

## Current perspectives

# Hygiene hypothesis: Fact or fiction?

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The hygiene hypothesis of asthma and allergy has recently received a swell of popularity and published supporting evidence, and has been extended to autoimmune conditions of childhood. Broadly stated, naturally occurring infections and microbial exposures might essentially immunize against the development of asthma and allergic and autoimmune diseases. If true, then reductions in nature's immunotherapy over the past century might be a major factor in the global increase of these conditions (eg, the higher prevalence of asthma and allergies in urban metropolitan areas compared with rural and farm communities) and might lead to new therapies for these conditions. Although such a unifying hypothesis has great appeal, currently it is only speculation about what might be at the end of the investigative road. How close are the current studies to establishing a causal relationship between microbial exposures and a reduction in allergic, asthmatic, and autoimmune disease prevalence? A systematic epidemiologic appraisal of the current hygiene hypothesis evidence can provide a critical analysis of what is currently known and an investigative blueprint for future studies that can ultimately prove causation and improve recommendations, interventions, and therapies. (*J Allergy Clin Immunol* 2003;111:471-8.)

**Key words:** Hygiene, allergy, atopy, asthma, autoimmunity, childhood, endotoxin, epidemiology, type 1 diabetes, celiac disease, inflammatory bowel disease, rheumatoid arthritis, microbiology

### THE DISCIPLINE OF EPIDEMIOLOGIC INVESTIGATION

This rostrum will use 10 established epidemiologic criteria to rate the validity of some of the main causal relationships of current hygiene hypothesis research (Table I). This analysis is linked to a compendium of future investigations that can fill in missing study pieces and lead current speculation in the direction of established fact (Table II). A schematic for specific cause-and-effect relationships within the hygiene hypothesis is also provided (Fig 1).

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#### Abbreviations used

AD: Atopic dermatitis

NHANES: National Health and Nutrition Examination Survey

Specific epidemiologic criteria need to be established before a strong causal relationship can be considered proven (Table I).

1. *Numeric association:* usually in the form of significant correlations or odds ratios in which significance is defined as a *P* value of .05 or less from the appropriate statistical test.
2. *Strength of evidence:* usually developed from prospective cohort studies in which the development from cause to effect can be examined in the same individual, and relative risk can be calculated. The type of study that has the greatest strength of evidence is the randomized controlled trial, in which the exposure is controlled and the subject is followed until an outcome event (or a sufficient time period specific to the outcome event) is observed.
3. *Sensitive and specific relationship:* increase in effect when cause is there and decrease when it is not.
4. *Dose-response relationship:* stronger than 3, when increased exposure to the causal agent across a range of levels leads to a proportional increase in outcome.
5. *Correct temporal relationship.*
6. *The relationship makes biologic sense.*
7. *The cause predicts the effect:* a stricter criteria than 3, whereby predictions can be made for specific individuals and not just for groups of individuals.
8. *Consistent findings under similar conditions:* additional studies in different cohorts and locales and by different investigators arrive at the same conclusions.
9. *Parsimony:* the relationship provides a straightforward explanation consistent with biologic mechanisms that conform with Occam's razor, and the significance of the relationship withstands moderating and confounding variables.
10. *Elegance:* the cause-and-effect relationship explains a variety of similar relationships.

The perfect cause-and-effect relationship, exemplified by known microbial pathogens and Mendelian genetic diseases, meet all of these criteria, even in the presence of confounding variables after appropriate adjustments have been made. There might be weaker causal relationships, such as the "web of causality" or the "2-hit model" in cancer that suggest that several things have to take place for

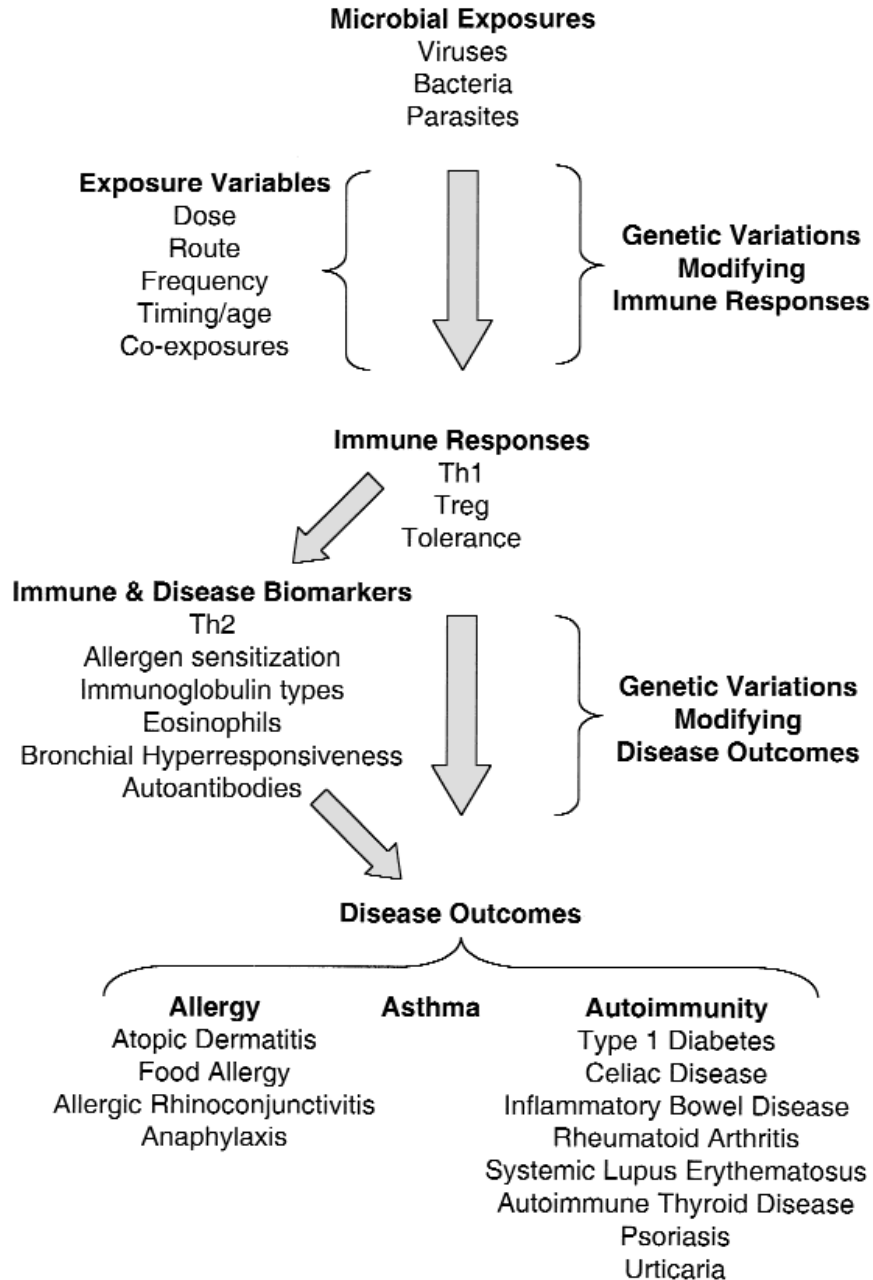


FIG 1. Cause-and-effect schematic for the hygiene hypothesis.

the causal chain to be activated. Although their predictive value might be more complicated, these complex causal frameworks still rely heavily on the 10 items listed above.

### ENDOTOXIN, ALLERGY, AND ASTHMA: UNDER THE MICROSCOPE

Bacterial endotoxin exposure in childhood is a good example of a naturally occurring exposure that might alter immune development and lead to less allergy and asthma. The long study of endotoxin provides a rich reservoir of information for building hygiene hypothesis paradigms.<sup>1</sup> Basic and clinical scientists have elucidated

the immune stimulatory potency of endotoxin, the specific cellular and molecular mechanisms leading to endotoxin-mediated activation, how endotoxin alters innate immune responses and instructs adaptive immunity, endotoxin's potential for benefit and harm, and genetic polymorphisms that can alter immune responses and disease outcomes. Important factors that are expected to influence the relationship of endotoxin exposure to disease outcomes include (1) timing of exposure relative to disease development (eg, early life exposure), (2) the nature of the stimulus (eg, dose and frequency), (3) coexposures, and (4) genetic variations underlying immune responsiveness to endotoxin.<sup>1</sup>

**TABLE I.** Epidemiologic criteria that establish causation applied to the hygiene hypothesis research areas discussed herein

	Endotoxin	Common colds	GI tract flora	GI tract pathogens	Autoimmunity
1. Numeric association	+	+	+	+	+
2. Strength of evidence	±	+	+		
3. Sensitive-specific relationship	+	+	+	+	
4. Dose-response relationship	+	+	±	+	
5. Temporal relationship	+	+	+		
6. Biologic plausibility	+	+	±	+	±
7. Cause predicts effect	+	+	+		
8. Consistent findings		±	±	±	±
9. Parsimony	+	+	+		
10. Elegance		+			

GI, Gastrointestinal; +, meets criteria; ±, suggests criteria.

**TABLE II.** Studies that can establish causation between microbial exposures and subsequent allergy, asthma, and autoimmunity

- *Longitudinal prospective studies:* for farm-nonfarm, rural-urban, and metropolitan–low-income inner-city comparisons. Ideally, birth cohort studies that include food allergies, AD, allergic rhinitis, and asthma, with measured microbial exposures. Aim for consistent measures of exposure and disease in the different studies to allow for comparisons between studies. Support ongoing prospective birth cohort studies to continue to the school years.
- *Biomarkers:* develop legitimate biomarkers of regulatory immune mechanisms in human patients that link to animal models of disease.
- *Genetic polymorphisms:* assessing the effect of genetic modifiers of immune responses to microbial exposures and their associations with disease outcomes. A longitudinal prospective birth cohort format that includes relevant measures of environmental exposures will provide the strongest evidence and allow for gene-environment interactions analyses.
- *Adulthood:* does this protective association before disease development hold true for adults and older children, or is this a phenomenon of early childhood?
- *Autoimmunity:* studies of autoimmune conditions (eg, type 1 diabetes, celiac and inflammatory bowel disease, juvenile rheumatoid arthritis, lupus, autoimmune thyroid disease, psoriasis, and urticaria) that mirror the stronger epidemiologic studies for allergic diseases and asthma. Much larger subject numbers will be needed because of the relatively low prevalence of these conditions.
- *Immune mechanisms:* a better understanding that accounts for the paradox of microbes mitigating autoimmune development but still have T<sub>H</sub>1-mediated autoimmunity. A better understanding of regulatory immune mechanisms, such as regulatory T lymphocytes in autoimmunity, in response to parasites and other microbes, and in asthma and allergy.
- *Microbes—friend and foe:* reconciling the paradoxical causal relationships between microbe exposures and these immune-mediated conditions. For example, are those who are susceptible to early wheezing or later occupational-type asthma from endotoxin exposure the same as those who are likely to benefit from early childhood exposure? Refined understanding of these issues is important to the safety concerns of future microbe-derived interventions.
- *Randomized, controlled intervention studies:* the strongest study design for satisfying the epidemiologic criteria. These studies are challenging to develop because early intervention might be the key, and there will be safety issues to resolve before such trials can be conducted in young children.

Some of the most recent studies on endotoxin exposure exemplify the epidemiologic weight of the current hygiene hypothesis evidence. A collaborative group of European investigators of farm and nonfarm communities in Germany, Austria, and Switzerland have reported numerous associations between greater endotoxin exposure and asthma and allergy outcomes in school-age children.

1. Greater endotoxin exposure (as measured in mattress dust) was associated with less allergen sensitization, hay fever symptoms, and atopic asthma, in a dose-responsive manner.<sup>2</sup>
2. Farm children were exposed to more endotoxin.<sup>2</sup>
3. Greater endotoxin exposure was associated with less endotoxin-induced TNF- $\alpha$ , IL-10, IL-12, and IFN- $\gamma$  production by blood cells in vitro, in a dose-responsive manner.<sup>2</sup>
4. The blood cells of farmers' children expressed high-

er amounts of CD14 and toll-like receptor 2, which are innate immune receptors for microbial compounds that include endotoxin.<sup>3</sup>

5. Recollection of frequent exposures to farm barns and unpasteurized milk in early life had the strongest associations with low asthma and allergy prevalence.<sup>4</sup>
6. High levels of endotoxin exposure was associated with an increased prevalence of nonatopic wheezing.<sup>2</sup>

These studies demonstrate the epidemiologic criteria of numeric association (criterion 1), and provide some information concerning dose response for endotoxin (criterion 4), immune biomarker links to the biologic paradigms (criterion 6), and clues to temporal relationships (ie, the importance of early life exposures [criterion 5]). One argument from these numeric associations is that living in a farm setting protects one from atopic asthma because early exposure to endotoxin alters relevant

immune development. Most large cross-sectional studies are vulnerable to reverse causation (ie, the potential for the causal links to be in the opposite direction of that hypothesized). For example, in children with atopic asthma, their mothers might wash their bedding more often and keep them from spending time in the barns to minimize the exposures that might worsen their symptoms, leading to lower levels of mattress dust endotoxin. The numerous associations of immune biomarkers with endotoxin exposure strengthen the links to biologic plausibility.<sup>1</sup> However, the biomarkers in these studies, except for allergen sensitization, have not been associated with disease outcomes (eg, asthma and hay fever). These large association studies are also vulnerable to “ecological fallacy” (ie, the attempt to apply cause and effect to an individual on the basis of associations made by using large groups of subjects). In other words, these studies can suggest association but do not prove cause and effect on an individual level.

Prospective birth cohort studies can provide stronger evidence of a causal relationship between early life endotoxin exposure and less allergy and asthma in later childhood by actually tracking the same group of individuals from exposure to disease development. In a large birth cohort study in metropolitan Germany (LISA study,  $n = 1884$ ), a significant association between endotoxin exposure in infancy and less subsequent atopic dermatitis (AD) was observed as a possible effect on the allergic march of early childhood.<sup>5</sup> Specifically, mattress dust endotoxin levels sampled 3 months after birth were assessed for associations with disease outcomes on the basis of parent questionnaires at ages 6 and 12 months (eg, history of physician-diagnosed AD, as well as itchy rash, cough, and wheezing). Significantly lower disease prevalence was observed only for physician-diagnosed AD at 6 months of age with the highest quintile of endotoxin exposure. Therefore dose-response associations, persistent temporal associations, and consistent findings for both physician-diagnosed AD and reported itchy rash were not observed for AD. However, a higher prevalence of respiratory symptoms at ages 6 and 12 months, including physician-diagnosed respiratory infection and parent-observed wheezing, was also observed. This latter observation is consistent with another birth cohort study of infants in Boston, Massachusetts, in which endotoxin was measured in dust samples from 4 household locations (bedroom, bedding, family room, and kitchen) within the first 3 months of life.<sup>6</sup> Higher endotoxin levels in only the family room dust samples were significantly associated with parental reports of repeated wheezing in the first year of life.

Continuing these birth cohort studies into the elementary school years will bring their study participants into an age range in which the natural history and outcome measures of asthma and allergen sensitization are stronger. For the Boston cohort, a progressive decline in the prevalence of parent-reported wheezing at ages 2, 3, and 4 years was observed in those with higher dust endotoxin levels, such that by age 4 years, the wheezing prevalence

trend was lower for the endotoxin-high versus endotoxin-low group.<sup>7</sup> This suggests a paradoxical temporal relationship between endotoxin exposure and wheezing conditions: greater exposure might increase wheezing in the first year of life and also decrease wheezing conditions after age 4 years.

Considered together and in the context of the epidemiologic criteria for establishing causation, the current farm-nonfarm endotoxin studies meet epidemiologic criteria 1 and 3 listed above and provide some limited information for criteria 4, 5, 6, 7, and 9 (Table I). These are strong hypothesis-generating observations for future longitudinal prospective farm-nonfarm studies (Table II). The current prospective birth cohort studies on endotoxin are beginning to satisfy criteria 2, revealing some associations in the first year or two of life between early endotoxin exposure and subsequent AD. Endotoxin exposure is also associated with more nonspecific respiratory illness, including wheezing. These studies are in their early stages to draw conclusions regarding asthma and respiratory allergic outcomes, but they have the potential to satisfy most of the listed criteria. The subjects and environments of these endotoxin birth cohort studies might differ significantly from the current farm studies in their levels of endotoxin exposure because the range of exposure might be higher for children raised on farms, in rural communities, or both.<sup>8</sup>

## THE CURE IS THE COMMON COLD

The German Multicentre Allergy Study shows the merits of prospective birth-to-7-years investigations in the study of causal relationships in asthma. The investigators in this natural history study assessed the relationship of infectious diseases in the first 3 years of life with asthma outcomes until or through age 7 years.<sup>9</sup> Colds with a runny nose had remarkable associations with less asthma. The associations of colds with a runny nose were strengthened by a dose-response relationship (more colds with less asthma), a temporal relationship (more colds and less reported wheezing at ages 4, 5, 6, and 7 years), and an effect on both reported and objective measures of asthma and atopy at age 7 years (physician-diagnosed asthma, parent-reported wheezing, bronchial hyperresponsiveness to histamine, and inhalant allergen sensitization). Bacterial, fungal, gastrointestinal, and urinary tract infections and antibiotic courses in the first 3 years were also enumerated, but no similar associations with asthma or allergen sensitization were found. This investigation meets most of the causal criteria (Table I).

A prospective birth cohort study in Tucson, Arizona (Tucson Children's Respiratory Study), is a complementary investigation in which children raised with more older siblings or attending daycare from an early age (ie, during the first 6 months of life) were more likely to have frequent wheezing at age 2 years but increasingly less likely at ages 6, 8, 11, and 13 years.<sup>10</sup> One possible reason for this finding is that routine exposure to other young children leads to more common colds. Daycare attend-

ance in the first 2 to 3 years of life has been well associated with more observed respiratory tract infections.<sup>11-14</sup>

One might argue that, together, these 2 prospective birth cohort studies provide consistent evidence (ie, criterion 8) for common colds in early childhood and a lower likelihood of asthma in the school years, even if the measures of respiratory virus exposure (ie, cold symptoms, older siblings, and daycare) are indirect. All of the causal criteria could then be considered met to varying degrees (Table I). These large prospective birth cohort studies that extend into the school ages demonstrate that although challenging to accomplish, they are feasible and provide powerful findings.

## A GUT FEELING

Similar associations have been found between gastrointestinal microbes and less allergy and asthma. In a large cross-sectional survey in the United States (Third National Health and Nutrition Examination Survey [NHANES]), serologic antibody evidence of previous hepatitis A, as well as *Toxoplasma gondii* and herpes simplex virus 1 infections (but not hepatitis B or C), were associated with less asthma, hay fever, and allergen sensitization.<sup>15</sup> This observation mirrored a similar study of Italian military cadets, in which cumulative exposure to hepatitis A, *Toxoplasma* species, and/or *Helicobacter pylori* (ie, seropositive to none vs 1 vs 2 or 3) was associated with an increasing effect on less atopy, allergic rhinitis, and allergic asthma.<sup>16</sup> In comparison, this Italian study observed no significant association between atopy and seropositivity to measles, mumps, rubella, chicken pox, cytomegalovirus, or herpes simplex virus 1. Meanwhile, in rural Africa, where parasitic infestation is common, *Schistosoma* species<sup>17</sup> or *Ascaris* species-hookworm<sup>18</sup> infestation was associated with less allergen sensitization and asthma. The paradox of less allergy and asthma in children infested with T<sub>H</sub>2-inducing parasites is bolstering interest in the potential disease-mitigating role of IL-10-producing cells, such as regulatory CD4<sup>+</sup>CD25<sup>+</sup> T lymphocytes, to override or bypass T<sub>H</sub>2 pathogenic mechanisms.<sup>19</sup> Regulatory CD4<sup>+</sup>CD25<sup>+</sup> T lymphocytes can block autoimmunity and transplant rejection in some animal models. They also develop in response to foreign antigens, such as infectious pathogens, although their role in these immune responses is less clear.<sup>20</sup> In a murine model of allergic asthma, in vitro-generated IL-10-producing T cells downregulated the allergic response.<sup>21</sup> The combination of IL-10 with IL-4 can induce B lymphocytes to produce IgG<sub>4</sub> instead of IgE, thereby modifying the T<sub>H</sub>2 response to be nonpathogenic.<sup>22,23</sup>

Prospective longitudinal birth cohort studies have reported differences in bacterial colonization of the gastrointestinal tracts of infants who have allergic disease. In one study, allergic infants (ie, AD, allergen sensitization, or both at age 2 years) had less enterococci and bifidobacteria and more clostridia and *Staphylococcus aureus* in stool samples obtained during at least one of 5 ages during the first year of life.<sup>24</sup> In a similar study, stool sam-

ples from atopic infants (who were allergen-sensitized at age 12 months) had more clostridia and less bifidobacteria.<sup>25</sup> The strongest type of prospective clinical investigation, the randomized controlled trial, is exemplified in a birth cohort trial of oral lactobacillus GG supplementation in the first 6 months of life (beginning several weeks before birth).<sup>26</sup> The prevalence of AD by age 2 years was reduced 2-fold in those receiving supplementation (23% vs 46%,  $P = .008$ ). In exclusively breast-fed infants in which the mothers received the lactobacillus GG, the effect was greater (15% vs 47%,  $P = .01$ ).<sup>27</sup> Biologically, clinical and basic investigators are trying to understand how the gastrointestinal tract, a reservoir for symbiotic bacteria, might also be a site for bacteria-induced immune modulation that can alter disease outcomes.

Because inhalant allergies and asthma develop later in childhood, these studies are similar to the prospective birth cohort studies on endotoxin exposure described above because their cohorts are too young to be used to draw conclusions about subsequent allergic rhinitis and persistent asthma. The ideal of continuing such longitudinal prospective birth cohort studies over at least 5 years is a challenging aspect of the investigation of these medical conditions. The associations with less AD and allergen sensitization in infancy, which are well-known risk factors for later childhood asthma and allergic rhinitis, substantiate the importance of longer and larger studies that continue into the school ages.

Considering the causal criteria, the current studies of gastrointestinal pathogens (eg, hepatitis A and ascaris) meet criteria 1, 3, 4, and 6 (Table I). The same research group has made very similar observations in 2 different cohorts and databases (US NHANES and Italian military cadets), which begins to satisfy criterion 8 (consistency of findings). These are strong hypothesis-generating observations for future longitudinal prospective studies. The current prospective birth cohort studies of gastrointestinal colonization with symbiotic bacteria further satisfy criterion 2, revealing some associations in the first year or two of life between differences in gastrointestinal flora (natural or lactobacillus supplemented) and subsequent AD. The lactobacillus supplementation study is particularly strong in its randomized controlled prospective design. For AD in infancy, this further achieves criteria 5 (temporal relationship), 7 (cause predicts effect), and especially 9 (parsimony) because rigorously controlled modification of a single variable was associated with significant differences in AD prevalence. Continuing such studies until the children reach school age can provide conclusive evidence for childhood asthma and allergic rhinitis (Table II).

## HYGIENE IN ESTABLISHED DISEASE: THE OPPOSITE CAUSE-AND-EFFECT RELATIONSHIP

Once asthma and allergic diseases are established in an individual, the cause-and-effect relationship is generally the opposite: microbial exposures worsen estab-

lished disease. Endotoxin exposure can again serve as a model example to illustrate this relationship. Endotoxin exposure at high levels in workplace settings is a common cause of occupational asthma.<sup>1,28</sup> Inhaled endotoxin challenge studies in human patients have well demonstrated that patients with asthma and allergic rhinitis are sensitive to endotoxin exposure, resulting in both bronchoconstriction and worsening of airways inflammation. Furthermore, some nonatopic nonasthmatic persons are hypersensitive to inhaled endotoxin, defining an endotoxin-sensitive asthma type.<sup>29</sup> In contrast, some people who are hyporesponsive to inhaled endotoxin have loss-of-function mutations in the gene encoding toll-like receptor 4 receptor (endotoxin receptor).<sup>30</sup> This is an example of scientific elegance, establishing a direct causal relationship between inhaled endotoxin exposure and both airflow obstruction and airways inflammation. Although the epidemiologic effect of these genetic polymorphisms in populations (eg, farm and nonfarm children and occupational settings) has yet to be established, it is strong evidence of differing cause-and-effect relationships between endotoxin and asthma dependent on timing, dose, and frequency of exposure, and individual differences in capacity to respond to the exposure.

Such duality in causal relationships between microbes and asthma-allergy challenges and helps to define the limits of  $T_H1/T_H2$  relevance to these diseases. The original paradigm of a reciprocal relationship between  $T_H1$  and  $T_H2$  might only be relevant to immune development before the establishment of these diseases. This is consistent with numerous studies documenting increased  $T_H1$  and  $T_H2$  cells in asthmatic airways<sup>31</sup> and AD skin lesions.<sup>32</sup> This implies that moderating influences on  $T_H1/T_H2$  immune development might have the greatest effect before disease development, with potentially little benefit for established disease. Understanding the plasticity of disease development, establishment, or both between these opposing paradigms will help to distinguish those who might benefit from those who might be harmed (Table II).

### HOW VALID IS THE HYGIENE HYPOTHESIS FOR AUTOIMMUNITY?

Several recent epidemiologic investigations suggest that autoimmune conditions of childhood might have similar causal relationships with asthma. An assessment of several Finnish medical registries revealed asthma to be more common in children with autoimmune diseases.<sup>33</sup> The cumulative incidence of asthma in Finnish children with celiac disease was 24.6%, that of rheumatoid arthritis was 10%, and that of type 1 diabetes was 5% compared with 3.4% when these autoimmune conditions were absent. This epidemiologic observation is congruous with the results of a study of the prevalence of childhood asthma (ie, >4 episodes of wheezing in the past 12 months in 13- to 14-year-old children) and type 1 diabetes (0- to 15-year-old children) in 28 different countries, that found a strong positive correlation of the preva-

lence of these 2 conditions.<sup>34</sup> High prevalence rates of both diseases were found for affluent English-speaking countries. A study of the adult data from the third US NHANES (n = 20,050) similarly found that the subpopulation with an autoimmune condition (ie, type 1 diabetes, thyroid disease, and/or rheumatoid arthritis) had a higher prevalence of physician-diagnosed asthma, hay fever, or both.<sup>35</sup> In this study, autoimmunity was not associated with allergen sensitization to at least one of 10 common aeroallergens.

Because autoimmunity is commonly considered to be  $T_H1$  mediated, these epidemiologic clues suggest that the immune pathogenic processes underlying asthma and autoimmunity are not mutually exclusive (ie, not  $T_H2$  vs  $T_H1$ ), might be shared, and might also extend the hygiene hypothesis to autoimmunity. In terms of establishing causal relationships, however, these registry studies are useful as a starting point, but are one of the weaker study types for establishing causality. They lack a direct link between cause and effect and do not meet epidemiologic criteria 2 through 10, with the possible exceptions of 6 and 8 (Table I). As a basis for comparison, the cross-sectional farm-nonfarm studies have more strength because they connect the endotoxin levels of specific individuals with their disease status and provide some evidence of a dose response.

Biologically, the influence of microbe-driven immune development on autoimmune disease outcomes is not as well understood.<sup>36</sup> For the hygiene hypothesis to be relevant to autoimmunity, there is a fundamental immune paradox. Bacteria and viruses typically induce  $T_H1$ -type immune responses; however, many autoimmune conditions appear to be mediated by autospecific  $T_H1$  cells. Autoimmune animal models reveal that  $T_H1$  autoimmunity is counterbalanced by the previously described regulatory T cells to reconcile this paradox. Their current identity is a subset of lymphocytes expressing CD4 and CD25 surface markers and producing IL-10 and TGF- $\beta$ , with other undetermined cell-cell interactions underlying their regulatory effects on conventional T-cell activation.<sup>20</sup> Autoimmune animal models also suggest hygiene hypothesis mechanisms because unclean mice are less prone to the development of spontaneous autoimmunity. However, the mechanisms by which microbes can reduce the likelihood of autoimmunity are less clear. For autoimmunity, hygiene hypothesis research is relatively nascent, and when compared with prevalent childhood conditions, such as asthma and allergic diseases, the relatively uncommon autoimmune conditions will require much greater numbers of subjects for epidemiologic investigations to be adequately powered (Table II).

### HYGIENE IN THE CITY?

Is there a hygiene hypothesis paradox in US inner cities, in which, despite presumably unclean living conditions, a disparate asthma burden has been a worsening problem for the inner-city poor? Recent studies from the US Centers for Disease Control found that asthma preva-

lence is higher for those living in US urban versus rural communities, but is not significantly influenced by ethnicity or poverty.<sup>37,38</sup> Asthma severity (eg, emergency department visits, hospitalizations, and death), however, is linked to poverty and African American heritage.<sup>37,39</sup> When considered with the microbe-asthma causal relationships described above, less hygienic conditions in the inner city are consistent with greater asthma severity, but inconsistent with greater asthma prevalence.

For hygiene hypothesis considerations, unclean inner-city living conditions might not be the equivalent of farm and rural living. For example, endotoxin levels in dust from the homes of low-income metropolitan Denver families were much lower than from farm homes, rural homes, and farm barns, and relatively close to those of non-low-income families in Denver.<sup>40</sup> Many other factors that contribute to the disparities experienced by inner-city asthmatic children is beyond the scope of this article.<sup>41</sup> Current hygiene hypothesis investigations suggest that prospective birth cohort studies of asthma development in inner-city children that test these causal relations would be very valuable (Table II).

## CONCLUDING REMARKS

This article does not provide a complete account of hygiene hypothesis-related research, published or ongoing. Instead, a systematic epidemiologic approach to the consideration of this body of evidence, with some relevant examples, has been provided. To date, the hygiene hypothesis argument for the prevention of allergy, asthma, and autoimmune diseases has not been developed strongly enough to provide any course of action for either prevention or therapy. The current evidence, however, is intriguing and can well substantiate an investigative blueprint from which essential studies can be targeted and developed, and causation between microbial exposures and subsequent allergy, asthma, and autoimmunity can be established (Fig 1). Some complexities in causal relationships are already apparent. For example, many different microbial exposures might contribute to a microbial burden of causation. There are opposing causal relationships between microbial exposures and these diseases: early exposure might prevent subsequent disease development, and exposure worsens established disease. Some individuals are unlikely to respond to microbial exposures, others are predisposed to experience harmful outcomes from microbial exposures, and even others might experience primarily benefit. A reciprocal relationship between  $T_H1$  versus  $T_H2$  immune development and allergy, asthma, and autoimmunity seems too simplistic because commonly classified  $T_H1$  and  $T_H2$  diseases coexist in the same individuals. Additional mechanisms of immune responses and development (eg, regulatory immune responses) are being fervently studied. The current studies already implicate these complexities in a classic gene-environment interaction in health and disease (Fig 1). Some of the investigational pieces that can lead to a full and refined understanding of cause-

and-effect relationships and important variables are listed (Table II).

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