
von Hertzen, 1999	Longitudinal (retrospect)	*M. tuberculosis*	63
von Mutius, 2000	Ecolocial	*M. tuberculosis*	62
Kosunen, 2002	Longitudinal (retrospect)	*H. pylori*	64
Matricardi, 2000	Cross-sectional	Hepatitis A, *H. pylori, T. gondii*	68
Matricardi, 2002	Cross-sectional	Hepatitis A, herpes simplex, *T. gondii*	69
Linneberg, 2003	Cross-sectional	Hepatitis A, *H. pylori, T. gondii**	70
Gerrard, 1976	Cross-sectional	Unspecified	71
Anderson, 1978	Cross-sectional	Unspecified	72
Samuels, 1961	Anecdotal data	Unspecified	74
Tyrell, 1967	Cross-sectional	Unspecified	75
Brown, 1966	Cross-sectional	Influenza virus A, B	76
Flynn, 1994	Cross-sectional	Unspecified	78
Illi, 2001	Longitudinal (prosp)	Unspecified	80
Kilpi, 2002	Longitudinal (prosp)	Varia	81
Exposure to LPS (endotoxin)			
Gereda, 2000	Cross-sectional	LPS in home dust	93
Gehring, 2001	Cross-sectional	LPS in home dust	94
von Mutius, 2001	Cross-sectional	LPS in home dust	95
Braun-Fahrländer, 2002	Cross-sectional	LPS in home dust	96
Exposure to commensals in the GI-tract			
Björksten, 1999	Cross-sectional	Gut commensals	102
Björksten, 2001	Longitudinal (prosp)	Gut commensals	103
Exposure to farm/country environment and pets			
Braun-Farhländer, 1999	Cross-sectional	Unspecified	114
von Ehrenstein, 2000	Cross-sectional	Unspecified	115
Riedler, 2000	Cross-sectional	Unspecified	116
Ernst, 2000	Cross-sectional	Unspecified	120
Kilpeläinen, 2000	Cross-sectional	Unspecified	118
Portengen, 2002	Cross-sectional	Unspecified	119
Kauffmann, 2002	Cross-sectional	Unspecified	121
Horak, 2002	Longitudinal (prosp)	Unspecified	117
Hasselmar, 1999	Longitudinal (prosp)	Unspecified	123
Ownby, 2002	Longitudinal (prosp)	Unspecified	124
Celedon, 2002	Longitudinal (prosp)	Unspecified	125
Perzanowski, 2002	Longitudinal (prosp)	Unspecified	126
Exposure to helminths			
Larrick, 1983	Cross-sectional	Unspecified	130
Behrendt, 1993	Cross-sectional	Unspecified	131
Hagel, 1993	Cross-sectional	Varia	132
Lynch, 1993	Cross-sectional	Varia	133
van den Biggelaar, 2000	Cross-sectional	Schistosoma haematobium	134
Scrivener, 2001	Cross-sectional	Varia	135
Huang, 2002	Cross-sectional	Enterobius vermicularis	136

* Seropositivity to enteropathogens (*Clostridium difficile, Campylobacter jejuni, Yersinia entrocolitica*) associated with increased risk of atopy.
M. tuberculosis, Mycobacterium tuberculosis; H. pylori, Helicobacter pylori; T. gondii, Toxoplasma gondii.

Exposure to bacterial, viral and protozoan pathogens

The re-emergence of interest in microorganisms, not as initiators or inciters of asthma, but as protectors against atopic diseases, began finally after the publication of the three pioneer studies on the inverse association between childhood infections and the subsequent development of asthma and atopy (59–61).

Single agent. In 1996, an inverse association between measles infection and atopy, defined by SPTs, was shown in a cohort of young adults in Guinea-Bissau (59), and in the next year, a similar inverse relationship between hepatitis A infection in childhood and occurrence of atopy, defined by SPTs and serum IgE antibodies, was found among Italian military cadets (60). Further, Japanese school children who responded

positively to tuberculin, were found to have lower serum levels of total IgE and Th2 cytokines [interleukin (IL)-4, IL-10, IL-13], higher serum levels of IFN-γ, and showed lower prevalence of atopy and asthma than children with a negative tuberculin test result (61). Two further studies of *Mycobacterium tuberculosis* suggested that this bacterium might have immunomodulatory capacity in the development of atopic diseases. The other study showed that the notification rate of tuberculosis was inversely associated at the regional level with the occurrence of asthma symptoms: an increase in the notification rate of 25 per 100 000 was related to a decrease of 4.7% in the prevalence of lifetime wheezing (62). The other study revealed that those individuals who had suffered from tuberculosis in childhood or adolescence ($n = 1162$) showed 30 years later lower prevalence of asthma/atopic conditions as compared with age, gender and geographically matched controls. However, such a suppressive effect by *M. tuberculosis* could be observed only in women, which was partly explained by the differences in the natural history of atopic diseases between the sexes and the occurrence of tuberculosis mostly in later childhood and adolescence in that study (63). Accumulating data also suggest that *Helicobacter pylori*, the bacterium living in the gastric mucosa, may also have immunomodulatory capacity with systemic effects and could play a role in the development of atopic diseases. In a cohort of 326 adults with a 21-year follow-up the occurrence of antibodies to *H. pylori* was inversely related to the occurrence of allergen-specific IgE antibodies (64). Irrespective of the well-characterized role of this bacterium in gastric pathology, a great majority (80–90%) of the infected persons remain asymptomatic over their lifetime (65). *Helicobacter pylori* may have been part of the indigenous microflora of humans for millions of years, but is being gradually eliminated as a consequence of the changes in modern life (66). With modern life, probably for the first time in human history, there are a large number of noncolonized persons, and the question has recently risen whether *H. pylori* in fact plays a beneficial role in human gastric physiology (67). All the agents referred above may, however, act only as markers of a greater overall load of infection burden, rather than having important effects as single agents *per se*.

Multiple agents. To date, few studies have explored the impact of pathogens (specified serologically or by other methods) on the development of atopic conditions. Matricardi et al. (68) found in a cross-sectional study of young adults that respiratory allergy was less frequent in individuals who were heavily exposed to orofecal and foodborne microbes including *Toxoplasma gondii, H. pylori* and hepatitis A virus. The authors proposed that hygiene and westernized diet might facilitate atopy by affecting the overall pattern of commensals and pathogens that stimulate the gut-associated lymphoid tissue (GALT) and thus contribute to the expression of atopic diseases. The results have been replicated in a comprehensive population study in the USA (69) and in Denmark (70). Recent data from our laboratory lend further strong support to these findings. In a comparison between adults in Finland and Russia, we found that atopy, defined by SPTs, was significantly more common among the Finns as compared with the Russians, whereas exposure to pathogens had occurred, as expected, markedly more frequently among the latter. Interestingly, a separate analysis of each of the 22 pathogens tested revealed that exposure to *H. pylori* alone explained roughly a half of the difference in atopy prevalence between the countries. When all the 22 pathogens were included in the analysis, nearly all of the difference in atopy prevalence between the countries could be explained (E. Vartiainen, unpublished results).

Unspecified agents. A plenty of anecdotal and epidemiological data are available to suggest an inverse association between infections, mostly respiratory, and atopy. The idea of such an inverse association is not new. Dating back to 1976, Gerrard et al. (71) studied the prevalence of atopic diseases among a normal white population ($n = 819$) and Metis Indians ($n = 275$) from Sasatchewan in Canada. They found that the prevalence of asthma and eczema was higher among the white as compared with the Metis population and was contrasted with the increased prevalence of helminth infestation as well as of other untreated viral and bacterial infections among the Metis population. Gerrard et al. (71) concluded that atopic diseases are, in part, the price paid by some members of the white community for their relative freedom from infectious and parasitic diseases. Anderson (72) who performed his studies in New Guinea, reported that respiratory infections occurred more frequently among children in the Highland, where asthma prevalence was very low as compared with children living in the coastal parts of the country where asthma occurred more frequently.

The classic studies on the remote island Tristan da Cunha have provided additional early evidence in favor of the hygiene hypothesis. The highly inbred population of this island has shown an extremely high prevalence of asthma and a high prevalence of atopy (73). Based on serological testing and the information obtained from a medical officer, the inhabitants on Tristan da Cunha had been exposed to intense respiratory infections very rarely. On the contrary, during their 2-year stay in the UK as immigrants because of volcanic eruption on their home island, they showed a strikingly high incidence of respiratory infections (74, 75). Brown et al. reported concordant findings among the Pacific island populations (76, 77), and more recently, by Flynn et al. (78, 79). Further, data from a longitudinal birth cohort study of

1314 German children with a follow up of 7 years suggested that repeated viral infections, particularly common colds 'runny nose' and infections of the herpes type, in early life may reduce the risk of developing asthma up to school age in a dose-dependent manner. The reduction rate of asthma, e.g. appeared to be approximately 50% in children with two or more episodes of common cold during the first year of life (80). Common respiratory infections in early life have also been shown to halve the subsequent risk for developing atopic eczema (81).

Discrepant results. However, not all studies have found an inverse association between infections and atopy, but few, in contrast, found a positive or no association (82–86) (Table 2). These studies, which have obtained data on infections using questionnaires or medical records, but have not, with one exception, used serological testing, have examined the role of measles (83), mycobacteria (86) or several viral and bacterial pathogens (82, 84, 85). The measles study has been criticized for a selection bias: differential misclassification of exposure has been proposed to explain the possible association found in that study, most probably because of underdiagnosis of measles particularly among healthy nonatopic children (87). Most studies have not given any information on the time point of contracted infections. It must be borne in mind that viral/bacterial infections may have bidirectional effects in the atopy/asthma-associated immunomodulation (50). Data obtained from animal models have shown that although particular microbial antigens possess immunomodulatory capacity and can prevent the development of Th2 inflammatory responses, the same antigens can also promote allergic responses. The outcome appears to be critically dependent on the timing between exposure to microbial antigens and allergic sensitization. Stimulation by bacterial antigens in an already established Th2 environment readily aggravates the allergic condition, whereas in a naive environment, Th2 responses may be prevented [reviewed by Renz and Herz (50)].

Table 2. Data not providing support to the hygiene hypothesis

First author, year	Type of study	Immunomodulatory agents(s)	Ref. no.
Uter, 2003	Cross-sectional	Hepatitis A, *H. pylori*, herpes simplex	82
Paunio, 2000	Cross-sectional	Measles	83
Bodner, 1998	Cross-sectional	Common childhood infections	84
Farooqi, 1998	Cross-sectional	Common childhood infections	85
Strannegård, 1998	Cross-sectional	Mycobacteria	86
Bolte, 2003	Longitudinal (prosp)	LPS in home (mattress) dust	97
Wickens, 2002	Cross-sectional	Unspecified (farming)	122
Palmer, 2002	Cross-sectional	Ascaris lumbricoides	137
Dold, 1998	Cross-sectional	*Ascaris* sp.	138

H. pylori, *Helicobacter pylori*; LPS, lipopolysaccharide.

Lipopolysaccharide

It has become clear that microorganisms need not to be even alive to confer protection against atopy (57, 88, 89). Lipopolysaccharide (endotoxin), the major cell wall component of gram-negative bacteria, is a potent inducer of IL-12 and IFN-γ production by macrophages and T-cells, respectively (90, 91), and has shown to have immunomodulatory capacity similar to many pathogens (92). Gereda et al. (93) examined whether chronic environmental exposure to LPS in home dust mitigates the development of allergen sensitization in young asthma-prone children (wheezing infants). They found that the homes of allergen-sensitized infants contained significantly lower concentrations of home-dust LPS than those of nonsensitized infants. Importantly, increased house dust-LPS concentrations correlated with increased proportions of IFN-γ-producing CD4+ T cells, but not with proportions of cells that produced Th2 cytokines. This study was the first to show directly that indoor LPS exposure in early life may protect against atopy by enhancing type 1 immunity. These findings by Gereda et al. have gained further support by two German studies performed among infants (94) and farmers' children (95), and, by a multinational study among school children (96).

Most recently, however, such an inverse association between exposure to LPS and the occurrence of atopy could not be confirmed. In a prospective German study nearly 2000 infants were followed from birth till the age of 2 years. Exposure to LPS, measured by LPS levels in mother's mattress dust, showed no protective effect on atopy development till the age of 2 years, LPS rather seemed to increase the risk of atopic sensitization, particularly in infants with parental atopy (97). The effect of LPS exposure may vary with the type, dose and route of concomitant allergen exposure, in addition to genetic cofactors of the children (97).

Exposure to commensals in the gastrointestinal tract

Not only pathogens but also commensal bacteria of the gut microflora appear to play a significant role in the development of atopic diseases. The involvement of both respiratory and gastrointestinal tract in this immunomodulation may not be unexpected as the bronchus-associated lymphoid tissue (BALT) and the GALT belong to the same common mucosal immune system (CMIS) (98); the overall stimulus and turnover rate by pathogens and commensals in the mucus-associated lymphoid tissues during critical periods of life is now widely considered as the decisive factor in the context of the hygiene hypothesis (99).

Large geographical variations in the composition of gastrointestinal microflora in infants have been observed (100, 101). A comparison between Sweden and Estonia revealed a more intensive colonization with lactobacilli among the Estonian infants, whereas the Swedish infants

showed increased numbers of clostridia, especially *C. difficile* (101). Björksten et al. (102) examined further whether atopic diseases among children are associated with differences in their intestinal microflora in Estonia with a low and in Sweden with a high prevalence of atopy at the age of 2 years. They found that the atopic children in both countries were significantly less often colonized with lactobacilli as compared with the nonatopic children, and concluded that differences in the indigenous intestinal flora might affect the development and priming of the immune system in early life. A further prospective study examined the relationship between intestinal microflora and the development of allergy in Swedish and Estonian children through the first 2 years of life. Major differences in the composition of the gut flora were found between infants who developed and those who did not develop allergy, and these differences were discernible before any clinical manifestation of atopy could be observed (103). The differences in the intestinal flora in early life were proposed to be associated with Western lifestyle including such factors as the high hygiene control during delivery and high consumption of industrially processed and sterilized food in early life (104, 105).

Lactobacilli have raised considerable interest in recent years as immunomodulatory agents. These commensals have been shown to stimulate the production of IL-12, IFNγ, IL-18 and, to a lesser extent, also IL-10 by human peripheral blood mononuclear cells (PBMCs) (106). The continuous low-grade stimulation by the indigenous microflora is considered necessary for the normal maturation of the immune system, and it has been proposed that quantitative and qualitative differences in gastrointestinal microbial colonization between infants born in Western and Eastern countries may partly explain the regional differences in atopy prevalence (28). The early use of antibiotics, which have also been shown to have direct effects on human immune functions (107), might be expected to affect adversely on the maturation process of the immune system, particularly in individuals genetically susceptible to atopy. Indeed, a positive association between the use of antibiotics during the first or two first years of life and the subsequent development of atopic diseases have been reported in several (85, 108–112), albeit not in all (80, 113) studies. Although the possibility of reverse causation (i.e. children who are genetically susceptible to atopic diseases, experience more frequently respiratory infections and therefore use antibiotic more frequently than children without genetic predisposition), must also be taken into consideration (85), later analyses suggest that the associations between the use of antibiotics and asthma/allergy are independent of the effect of respiratory infections (110).

Exposure to farm/country environment and pets

A large body of evidence has rapidly accumulated to suggest that growing up on a farm may reduce the risk of developing atopic diseases. Swiss school children showed to have atopy, measured by radioallergosorbent test, and symptoms of hay fever significantly less frequently than children from the same area who have not been growing up on a farm (114). This inverse association between exposure to farm/country environment in childhood and the subsequent development of atopic disorders has been confirmed by further studies in Germany (115), Austria (116, 117), Finland (118), Denmark (119), Canada (120) and France (121) There is also some evidence to suggest that exposure to country living also in later childhood or even in adulthood 'ever living in the country' may confer protection against asthma and atopy implicating that immunomodulation may not be restricted to exposure in the first years of the life only (121). Importantly, a study from New Zealand has shown that the results obtained from Europe and Canada are not necessarily applicable to other continents and countries. In that study, children currently living on a farm had more hay fever, allergic rhinitis, eczema, asthma and wheeze, albeit no more atopy, as compared with children not living on a farm. The discrepant results in this study were partly explained by small, if any, differences e.g. in animal contacts, socioeconomic status and in the use of coal and wood fuels between farm and nonfarm residents in New Zealand (122).

The factors in farming families that could explain the protective effect against atopy might include, as stated earlier (36), larger family size, frequent heating with wood, more pets, less maternal smoking, more dampness and different dietary habits. However, none of these factors have been shown to explain the differences to any major extent. By contrast, contact to livestock and poultry has been found to explain much of the inverse association between farm environment and atopy (36, 115, 116). These data suggest that exposure to a wide spectrum of microorganisms and bacterial products, such as LPS, may be responsible for the lower prevalence of atopic diseases among individuals grown up on a farm or rural area. This also appears to be true for exposure to pets, which may serve as a secondary source of transmission of a variety of microorganisms (123–126).

Exposure to helminth infections

Parasitic helminth infections are still the most prevalent and persistent of all childhood infections globally (127), and the ability of chronic helminth infection to suppress the development of allergic diseases is generally perceived (127, 128). Recent research of helminth infections in relation to asthma and atopy has shed new light on the possible mechanisms operating in the development of atopic (Th2) diseases as well as autoimmune (Th1) diseases. As the prevalence of type 1 diabetes has risen in parallel to asthma and atopy during the last decades, it is evident that the Th1/Th2 paradigm of mutual antagonism alone cannot explain these trends satisfactorily.

The immune system of people living in Western societies is no longer challenged as it was earlier by a variety of infectious diseases of viral, bacterial, fungal or parasitic origin, and the major defense mechanisms for parasitic infections, IgE antibodies, is still present and may now be readily directed to innocent environmental particles (129). Larrick et al. (130) in 1983 were among the first to suggest an inverse association between parasitic infestations and the occurrence of atopic diseases. They found highly elevated serum IgE levels and a low, if any, occurrence of atopy (assessed by SPTs) in Latin American Indians among whom intestinal parasites occurred very commonly. Behrendt et al. (131) found in a comparative study among 2054 preschool children in former East and West Germany that total serum IgE levels were significantly higher in East Germany when compared with the Western part of the country, and this difference persisted when children with positive allergy-related variables were excluded from the analysis. Parasitic infections appeared to be the major factor explaining this difference. A protective role by intestinal parasitic (helminth) infection against the development of allergic diseases has been reported by several studies (132–136), although some authors have reported an opposite result (137, 138). The evidence of an inverse relationship between helminths and atopic disease applies mostly to asthma, whereas for e.g. atopic eczema, the direct evidence is still weak (139).

An interesting piece of evidence of the mechanisms underlying the inverse relationship was obtained from the study by van den Biggelaar et al. (134). They showed among 520 school children in Gabon, Africa, where helminth parasitism is endemic, that the occurrence of both total serum IgE and specific IgE (e.g. to home dust mite) were very high. However, SPT reactivity to the mite allergen could be demonstrated in only less than one-third of these IgE positive children. This was shown to be associated with high production of IL-10 by PBMCs stimulated by parasitic antigen, resulting in dampening of allergen-induced responses *in vivo*. The authors concluded that helminth-induced IL-10 lowers the risk of developing skin-reactivity to an allergen. These regulatory T cells, by releasing anti-inflammatory cytokines, may play an important role in immunomodulation and prevention of atopic diseases. The presence of such an anti-inflammatory network during chronic helminth infections has been proposed to be the major factor in the lower prevalence of allergic diseases in chronically helminth-infected populations (54). It appears that not only helminths but also *Mycobacterium vaccae*, even as a killed suspension, (140), hepatitis C virus (141) and probably a variety of other microorganisms, also including commensals of the gastro-intestinal tract (142), induce normally the production of IL-10 and TGFβ by regulatory T cells thus mitigating airway inflammation and promoting tolerance to respiratory allergens. In the absence of such infections, a predominance of Th2 cells – which are ontogenically related to the regulatory T cells – (142) or a predominance of Th1 cells may develop instead. The secretion of the anti-inflammatory cytokines, particularly IL-10, by regulatory T cells appears now to hold the key to counter-regulation of both atopic and autoimmune diseases (143).

Milestones in the evolution of the hygiene hypothesis from 1989 to the present are outlined in Fig. 1. Figure 2 illustrates the major factors involved in the current concept of the hygiene hypothesis.

Gene–environment interactions

Although the hygiene hypothesis emphasizes the impact of environment in the development of atopic diseases, the significance of hereditary factors cannot be ignored. The complex interaction between genes and environment in the development of asthma is recognized, and the role of hereditary factors as important determinants of atopic diseases has been shown in twin studies and in studies among highly inbred populations (147). Genetic factors must be involved also in the marked differences in the occurrence of atopic diseases between different ethnic groups living close by each other in similar environments in Western societies. Particularly for asthma, heritability in some populations has been shown to be very high, up to 75% (148). The longitudinal studies in Germany after the reunification have provided evidence that environmental and lifestyle factors may be more important in the development of atopy and hay fever than in the development of asthma (149). Similarly in many African countries, the prevalence of atopy has risen substantially, whereas the prevalence of asthma and bronchial hyperresponsiveness have remained almost unchanged (147).

An interesting hypothesis has been put forward suggesting that populations originating in 'hostile' tropical environments have immune responses with a more Th2 inflammatory profile than populations that have moved and resided for millennia in temporal environments (150). This hypothesis is based on data obtained from epidemiological studies of tropical diseases, data on the relative prevalence of inflammatory alleles in different populations, and data on disease patterns among immigrant populations. For example several studies, both molecular and epidemiological, have shown that atopic disorders are more common in African-Americans than in white Americans (150, 151). A prediction that follows from these data is that also native Europeans have a lower *genetic* risk of atopic disease than immigrant populations originating in the Tropics. In Western societies, strong environmental risk factors, including reduced exposure to microorganisms and parasites, may rather predispose to atopic diseases (150). The highest prevalence of asthma symptoms in affluent English-speaking countries (7, 9) may partly be explained

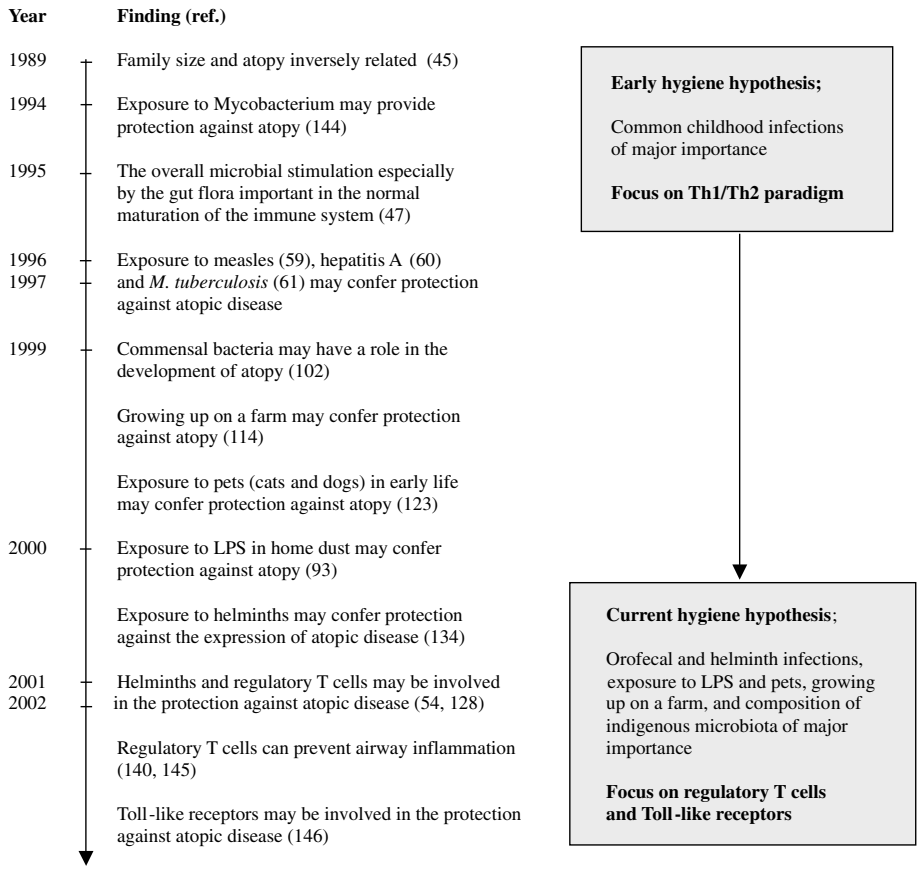

Figure 1. Milestones in the evolution of the hygiene hypothesis from 1989 to 2003.

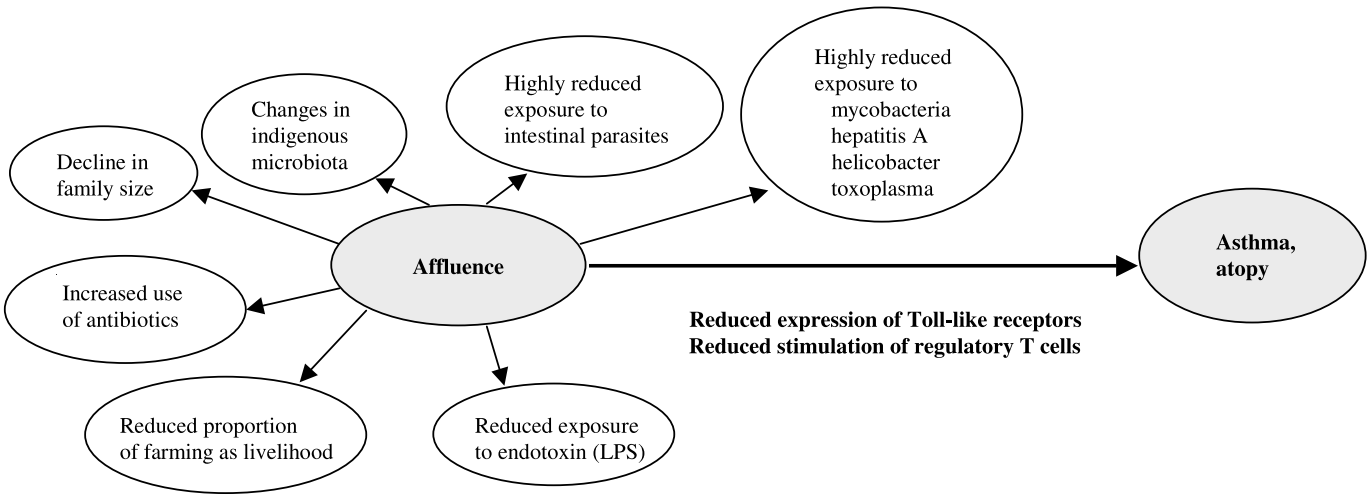

Figure 2. Major factors involved in the current concept of the hygiene hypothesis.

by the genetic heterogeneity of these populations; English-speaking communities have a far broader genetic diversity in terms of ethnic backgrounds than those communities with low prevalence of atopic disorders (152).

Concluding remarks

It has become evident that something is lacking from our affluent societies that has the capacity to protect against the development of atopic (and autoimmune) diseases

(129). Man has co-evolved with parasites and the constant pressure by bacterial and viral infections (153), and in affluent Western countries, lack of such pressure may have resulted in impaired maturation of the immune system in susceptible individuals. An 'unhygienic' environment may provide protection against atopic disorders by inducing, in addition to Th1 responses, also an additional immunological regulatory network (142). Not only intestinal helminth parasites, which have the propensity to persist and establish chronic infection, but also several other pathogens and apparently also many commensals are able to elicit the anti-inflammatory (regulatory) network including the regulatory T cells that secrete IL-10 and TGFβ, the anti-inflammatory cytokines, that inhibit deleterious immunopathologic responses (154). Accumulating evidence also suggests that the Toll-like receptors (TLRs) expressed mainly on dendritic cells and macrophages may have a crucial role in this regulatory cascade (155). For example, differences in the expression of TLRs have been found between farmers' and nonfarmers' children (146).

The longitudinal studies in Germany and Africa and the studies of family size and day care attendance suggest that environmental factors may be more important in the development of atopy and allergic rhinitis than in the development of asthma. The 'window' period for different atopic disorders may differ, and, particularly for atopy, may not be restricted to early life only. During such 'window' periods, a high overall infection turnover and a proper colonization by gut commensals may be decisive in the development of the regulatory network (54, 99).

As to the therapeutic use of immunomodulatory bacteria or bacterial components, few intervention studies have thus far been performed. Most data have been obtained from trials with probiotic bacteria. Lactobacillus, given pre- and postnatally, has been shown to reduce the occurrence of atopic eczema up to 2 years of age in genetically predisposed individuals (156), whereas no effect on allergic symptoms was found among teenagers and young adults with pollen and food allergy after the use of Lactobacillus for nearly 6 months (157). In addition, intradermal administration of killed *M. vaccae* suspension, twice a day for a week, has been shown to improve moderate-to-severe atopic eczema in children (158). The issue of using microbial products in prevention and treatment of atopic disease has been thoroughly reviewed recently (159), and is beyond the scope of this review.

More data from intervention studies are urgently needed. Irrespective of the abundant literature of the inverse association between exposure to microorganisms/helminths and the occurrence of atopic diseases, no definite conclusions of the causality of this association are yet warranted. Nonetheless, the notion by Gerrard for nearly three decades ago has proved to be true; atopic diseases appear, at least in part, to be the price paid for our relative freedom from infections and parasitic diseases in affluent societies.

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