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Influences in allergy: Epidemiology and the environment

Erika von Mutius, MD, MSc *Munich, Germany*

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Many decades after the first descriptions of hay fever in the 19th century, we still struggle with the formation of a cogent conception embracing all the many fragments of insight into the various aspects of allergic and asthmatic diseases. Although in daily practice we can grasp and allocate most of the clinical manifestations, in research the fundamental structures are only in part recognized. We therefore presume that the underlying mechanisms are complex and multifaceted. We have explored in countless directions while branching into ever more detailed analyses of phenomena, all of which are associated with the conditions of interest. But how can we rearrange these pieces to advance toward a broader understanding of asthma and allergies? Epidemiology as a scientific tool to study the occurrence, the natural course, and the determinants of a condition in various sociocultural environments might contribute to the edifice of such a scaffold. This review does not intend to present a comprehensive digest of the relevant literature: the reader can refer to other “state of the art” reviews. The intention is to discuss some conceptual cornerstones and to illustrate them with a few examples. (*J Allergy Clin Immunol* 2004;113:373-9.)

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Abbreviations used

aOR: Adjusted odds ratio
 α_1 -AT: α_1 -Antitrypsin
 Pi: Protein inhibitor

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HOST FACTORS

One dimension is likely to relate to an affected subject's genetic background because there is considerable evidence of a significant hereditary component in the pathogenesis of asthma and allergic diseases. It is well documented that a family history of atopic diseases is a strong risk factor for the development of asthma, hay fever, and atopic dermatitis.¹⁻⁴ A stronger concordance of atopic illnesses between homozygotic twins compared with dizygotic twins⁵⁻⁷ further supports this notion. During the last 10 years, there has been substantial effort devoted to genetic studies of asthma and allergic diseases on the basis of candidate gene approaches and genome-wide linkage studies in human and animal models. Up to now, despite significant findings regarding susceptibility regions and genes in some cases, these studies have still resulted in only a very limited understanding of asthma and allergic diseases. Some of the reasons for the failure to replicate the detection of particular loci across studies and for the modest contribution of each of these susceptibility loci might relate to heterogeneity factors between studies that might be difficult to detect and take into account in small-scale studies. Enlarging the number of participants in a given study has thus been proposed to increase the power to detect even small effects. Alternatively, identifying the factor or factors that conceal the underlying effect and thereby unmask the true relation

From University Children's Hospital, Munich.

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Reprint requests: Erika von Mutius, MD, MSc, University Children's Hospital, Lindwurmstr. 4, D 80337 Munich, Germany.

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between genetic susceptibility and development of a certain condition will result in a more profound understanding of the primary biologic mechanisms.

Some recent data discussing the potential contribution of 2 candidate genes encoding IL-4 and IL-13, 2 cytokines implicated in the pathogenesis of asthma and airway hyperresponsiveness, might be of interest in this discussion. In a Dutch study of families ascertained through a proband with asthma significant associations of atopy and asthma-related phenotypes with several IL-4 receptor α (*IL4RA*) polymorphisms were observed.⁸ More interestingly, a significant gene-gene interaction of a certain polymorphism in *IL4RA* with a promoter variation in *IL13* was detected. Individuals with the risk genotype for both genes were at almost 5 times greater risk for the development of asthma compared with individuals with both nonrisk genotypes, suggesting that the combination of susceptibility loci in a pathway central to the underlying pathomechanism might better reveal the genetic contribution to a certain condition than the investigation of a single nucleotide polymorphism.

More than 2 genes could affect a subject's susceptibility for an illness, but in many circumstances these genes will not have been identified. Biologic systems are often composed of complex regulatory networks fine tuning the level of important gene products. Therefore an individual's susceptibility for a certain condition might alternatively be assessed by measuring levels of gene products intermediate in the pathway to the inception of the condition. Recent work investigating the role of α_1 -antitrypsin (α_1 -AT) for the preservation of normal lung function might illustrate this point.

α_1 -AT is a major antiprotease of the lung, protecting the lower respiratory tract against the proteolytic matrix destruction caused by neutrophil elastase.⁹ The pleomorphic protein inhibitor (Pi) α_1 -AT locus codes for at least 17 deficiency alleles, with Z and S mutations being the most common in Northern Europe.^{9,10} Children with an impaired protease-antiprotease balance might be particularly vulnerable to ambient pollution, such as environmental tobacco smoke exposure. In a large cross-sectional survey, school-aged children were studied according to the ISAAC phase II protocol, including parental questionnaires, pulmonary function testing, and allergy testing.¹¹ Blood samples were obtained to measure plasma levels of α_1 -AT and to genotype for PiZ and PiS alleles. Children with levels of α_1 -AT of less than 116 mg/dL showed significant, albeit small, decrements in baseline lung function. When exposed to environmental tobacco smoke, pronounced decrements of pulmonary function, particularly in measures of mid- to end-expiratory flow rates, were seen in these children compared with that seen in exposed children with normal levels of α_1 -AT (maximum mid-expiratory flow [1/sec], 73.7 ± 8.6 vs 99.9 ± 1.7). Almost half of all subjects with low α_1 -AT levels had neither PiZ nor PiS genotypes, and their specimens showed normal migration in the electrophoresis. Other DNA polymorphisms in regulatory sites might account for the reduced α_1 -AT concentrations.¹² Mea-

surements of blood levels might be more appropriate than genetic studies identifying only single deficiency alleles to assess an individual's antiprotease balance and her or his response to hazardous environmental stimuli. In fact, several other studies investigating the role of PiZ and PiS genotypes have yielded rather conflicting results.¹³⁻¹⁵

ENVIRONMENTAL EXPOSURES

Another dimension of the conceptual scaffold is likely to relate to an affected subject's history of environmental exposures. Such exposures and potential determinants are manifold over an individual's life span. The more colloquial term *risk factor*, which has been widely used to illustrate these potential associations, implies the concept of an adverse, hostile environment affecting human health. The epidemiologic discipline was founded on such concepts because it emerged in the context of various infectious epidemics affecting large proportions of the population. The epidemiologic approach to chronic diseases of modern times might, however, require other concepts. Although it is conceivable that changes in lifestyle in our rapidly evolving societies affect the incidence of concomitantly emerging diseases, the reverse is equally plausible, namely the loss of protective environmental exposures. One might argue that before the beginning of the 20th century, environmental exposures have been rather unaltered over long periods, with the exception of newly emerging epidemic diseases, such as the plague. In these surroundings host factors, including the population's genetic and epigenetic make-up, have been shaped, adapting to the environment. In these settings certain biologic mechanisms might have been necessary to ensure health and survival, mechanisms that might be out of place in the absence of such exposures.

A characteristic of affluent societies of modern and postmodern times is the aging of populations and the reduced birth rate. For example, in Germany the rate of live births almost halved from 17.3/1000 in 1960 to 9.3/1000 in the year 2000.¹⁶ Yet crowding and the number of siblings has consistently been related to the development of hay fever and allergic sensitization,¹⁷⁻¹⁹ whereas the association with asthma remains less clear. Many studies allowing a separate analysis of the influence of older and younger siblings found a stronger relationship with the presence of older siblings than with younger brothers and sisters.¹⁹ In this context David Strachan¹⁷ was the first to propose the "hygiene hypothesis," stating that "infection in early childhood, transmitted by unhygienic contact with older siblings, or acquired prenatally from a mother infected by contact with her older children" might prevent the development of allergic diseases.

This suggestion has initiated intense debates because a large body of evidence exists to support the notion that infections, particularly viral infections, trigger asthma exacerbations.²⁰ Furthermore, a high burden of infections through older siblings or daycare has been related to the development of recurrent wheeze early in child-

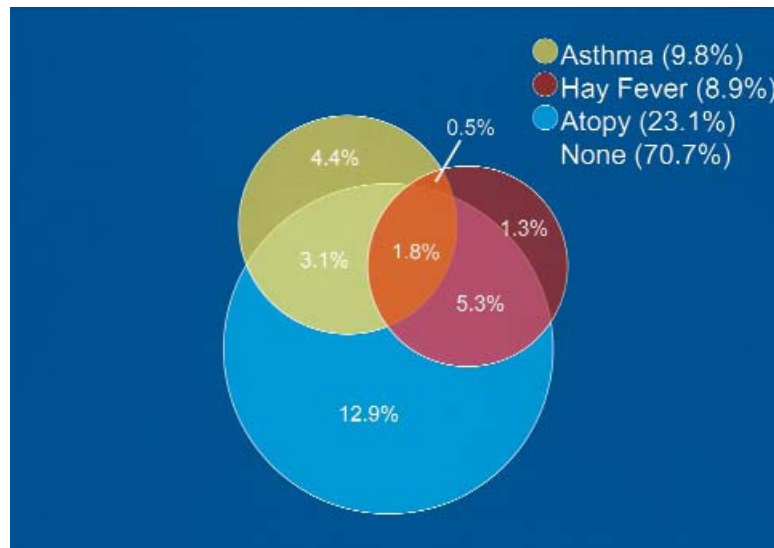


FIG 1. Venn diagram of asthma, hay fever (seasonal allergic rhinitis), and atopy among 9- to 12-year-old schoolchildren in Munich, Germany.

hood. A third conceptual dimension might have to be embraced that relates to the natural course of allergic diseases to reconcile these seemingly discrepant views.

NATURAL HISTORY OF ATOPIC ILLNESSES

It has long been reasoned that the increased production of specific and total IgE is the hallmark of atopic diseases, such as asthma, allergic rhinitis, and atopic dermatitis. Although in clinical populations of more severely affected patients this observation is compelling, on a general population level, asthma, atopic eczema, hay fever, and IgE levels appear rather as distinct phenotypes. For example, in the German ISAAC II population of school-aged children in Munich, more than 23% of subjects had positive skin prick test responses, but less than 10% of the children had asthma or hay fever,²¹ as shown in Fig 1. Only partial overlap between clinical diagnoses and the presence of IgE antibodies was seen.

Moreover, the natural course of the various atopic illnesses differs. Whereas the peak incidence of wheeze and atopic dermatitis is seen in the first years of life,^{22,23} the manifestation of hay fever peaks around school age and adolescence.²⁴ Although the ages at first manifestation for wheeze and atopic eczema are similar, striking differences in the geographic distribution of atopic dermatitis and asthma have become apparent. The spatial distribution of atopic dermatitis on a worldwide scale differs markedly from the geographic distribution of wheeze and allergic rhinoconjunctivitis.²⁵ The remarkable differences in the prevalence of hay fever, atopy, and asthma seen between Eastern and Western Europe are not found for atopic dermatitis.^{21,26} Children exposed to farming environments early in life have markedly reduced prevalences

of asthma, hay fever, and atopy, yet the prevalence of atopic dermatitis is unaffected by early childhood farming exposures.²⁷⁻²⁹ Finally, many of the risk factors associated with asthma and wheeze cannot be identified in studies of atopic dermatitis.^{26,30} Therefore the search for determinants proprietary to each condition will matter in the identification of the specific risk or protective factors.

Etiologic heterogeneity, the situation in which a particular clinical expression of a disease can be produced by several causes, might further misguide the quest for genetic or environmental influences. This obstacle must particularly be recognized in the epidemiology of asthma and wheeze. Since the landmark studies in Tucson, Arizona, many cohorts have consistently identified distinct wheezing phenotypes.^{23,31} Most importantly, an infantile wheezing phenotype that appears only transiently in the first 1 to 3 years of life and disappears thereafter can be disentangled from other either later occurring or persistent wheezing entities because of its good prognosis and its separate risk profile. Most more severe manifestations of wheeze at school age in western countries are related to atopic sensitization, but on a global level, the population-based proportion of asthma cases that are attributable to atopy has been estimated at less than one half.³² These observations emphasize the variety of mechanisms (particularities of airway-associated features, immune responses, or both) likely to underlie the top of the iceberg presenting as a more or less uniform clinical condition. For atopic dermatitis, the identification of distinct phenotypes appears even less obvious (unpublished data). However, the comorbid manifestation of wheeze, atopic dermatitis, reduced lung function, and polyvalent sensitization toward all types of inhalant and food allergens might represent a single distinct disease entity with specific underlying pathomechanisms.³³

TEMPORALITY

When attempting to detect causal relationships between certain environmental exposures and the various phenotypes discussed above, no undisputable checklist of criteria exists. The standards of epidemiologic evidence offered by Hill,³⁴ such as (1) strength of the association, (2) consistency of findings, (3) specificity of effects, (4) biologic gradient (ie, a monotonic dose-response relationship), (5) plausibility, (6) coherence of the interpretation, (7) experimental evidence, (8) analogy, and (9) temporality, are all fraught by exceptions and reservations.³⁵ The only *conditio sine qua non* is temporality, which refers to the necessity that a cause precedes the effect in time. This criterion is inarguable but often disregarded. In most cross-sectional surveys temporal sequences of events are not taken into account. Even if attempts are made to consider the timing of the exposure, retrospective assessment of the relevant factors can be fraught with recall bias. Temporality urges us to bear in mind our knowledge about the natural course of a certain condition when searching for potential determinants.

A peculiarity of the temporal dimension materializes in pediatric populations. A hallmark of childhood years is the maturation of many physical and intellectual skills with varying preprogrammed advancement. It is conceivable that such periods of maturation are exceptionally vulnerable to extrinsic (and intrinsic) influences, thereby adding a developmental aspect to the discussion. For example, the window of opportunity for environmental factors potentially influencing the maturation of certain immune responses might open and close at other ages than for certain features of maturing airways or skin. The programming of growing biologic systems in mammals does not, however, start at birth. Antigen-specific immune responses are already detected in cord blood dependent on maternal exposure to the respective antigen, and proliferative responses and cytokine production have been demonstrated as early as 22 weeks of gestation.³⁶ In many population-based surveys a history of maternal atopy had a stronger effect on the offspring's risk of atopy than a paternal history, suggesting independent maternal effects. These influences might relate to in utero environments in fetal life but could also comprise imprinting and epigenetic phenomena, as discussed in much more detail by Dr Vercelli in another contribution to this series. Our inability to identify the determinants of the increase in the prevalence of asthma despite identification of some individual risk factors, such as body mass index,³⁷ might be founded on epigenetic effects. It is furthermore intriguing that in recent studies the prevalence of childhood asthma reached a plateau in the 1990s, approximately 1 generation after the substantial rise in prevalence described in the 1960s and thereafter.³⁸⁻⁴¹ For the epidemiology of childhood asthma, the observance of the developmental aspects and the natural course of the different wheezing phenotypes has hitherto been crucial for a better understanding of the role of various environmental exposures, particularly those pertaining to the hygiene hypothesis.

MICROBIAL EXPOSURES IN THE DEVELOPMENT OF WHEEZE, ASTHMA, AND ALLERGIES

Infant wheezing

In infancy most wheeze is positively associated with viral infections. Infants in daycare settings are at higher risk of wheezing⁴²⁻⁴⁵ compared with their nonexposed peers. In a large population-based survey enrolling all newborns of the main birth clinics in Oslo, Norway, the presence of older siblings (adjusted odds ratio [aOR], 1.8; 95% CI, 1.3-2.6) and attendance to daycare at age 1 year (aOR, 1.6; 95% CI, 1.1-2.4) were positively associated with episodes of airway obstruction at age 2 years.⁴³ Similar effects were seen for a history of lower respiratory tract infections (bronchiolitis, bronchitis, and pneumonia: aOR, 11.6; 95% CI, 8.3-16.2), otitis media (aOR, 1.4; 95% CI, 1.0-1.9), and croup (aOR, 2.3; 95% CI, 1.3-3.9) during the first year of life.

Likewise, in a large population-based cohort of children followed to 2 years of age in the United States, children at daycare were at twice the risk of lower respiratory tract illnesses when taken to daycare instead of being looked after at home.⁴² The rate ratio for wheezing-associated respiratory illness was 2.3 (95% CI, 1.8-3.0) when comparing infants in daycare with nonexposed children.

Evidence for viral respiratory infections has been found in up to 70% of wheezing episodes in the first year of life.⁴³ These epidemiologic observations have been confirmed on the basis of emergency visit studies detecting viruses in nasal wash specimens of infants less than 2 years of age.^{46,47} The authors demonstrated that in wheezing patients 70% to 82% of the cultures were positive for virus compared with 20% of the cultures of a nonwheezing control group. The predominant pathogen in the washes of these symptomatic infants was respiratory syncytial virus, as recognized by a positive antigen test result in 68% of the samples. Rhinoviruses were detected by means of RT-PCR in equal proportion (41%) among wheezing and control infants in the latter study.⁴⁷

Asthma and wheeze at school age and in adolescence

Although daycare attendance and viral infections of the upper and lower respiratory tract increase the risk of wheezing in early life, the opposite is seen at school age or adolescence. Several studies have assessed daycare attendance in the first years of life and related the effects of such exposure to wheeze and allergies later in life. In East Germany the development of asthma and allergies at the age of 5 to 14 years was reduced if a child from a small family entered a daycare nursery at between 6 and 11 months of age compared with entry at between 12 and 23 months of life or after the second birthday.⁴⁸ Findings from the Tucson Children's Respiratory Study showing that children in daycare in the first 6 months of life were at significantly reduced risk of atopy and wheezing at school age and adolescence⁴⁹



FIG 2. Village in rural areas of Austria, where the ALEX study has taken place. Note that the differences in the prevalence of asthma, hay fever, and atopy were observed between children living in a farmhouse and neighboring children living in the same village but not in a farmhouse.

corroborate these observations. It seems intriguing that the Boston cohort reported very similar results over a period of 7 years,⁵⁰ with a significant reduction in the risk of wheezing at age 7 years.

The environmental factors contributing to the exposure encountered by children in daycare settings have not all been elucidated. A facilitated spread of viral infections might play a role because observations from the MAS birth cohort documented a decreased asthma risk with recurrent episodes of rhinitis in the first year of life. Likewise, herpes infections in the first 3 years of life were associated with less asthma and airway hyperresponsiveness.⁵¹ Other types of infections might also be involved: positive serologies to hepatitis A, *Toxoplasma gondii*, and *Helicobacter pylori* have all been linked to lower rates of asthma and allergies.^{52,53}

Microbial exposures also occur in the absence of infection. Viable germs and nonviable parts of microbial organisms are ubiquitous in nature and are found in varying concentrations in our many indoor and outdoor environments. These microbial substances are recognized by the innate immune system in the absence of overt infection and induce a potent inflammatory response.⁵⁴ It is of particular interest in this context that one type of microbial exposure, endotoxin, a cell-wall component of gram-negative bacteria, is rather abundant in daycare settings, as reported recently from Brazil.⁵⁵

Microbial products are particularly abundant in farming environments where animals such as cattle, pigs, and poultry are kept. Children growing up on farms have a significantly lower prevalence of atopic sensitization, hay fever, and asthma compared with children from the same rural areas but not raised on farms, as found in many parts of the world (Fig 2).^{27-30,56} For example, in a Swiss study of 6- to 15-year-old schoolchildren, the odds of having seasonal symptoms of hay fever (aOR, 0.34; 95% CI, 0.12-0.89) and of development of atopic sensitization, as measured by means of RASTs (aOR, 0.31;

95% CI, 0.13-0.73), were strongly decreased in children raised on a farm compared with their peers from the same rural area whose parents were not farmers.²⁷ Moreover, the protective effect of a farming environment was not limited to atopic asthma; the prevalence of asthma not associated with allergic sensitization was also significantly less among children raised on farms.⁵⁶

Contact with livestock, poultry, and “farm milk” was found to explain much of the relationship between farming and atopy.⁵⁶ Again, the timing of exposure was crucial for this protective effect to occur. Only children exposed to animal stables or unpasteurized milk in the first year of life, as compared with later exposure, had a significantly reduced prevalence of asthma, whereas early and persistent exposure was relevant for the protection from atopy and hay fever. When additionally considering a maternal exposure to farm animals during pregnancy and lactation, the odds of atopic sensitization were reduced to an aOR of 0.18 (95% CI, 0.06-0.56), the odds of hay fever to an aOR of 0.07 (95% CI, 0.005-0.91), and the odds of asthma to null.⁵⁶ This maternal influence was independent of the infant’s exposures.

A large variety of gram-negative and gram-positive germs, as well as a diversity of molds and fungi, is found in stables. In addition, nonviable parts of microbes, such as endotoxin and muramic acid, a component of peptidoglycan, are found in abundance not only in stables but also in indoor environments of farm houses (unpublished data).⁵⁴ Recent reports have suggested that the environmental exposure to endotoxin, as assessed in mattress dust levels, significantly decreases the risk of hay fever, atopic sensitization, and atopic asthma in childhood,^{54,57} thus particularly affecting the atopic phenotypes. In contrast, levels of muramic acid were not related to hay fever and atopy but were inversely related to nonatopic wheeze and asthma in these populations. The protective effects were also observed among children from nonfarming families, indicating that the relatively low levels of expo-

sure observed in nonfarm environments already favorably influence the development of childhood asthma and atopic diseases. This contention is supported by a number of recent studies inversely relating indoor exposures to endotoxin to atopy also in urban environments.⁵⁷

Gene-by-environment interactions

Even in these highly exposed farm children, the genetic dimension cannot be forgotten. Susceptibility to various microbial exposures might depend on the make-up of different elements of the innate immune response. The toll-like receptors represent a highly conserved family of innate immune recognition receptors, with microbial molecules as ligands. Polymorphisms in genes encoding these receptors might therefore modulate the protective effects observed in farming populations and thereby modify the exposure effects. We recently reported that a polymorphism in the gene encoding toll-like receptor 2 significantly interacts with a farming environment.⁵⁸ Only children with a T allele for *TLR2*-16934 were susceptible to the protective factors on the farm, whereas children homozygotic for the A allele had prevalences of asthma and atopy comparable with those of nonfarm children. Furthermore, among nonfarm children, no effect of *TLR2* polymorphisms was seen, suggesting the presence of a strong gene-by-environment interaction. Thus the importance of the genetic contribution would have been missed if the relevant environmental exposure had not been taken into account. Conversely, the relevant exposure for the protection from asthma and allergies, namely microbial ligands for the toll-like receptor 2, would not have been recognized without inclusion of the genetic dimension.

CONCLUSIONS

The epidemiologic investigation of complex diseases such as asthma and allergies should advance in several dimensions. Knowledge about the natural course and the developmental aspects of the various facets of a certain illness is desirable to detect potential windows of opportunity for environmental influences. Yet environmental factors are likely to exert their effects in the context of the host characteristics that convey or refuse susceptibility toward these influences. No uniform response will occur, depending on the environmental context of a certain exposure and the genetic make-up of an exposed individual. By taking temporality and gene-by-environment interactions as well as epigenetic effects into account, a better understanding of the complex causes of asthma and allergies can be achieved.

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