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The many faces of the hygiene hypothesis

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About 15 years have gone by since Strachan first proposed the idea that infections and unhygienic contact might confer protection against the development of allergic illnesses. The so-called hygiene hypothesis has ever since undergone numerous more or less subtle modifications by various researchers in the fields of epidemiology, clinical science, and immunology. Three major tracts have developed exploring the role of overt viral and bacterial infections, the significance of environmental exposure to microbial compounds, and the effect of both on underlying responses of the innate and adaptive immunity. To date, a truly unifying concept has not yet emerged, but various pieces of a complex interplay between immune responses of the host, characteristics of the invading microorganism, the level and variety of the environmental exposure, and the interactions between a genetic background and a range of exposures becomes apparent. These influences are discussed as determinants for a number of complex allergic illnesses in this review, while we attempt to pay attention to the importance of different phenotypes, namely of the asthma syndrome. Even if today practical implications cannot directly be deduced from these findings, there is great potential for the development of novel preventive and therapeutic strategies in the future. (*J Allergy Clin Immunol* 2006;117:969-77.)

Key words: Allergy, asthma, infection, innate, microbial, virus, Toll

Abbreviations used

COAST: Childhood Origins of ASThma study
PRR: Pattern recognition receptor
RSV: Respiratory syncytial virus
TLR: Toll-like receptor

In recent years, widespread attention has been given to the advancement of one field in allergy research that investigates the potential link between exposures to microbial sources and the development of allergic illnesses. The theory attempting to encompass the various elements of this complex relation has been coined the *hygiene hypothesis*. A large scientific and lay audience has been confronted with these ideas over the last years, and in the course of numerous deliberations, new angles and aspects of the hypothesis have been proposed. At least 3 distinct claims on the true nature of the hygiene hypothesis have been brought forward. These contentions relate to the potential role of overt and unapparent infections of human subjects with viruses and bacteria, the relevance of noninvasive microbial exposures in the environment, and the influence of such exposures and infections on a subject's innate and adaptive immune response.

Before attempting to address these various aspects of the hygiene hypothesis, it seems justified to highlight the complex nature of the problem. The grid in which the pieces of this jigsaw must be assembled is at least 4-dimensional, comprising the affected phenotype, time, the environment, and genetic susceptibility. Although in clinical practice manifestations of allergic illnesses sometimes appear rather uniform, the underlying mechanisms and causes might be manifold. For example, urticaria is an easily recognizable skin condition, but the variety of factors eliciting these appearances ranges from infectious stimuli to allergic mechanisms to neoplastic illnesses. It seems reasonable to assume that not a single cause but many will underlie the clinical manifestation. Likewise, there is increasing evidence over recent years to support the notion of a heterogeneous nature of the asthma syndrome.

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A number of studies have clearly shown that the effect of a given exposure depends on the timing. At least over childhood and adolescence the human organism is in a constant stage of development and maturation. It is conceivable that these predefined processes display windows of accessibility and vulnerability to extrinsic influences only at certain stages of development. Moreover, prenatal factors might play a significant role, either through mechanisms acting *in utero* or as epigenetic modulation of subsequent developmental trajectories. Our journey into the discovery of the relevant genes for allergic diseases has just begun, and we are at the very beginning of a fascinating field of research. The first glimpses into this novel field suggest that no single gene will be responsible for the clinical manifestation of allergic illnesses. Rather, alterations in many genes interacting with environmental influences at various time points of development are expected to contribute to the mechanisms underlying the various atopic conditions.

An interesting debate about the immunologic mechanisms potentially underlying the protection against allergies mediated by living in a less hygienic environment is ongoing. One mechanism frequently associated with the hygiene hypothesis is the skewing of the T_H1/T_H2 balance away from allergy-promoting T_H2 cells toward T_H1 cells.¹ The link between the T_H1/T_H2 balance and allergic diseases is mediated in part by IgE: T_H2 cells, by secreting IL-4 and IL-13, promote immunoglobulin class-switch recombination to IgE (for review, see Geha et al²).

However, there are some conflicting data that cannot be disregarded. Not only T_H2 -related diseases, such as allergies, are on the increase over the last decades but also inflammatory diseases, such as Crohn's disease and diabetes mellitus.^{3,4} Populations with a high incidence of helminthic infections favoring a T_H2 type of immune response are protected from allergic diseases.⁵ Furthermore, *in vitro* and animal data show that activation of the innate immune system does not necessarily promote a T_H1 response; depending on the experimental conditions, T_H2 responses might result also.⁶ Finally, on *in vitro* restimulation with LPS of peripheral blood leukocytes of children living in an environment with high microbial load, both T_H1 - and T_H2 -related cytokines were found to be downregulated.⁷

Thus although mechanisms related to T_H1/T_H2 balance undoubtedly are of primordial importance for the development of allergies, they might not suffice to explain the effects related to the hygiene hypothesis. Mechanisms operative at other steps of allergy development have to be considered when trying to understand the effects of microbial exposures. Serum levels of IgE, as of any other Ig isotypes, are not only determined by means of class-switch recombination to this isotype but also by factors regulating terminal differentiation of already switched B cells and the rate of secretion of IgE. IL-6, a proinflammatory cytokine, is one prominent factor governing these processes. Furthermore, T regulatory cells, in interaction with dendritic cells, occupy a central role in controlling immune responses, and their importance for the development of allergies has been well documented.^{8,9} Finally,

also mechanisms inherent to the innate immune system related to endotoxin tolerance can contribute to the effects elicited by exposure to microbes, as will be discussed later.

INFECTIONS AS INTERMEDIARY OF THE HYGIENE HYPOTHESIS

Viral infections and asthma

Before examining the role of various viral infections for the inception of asthma and wheeze, we must define the phenotype as precisely as possible. On a chronologic scale, the first phenotype that can be delimited relates to the transient wheeze of infancy, which is manifest in the first 1 to 3 years of life and then disappears.¹⁰ Antenatally acquired decrements in lung function, exposure to tobacco smoke through the mother *in utero*, and low birth weight are likely to causally contribute to disease expression.^{11,12} Viral infections of the upper respiratory tract are the most prevalent and potent triggers of transient wheezing in infancy¹³ but are unlikely to be causal determinants of this condition.

Beyond infancy, wheezing phenotypes are much harder to unequivocally classify. Persistence of symptoms from infancy to school age has been proposed,¹⁰ whereas others have classified wheeze at school age either as current symptoms or as a diagnosis of asthma on the basis of parental report. Conflicting results have emerged from these analyses. Although infections of the lower respiratory tract early in life have been identified as risk factors for persistent wheeze and asthma,^{14,15} nasal symptoms and day-care attendance early in life have been inversely related to the same outcomes at school age.¹⁶⁻¹⁸

The inconsistency might in part be attributable to the disregard of an important discriminating feature, namely atopy. Children with persistent wheeze into school age can have signs of atopy or lack any specific IgE antibody production (Fig 1). In a general population birth cohort in the United Kingdom, nonatopic wheeze was as prevalent as atopic wheeze at age 10 years, and each phenotype affected around 10% of enrolled children.¹⁹ The risk factor profile differed significantly between both conditions. Nonatopic wheeze was mainly associated with recurrent chest infections at age 2 years, whereas atopic wheeze was related to allergic illness in the child and the family. It has been known for a long time that virus-associated wheeze has a milder course and a better prognosis than allergic asthma.²⁰ Thus the inverse association between frequent wheeze at school age and day care or nasal symptoms early in life might be attributable to the strong link of viral infections with the milder form of nonatopic wheeze, thereby falsely suggesting a protection against all forms of wheeze.

It is, however, also conceivable that a child's increased exposure to viral infections through day care or contact with other children can influence the phenotypic expression. In cross-sectional surveys recurrent respiratory tract infections during the first 3 years of life have been shown to be negatively associated with atopy among asthmatic children at school age.^{21,22} Thus increased exposure to

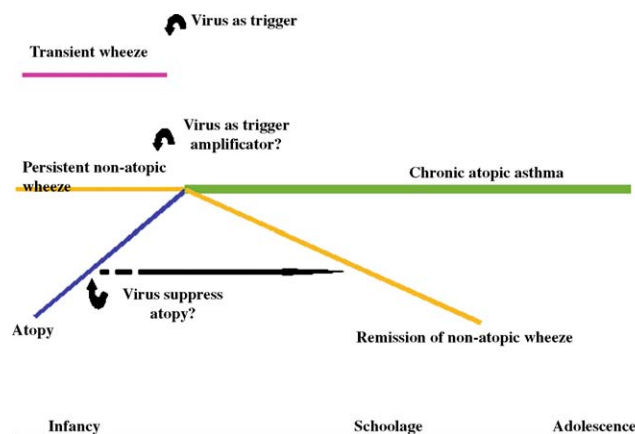


FIG 1. Schematic depiction of the role of viral infections in different wheeze phenotypes.

viruses in a child's environment might foster a milder form of wheezing by suppressing the atopic component. This notion is further supported by the studies investigating the effects of day care and rhinitis exposure early in life, which all showed a protection against atopy in the exposed children.^{16,17,23}

These epidemiologic observations rather crudely assessed the exposure to respiratory viruses without taking any details, such as the type of infecting virus, its virulence, the severity of the infection, and the viral load, into account. Among respiratory viruses, some might exert more deleterious effects than others. The ensuing discussion has mainly centered around 2 viruses that have been associated with asthma and wheeze in a large number of studies, namely rhinovirus and respiratory syncytial virus (RSV).

Rhinovirus has been detected in 80% of nasal aspirates of school-age asthmatic children within 4 days after parents reported episodes of wheezing.²⁴ A recent birth cohort of high-risk infants (Childhood Origins of ASThma [COAST] Study) has extended this work to younger age groups and confirmed that also in infants and toddlers up to the age of 3 years rhinovirus was the main isolate from nasal lavage specimens taken during symptomatic periods.²⁵ Infantile rhinovirus illnesses were the ones most significantly associated with the prevalence of wheezing in the third year of life. Interestingly, infants with wheezing rhinovirus illnesses were 2 to 3 times more likely to wheeze in year 3 compared with infants who wheezed with RSV infections. Furthermore, third-year wheezers were not infected more often during the first year of life but clearly had more severe symptoms of illness, suggesting that it is not the repeated infectious insult that elicits the wheeze, but rather the viral infection unmasks the underlying disposition.

These data put previous findings regarding RSV infections and their relation to asthma development into perspective. Only half of the high-risk infants of the COAST study who were infected with RSV wheezed, again suggesting that host factors are likely to play a significant role. Once an RSV infection has been accompanied by wheeze and bronchiolitis, the risk of subsequent wheeze is increased until school age and early

adolescence.^{26,27} Most studies did not find an association between RSV infection and the development of atopy. Furthermore, in the Tucson Children's Respiratory Study confirmed RSV had a similar effect on subsequent wheeze as other confirmed viral infections.²⁶ It is therefore plausible that RSV is just one of many respiratory viruses that are associated with the nonatopic persistent wheeze phenotype, as discussed above. The effect of each particular virus will depend on its own characteristics, the elicited immune response, and the interacting host factors. In addition, various genetic factors are likely to significantly modulate the immune responses to viruses, as recently shown in the COAST Study.²⁸

It has been suggested that viruses influence the differentiation of T cells upregulating T_H1 responses.²⁹ Viruses might also stimulate IL-10 production in T regulatory cells.³⁰ But viruses differ with respect to their invasive properties and the elicited immune response. Rhinovirus, for example, infects only small areas of the epithelial layer, with little or no mucosal damage. Even with large inoculating doses of virus, less than 10% of cells become infected. In contrast, RSV damages large groups of epithelial cells, resulting in edema, shedding of death cells, and increased mucosal permeability.³¹

The response to an invading virus depends on a host's immunologic setup (Fig 2). Deficiency in IFN- γ responses before the onset of bronchiolitis has been shown in human subjects and mice, suggesting an impaired viral defense before infection.^{32,33} Infants at risk of allergies might be particularly deficient in IFN- γ responses. Although initial studies have supported this concept,³⁴ long-term follow-up of such infants into school age has not confirmed the initial observations.³⁵ In fact, none of the early immunologic parameters, including T_H1 and T_H2 cytokines, were significantly predictive of allergic disease at age 6 years.³⁵ In turn, neonatal antigen-presenting cells fail to upregulate MHC class II and costimulatory molecules,³⁶ thereby providing insufficient stimuli to responding cells. Whether this deficiency differs between infants at risk and control subjects remains to be elucidated. Finally, it is unknown whether differences in antibody concentrations and classes exist between these groups.

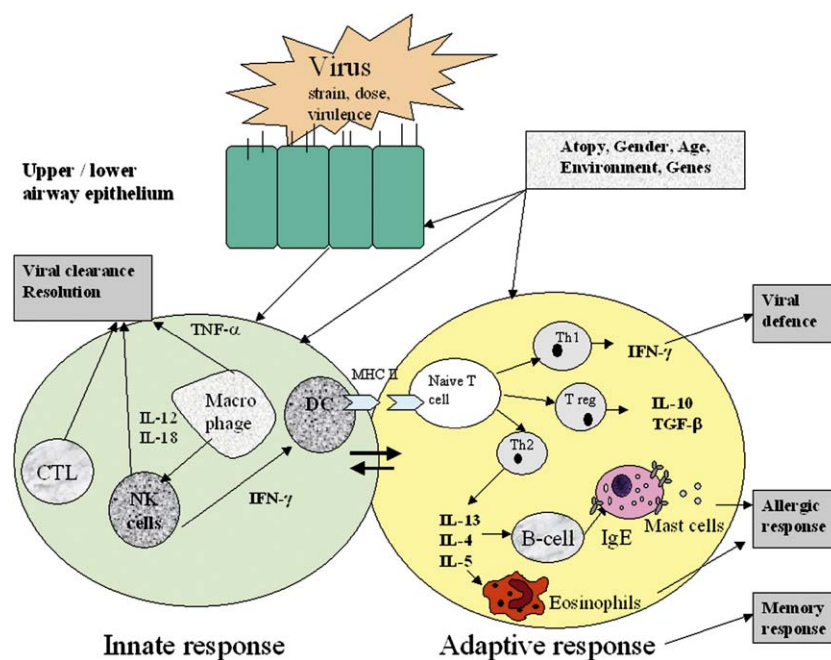


FIG 2. Interplay of the host's innate and adaptive immune responses to viral infections. CTL, Cytotoxic T lymphocyte; DC, dendritic cell; NK, natural killer cell.

In children at risk of allergy development, very little is known about the immunologic predictors of virus-induced wheeze. Gern et al³⁷ have recently shown that in infants with a family history of allergies, asthma, or both, mononuclear cell PHA-induced IL-13 and virus-induced IFN-γ responses at birth were indicative of the risk for wheezing in the first year of life.

In older children viral infections might interact with airway inflammation in several ways. Damage to the airway epithelium and consequently enhanced absorption of aeroallergens might lead to increased airway inflammation.³⁸ Furthermore, induction of proinflammatory cytokines (IL-6, IL-1β, and TNF-α), chemokines (IL-8, monocyte chemoattractant protein 1, and macrophage inflammatory protein 1α), adhesion molecules (intercellular adhesion molecule 1), and leukotrienes enhance cellular recruitment, activation, and inflammatory responses.^{39,40} This scenario is likely to occur among atopic and nonatopic wheezers. Finally, in asthmatic individuals viral clearance might be inhibited, thereby leading to more severe infections. However, studies in human subjects and mice do not support this notion.^{41,42}

Differences in specific characteristics of each virus, resulting in distinct immunologic changes, might play a role. The studies to date present a multifaceted story, demonstrating either T_H2 or T_H1 responses after RSV infection in human and murine models.^{43,44} For rhinovirus infections, increases in IL-10 levels, indicating a role for regulatory mechanism through, for example, regulatory T cells, were proposed, which might lead to an atopy-protective effect by this virus.^{45,46} Furthermore, a deficiency in IFN-β, impaired apoptosis, and increased virus replication were demonstrated in rhinovirus *in vitro* models,⁴⁷

opening the possibility for type I IFNs in the treatment or prevention of virus-induced asthma exacerbations. Some studies point out epidemiologic and immunologic similarities between bronchiolitis caused by influenza and RSV and suggest that host factors are more important than the nature of the infecting virus in the development of severe forms of bronchiolitis caused by influenza and RSV.⁴⁰

Other viral infections and allergic illnesses

There is conflicting evidence relating results of serologic studies investigating hepatitis A and relating these findings to the prevalence of hay fever and atopic sensitization in population-based studies. A number of surveys have shown protection from allergy with a positive serology to hepatitis A,⁴⁸⁻⁵⁰ whereas others could not confirm these results.⁵¹⁻⁵³ A positive serology to hepatitis A might thus be a marker of other unhygienic environmental exposures rather than a true culprit of the association, although the immunologic characteristics of hepatitis A might suggest a modulating effect. The receptor for the hepatitis A virus is T-cell immunoglobulin- and mucin-domain-containing molecules (TIM-1). This receptor and its ligand, TIM-4, belong to a family of proteins that are involved in the regulation of CD4 T-cell differentiation, airway inflammation, and airway hyperresponsiveness.⁵⁴

Few other viruses have been specifically investigated in the context of epidemiologic studies investigating the determinants of allergic diseases. Two reports have suggested a protective role for herpes infections,^{16,48} but confirmation in other populations is awaited. Also, infections with EBV and cytomegalovirus have been inversely related to specific IgE levels.⁵⁵

TABLE I. Influence of different exposure on the phenotype

Exposure	Influence on phenotype	
Respiratory viral infections	Rhinovirus	Trigger of wheezing episodes, risk for wheeze (?), protection against atopy (?)
	RSV	Risk for wheeze, mostly no effect on atopy
Other viral infections	Hepatitis A	Conflicting data for allergic illnesses
	Herpes	Potentially protective against atopy
	EBV	Potentially protective against atopy
Bacterial infections	Salmonellosis	Potentially protective against atopy
	<i>Helicobacter pylori</i>	Potentially protective against atopy
	Mycobacteria	Immunomodulation, conflicting results regarding atopy
Parasites		Potentially protective against atopy with high-intensity infections
Microbial exposure	Endotoxin	Protective against atopy, risk for wheeze
	Muramic acid	Potentially protective against wheeze
	Fungi	Weak association with atopic wheeze

Bacterial infections

There are few studies investigating the association between the occurrence of bacterial infections and the development of asthma and allergies. Although in a large Norwegian survey otitis media in infancy was negatively associated with allergic sensitization in school-age children of atopic parents,⁵⁶ no relation between a number of bacterial infections, including otitis media, and asthma was seen in the prospective Multicentre Allergy Study (MAS) cohort.¹⁶ In Sardinia children who had been hospitalized with salmonellosis had a lower prevalence of allergic rhinoconjunctivitis and asthma than children who had been hospitalized with nonbacterial enteritis,⁵⁷ but these findings need confirmation in other populations. In turn, a number of studies have investigated the potential protective effect of infection with *Helicobacter pylori* by measuring IgG antibodies toward this pathogen. Inverse associations with sensitization toward aeroallergens were seen in 2 studies,^{51,58} and in a composite score of seropositivity to hepatitis A, *Toxoplasma gondii*, and *H pylori*, the latter also contributed to an inverse relation with atopic sensitization, allergic rhinitis, and asthma.^{50,59} Interestingly, a dose-response relation was observed in these studies: the more infections these subjects had encountered, the lower the observed prevalence of atopy, allergic rhinitis, and asthma. These findings clearly suggest it is not one microorganism that accounts for the observed protection but most likely a number of agents.

The potential beneficiary role of mycobacteria exposure has been heavily discussed. The deliberations were grounded in literature suggesting a strong inverse association between BCG vaccination and the prevalence of allergic illnesses in Japan.⁶⁰ Subsequent work has, however, refuted such a role of BCG immunization for the development of asthma and allergies in Western populations.⁶¹⁻⁶⁴ The interest in mycobacteria is, however, continuing because these microorganisms show some remarkable potentially immunomodulatory characteristics. In murine models of allergic asthma, treatment with mycobacteria resulted in a suppression of several allergic features. The precise mechanisms of this suppressive effect are under investigation. Mycobacteria have been associated with increased T_H1 immune responses, primarily

an augmented IFN- γ secretion,^{65,66} but others were unable to reproduce these findings.^{67,68} Therefore other mechanisms, such as the induction of regulatory T cells and IL-10-dependent mechanisms, might be responsible for the observed effects.^{69,70}

Parasites

It seems unlikely that in westernized societies parasitic infections will play a major role in the protection against asthma and allergies. There is, however, good evidence to suggest that in endemic areas, such as in Africa or Latin America, parasitic infections are strongly inversely related to the development of atopy.^{71,72}

ENVIRONMENTAL EXPOSURES AS INTERMEDIARY OF THE HYGIENE HYPOTHESIS

Over recent years, there is accumulating evidence to suggest that not only overt infections of the human body but also environmental exposures to nonviable microbial products might pertain to the hygiene hypothesis. In this context the various exposures of children growing up on farms can be seen as a natural experiment. These children are in fact exposed to a large number of diverse microbial environments in animal sheds and hay lofts and through harvesting activities and certain foods. Neighboring children in the same villages but not exposed or much less exposed to these sources of microbial contamination serve as natural control subjects. A large number of studies has documented that children raised on farms have a lower prevalence of hay fever and atopic sensitization in childhood, as well as in adulthood, whereas effects on asthma are more consistent for the atopic than the nonatopic phenotype.⁷³⁻⁸² Important exposures in these environments have been identified as animal sheds,^{73,83} hay lofts (unpublished data), and the consumption of unpasteurized cows' milk.⁷³ The timing of the exposure played a crucial role because the protection was strongest when the exposures occurred in the first years of life compared with in later years.^{73,84} Interestingly, a clear maternal effect was also seen because the mother's exposure to animal sheds

during her pregnancy had a protective effect on the offspring.⁷³ This beneficial exposure is likely to be mediated, at least in part, by the innate immune system (unpublished data). In contrast, other potential determinants, such as the use of antibiotics and antipyretics, parasitic infections (unpublished data), and severe infectious illnesses early in life, do, in our hands, not contribute to the observed beneficial farming effect.

Some microbial exposures have been measured in these environments. Higher concentrations of bacterial products, such as endotoxin from gram-negative bacteria and muramic acid from all bacteria, were found in the mattresses of farm children compared with concentrations found in the mattresses of nonfarm children.^{7,85} Likewise, higher levels of fungal exposures were found in these environments.⁸⁶ Although endotoxin was inversely related to atopy and related phenotypes, it was a risk factor for nonatopic asthma, increased airway hyperresponsiveness, and low lung function.^{7,87} The levels of muramic acid were inversely associated with the nonatopic wheezing phenotype.⁸⁵ Fungal levels, in turn, had a weak association with atopic wheeze.⁸⁶ However, similar findings have also been reported from Norway.⁸² Endotoxin levels have also been measured in nonfarming environments, and most studies have found an inverse association with atopic sensitization but an increased risk of wheezing, supporting the findings from the farming environments.⁸⁸

Overall, the findings support the notion that nonviable products can stimulate immune responses in ways to protect against allergies. The question arises whether the immunologic mechanisms underlying the protective effect of infections and environmental exposure might be similar and what might be the common immunologic denominator of viable infectious microbes and microbial components present in the environment. Innate immune pathways might provide an answer. Pathogen-associated molecular patterns, evolutionarily highly conserved structural components of microbes, are recognized by similarly conserved receptors of the hosts' innate immune system, the pattern recognition receptors (PRRs).⁸⁹ Examples for pathogen-associated molecular patterns are the bacterial compounds cited above, LPS (endotoxin), and muramic acid, a component of peptidoglycan that is part of the cell wall of most bacteria. Examples for human PRRs are the human Toll-like receptors (TLRs) and CD14. To date, 10 functional TLRs have been described in human subjects. The cellular signaling cascade ensuing engagement of TLRs initiates innate host defense mechanisms,^{90,91} but it also provides signals required for initiating and modulating the adaptive immune response.^{92,93} In the context of the development of allergies, TLR4, serving as receptor for LPS,⁹⁴ and TLR2, which recognizes, for example, peptidoglycan of gram-positive bacteria,⁹⁵ have received the most attention. Polymorphisms in the genes for TLR4 and TLR2 have furthermore been shown to interact with these environments, modulating the allergy protective effect.⁹⁶ Other TLRs include TLR9, mediating responses to bacterial DNA through the recognition of cytosine-guanine pairs in the bacterial

DNA (CpG motifs),⁹⁷ and TLR5, recognizing flagellin (see review by Akira et al⁹⁸ in the current issue).

One could doubt whether microbial compounds, such as LPS, present in the environment and, in contrast to an infection, not eliciting any clinically apparent host response, will have any effect on the host's immune system at all. However, the observation that peripheral blood leukocytes of farmers' children, who live in an environment with a high load of endotoxin,⁷ express increased levels of TLR2 and CD14 when compared with that of less exposed children⁹⁹ strongly argues for a modulation of the innate immune response by microbial compounds, even in the absence of infection. How such activation of the innate immune response results in a reduced risk of allergies is an intriguing question.

Engagement of TLRs triggers a signaling cascade, resulting in host defense mechanisms, inflammatory responses, and signals for initiating adaptive immune responses. Interrupting one important pathway of TLR-triggered cellular activation in MyD88 knockout mice results in increased IgE levels and decreased T_H1-related immune responses.¹⁰⁰ This and other observations support the hypothesis that exposure to microbes skews the T_H1/T_H2 balance toward T_H1 responses. Indeed, many epidemiologic studies have reported that exposure to microbial compounds not only prevents clinical manifestation of allergic disease but also reduces IgE levels. However, as noted earlier in this review, changes in addition to alterations in the T_H1/T_H2 balance have to be invoked for a more comprehensive understanding of the effects of exposure to microbial compounds.

Although regulatory T cells have been shown to play a crucial role in allergic diseases and to underlie the clinical effects of specific immunotherapy,¹⁰¹ their contribution to the effects of TLR stimulation on the development of allergies is not yet fully elucidated. It seems that the suppressive regulatory activity of regulatory T cells can both be enhanced by activation through TLRs^{102,103} or downregulated.^{104,105}

Finally, activation through PRRs by microbial components might activate anti-inflammatory mechanisms of the innate immune response related to the phenomenon of endotoxin tolerance. This denotes the old observation that repeated or prolonged exposure to endotoxin results in some kind of refractory state, with reduced responsiveness on re-exposure.^{106,107} Interestingly, molecules contributing to endotoxin tolerance, such as IL-1 receptor-associated kinase—monomyeloic (IRAK-M)¹⁰⁸ or suppressor of cytokine signaling-1 (SOCS-1),¹⁰⁹ are upregulated by LPS. Again, the possible relevance of such results based on *in vitro* and animal experiments for the effects observed in human subjects in the setting of epidemiologic studies remains to be established.

Of note, activation of the innate immune response does not universally result in beneficial effects, as seen in the farm studies.^{7,110} Although it has been shown in mice that TLR4, as well as TLR2, agonists can decrease several allergen-induced parameters, such as pulmonary eosinophilia, IL-13 in bronchoalveolar lavage fluid, total serum

IgE, and airway hyperresponsiveness,¹¹¹ no effects or worsening of asthmatic symptoms after exposure to TLR ligands, such as LPS, have been reported.¹¹²

PRACTICAL IMPLICATIONS OF THE HYGIENE HYPOTHESIS

To date, there are no practical implications that can be deduced from the hygiene hypothesis for the prevention of asthma and allergies. Many authors have been concerned about a potential harm associated with the prescription of antibiotics, and a number of studies found a positive association between antibiotics and asthma. These associations are, however, likely to be attributable to reverse causation. Because in many countries asthma, particularly at a young age, is still treated with antibiotics, an association between the use of these drugs and asthma must become positive. Only surveys taking the indication of antibiotic prescription or the antedating of drug use before the beginning of asthma into account will therefore be able to adequately address this question. Such studies do thus far not show a convincing effect of antibiotic use on the development of asthma and allergic illnesses.¹¹³

The first attempt to introduce one concept of the hygiene hypothesis into clinical application is the administration of probiotics for the prevention of allergies. Although the first results showing a substantial reduction in the risk of development of atopic eczema are promising,¹¹⁴ confirmation in other populations is needed before a wide use of these products is justified. Numerous research groups are working on other future applications of immunomodulatory compounds derived from mycobacteria,¹¹⁵ helminths,⁷¹ and bacteria (CpGs).^{116,117} These are promising avenues into the future.

CONCLUSION

The hygiene hypothesis has seen many modifications by research in epidemiology, clinical science, and immunology. Three major trajectories have been proposed discussing the role of overt viral and bacterial infections, the significance of exposure to microbial compounds in the environment, and the interaction of both with underlying innate and adaptive immune responses. A truly unifying concept is still missing, but various pieces of a jigsaw containing a host's immune response, characteristics of the invading microorganisms, the level and variety of the environmental microbial exposure, and a genetic background become apparent. Practical implications cannot directly be deduced from these findings, but we see great potential for novel preventive and therapeutic strategies in the future.

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