Allergen Tolerance Versus the Allergic March: The Hygiene Hypothesis Revisited

Kevin Tse, MD, MAS, and Anthony A. Horner, MD

Corresponding author
Anthony A. Horner, MD
Department of Medicine and the Sam and Rose Stein Institute for Aging, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0663, USA.
E-mail: ahorner@ucsd.edu

Current Allergy and Asthma Reports 2008, **8:**475–483
Current Medicine Group LLC ISSN 1529-7322

In addition to genetics, several environmental variables appear to impact allergic risk. Meta-analyses of epidemiologic studies presented in this article demonstrate a correlation between specific ambient exposures (eg, livestock, pets, endotoxin, and unpasteurized milk ingestion) and reduced allergic risk during childhood. Additional laboratory investigations discussed in this review characterized the intrinsic immunostimulatory activities of living environments. Considered together, results of these investigations suggest a novel paradigm by which early-life home exposures to microbial products and other allergen-nonspecific immunostimulants modify allergic risk.

Introduction

Over the past half-century, allergic diseases have become far more common in industrialized countries, whereas atopy rates remain low in most of the Third World [1•,2,3]. Although reasons for these trends remain speculative, the rapidity with which allergic disease prevalence rates have increased in affected countries strongly suggests environmental factors have had a dominant role. Therefore, there is a great deal of interest in determining which ambient exposures are responsible for the low and high allergic disease prevalence rates of poor and affluent countries, respectively.

Allergen exposures are clearly required for the development of T-helper type 2 (Th2)-biased hypersensitivities. For some allergens (eg, cockroach and house dust mite), the risk of developing hypersensitivities increases considerably when the home allergen burden increases above quantifiable threshold levels [4–6]. However, for other allergens (eg, dogs and cats), increased levels of home exposure appear to be linked to a decreased risk of sensitization to the allergen of interest and to other unrelated allergens [6,7]. These and other lines of investigation suggest that aside from allergens themselves, living environments contain additional molecules that influence the immunologic balance between allergen-specific tolerance and hypersensitivity.

Epidemiologic studies have found that environmental variables linked to lifestyle (eg, urban vs rural living) [8], diet [9], exposures to diesel exhaust and other man-made pollutants [10•], and infectious and noninfectious exposures to microbes [1•] influence allergic risk. One consistent finding derived from these studies is that children reared on farms are less likely to develop allergic diseases than children reared in cities [8,11•,12]. In a previously published meta-analysis of nine pertinent studies, we found an odds ratio (OR) of 0.74 with 95% CIs of 0.61 to 0.91 for allergic stigmata in children reared on farms compared with children reared in nonfarming environments [13]. However, the reasons why farm living reduces allergic risk remain speculative. As houses located on farms, particularly those with livestock, are rich in microbial content [8,14–16], it has been suggested that microbial stimulation educates host immunity in a manner that prevents dysregulated immune responses to ambient allergens. This theory—the "hygiene hypothesis"—is also supported by investigations in which other variables linked to microbial exposure, including pet ownership, family size, daycare attendance (community-acquired infections), vaccination status, antibiotic use, animal exposure, and infectious disease history, were found to influence allergic disease risk [1•,8,17].

Testing for Associations Between Early-Life Exposures and Allergic Risk: Meta-Analysis Techniques

Ever since children reared in farming communities were shown to have reduced atopy rates [8,11•,12], researchers have tried to identify specific farm-associated exposures

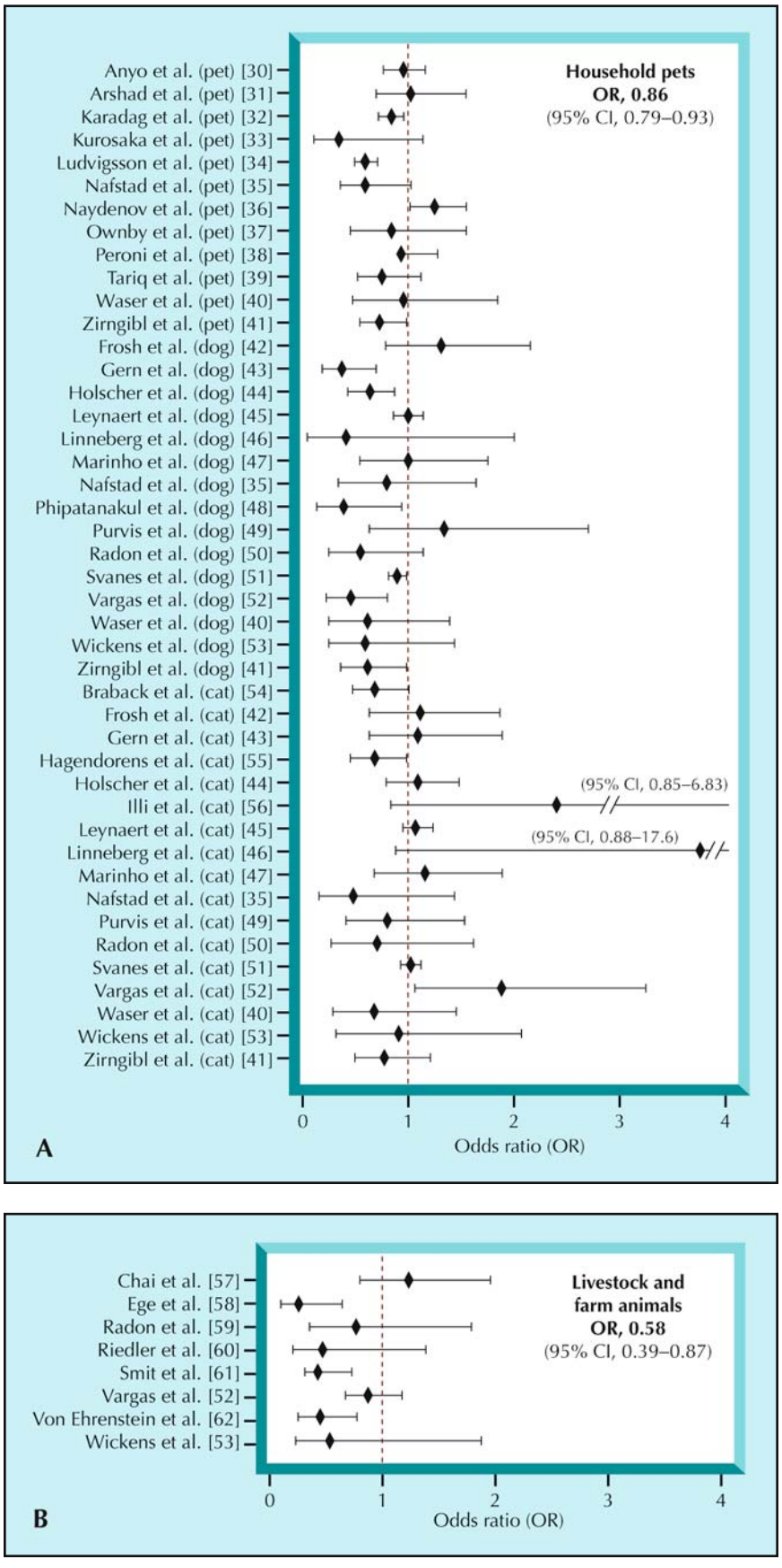
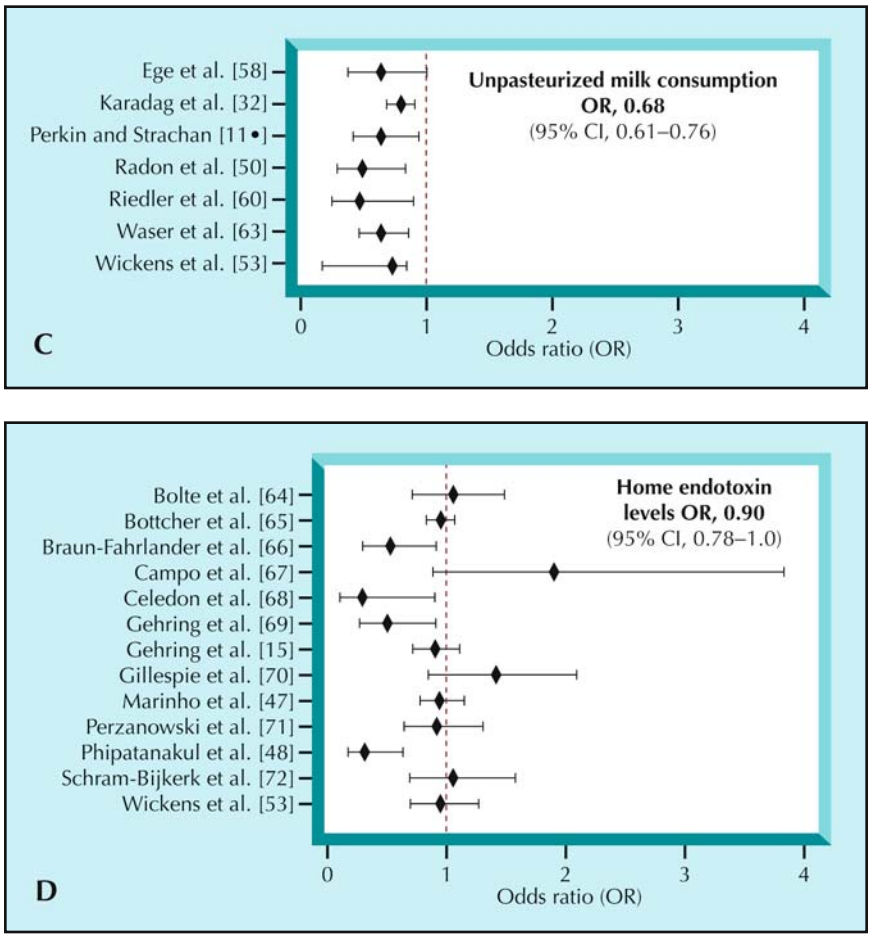


Figure 1. Associations between farm-related exposures and allergic risk during childhood. **A**, Household pet exposure. **B**, Livestock and farm animals. **C**, Unpasteurized milk consumption. **D**, Home endotoxin levels. Individual studies are identified by the first author and reference number in this paper's bibliography. Odds ratios (ORs) and 95% CIs are represented by black squares with error bars. If individual studies offered more than one relevant OR with CIs, summary ORs/CIs were calculated and presented in the figure. Additional between-study summary ORs and CIs were determined for investigations identified within each meta-analysis. All ORs and CIs were calculated by random-effects modeling.



that may protect against the genesis of allergic diseases in rural and urban settings. Although results have not always been consistent, many epidemiologic studies suggest that regular exposure to livestock and pets, unpasteurized milk consumption during childhood, and elevated home endotoxin levels protect against the allergic march. To better assess the real impact of these exposures on pediatric allergic risk, we conducted meta-analyses of all relevant studies published between 1966 and 2008.

Initially, MEDLINE searches were conducted to identify pertinent articles using the following search commands: "atopy," "allergy," "asthma," "eczema," "wheeze," or "rhinitis" and "endotoxin," "dog," "cat," "livestock," or "unpasteurized milk." This search identified 6758 articles of potential relevance for these meta-analyses. All abstracts were reviewed independently by two investigators. Abstracts obviously unrelated to the topic at hand were discarded. For the rest, copies of full articles were retrieved and reviewed. To be included in these meta-analyses, investigations were required to meet the following criteria: 1) they had to have assessed for associations between relevant environmental exposures during childhood and atopic risk, 2) they were considered of compatible design with other studies included in the analyses, 3) results were reported as ORs to facilitate the execution of meta-analyses, and 4) the design and quality of their datasets were deemed adequate and appropriate based on descriptions provided in the text of the paper.

Of the initial 6758 papers identified in the MEDLINE database with our search commands, 6712 were excluded because they were considered irrelevant, incompatible for comparative analyses with other selected studies, and/or did not report results as ORs. Forty-six reports were considered appropriate for the conduct of meta-analyses to determine whether correlations exist between childhood incidence/prevalence rates for allergic manifestations and pet ownership (Fig. 1A), regular contact with livestock (Fig. 1B), unpasteurized milk consumption (Fig. 1C), and home endotoxin levels (Fig. 1D).

Using ORs and CIs reported within individual investigations, we calculated pooled-effects estimates (ORs and 95% CIs) using fixed- and random-effects models. Heterogeneity, a value that uses a chi-square test to determine goodness of fit, was calculated for each fixed-effects model. Significant heterogeneity was found between studies ($P < 0.1$) with fixed-effects modeling. Therefore, random-effects models were selected to calculate summary ORs and CIs presented within this paper, as these estimates tend to be more conservative, taking into account between-study and within-study sampling variability. Fixed-effects and random-effects models were run with the R statistical software package, which is in the

public domain and available online (http://www.r-project.org), using the "rmeta" command.

Pet Ownership and Allergic Risk

Twenty-seven studies were considered appropriate for inclusion in a meta-analysis of associations between pet ownership and allergic stigmata development (Fig. 1A). In these investigations, atopic wheeze, eczema, allergic rhinitis, and/or conjunctivitis symptoms were the criteria for defining atopy. Several studies found no effect or a small positive effect of regular pet exposures on allergic risk; two studies found a marked increase in the incidence of allergic stigmata for individuals reared in homes with cats. Nonetheless, the combined OR for all 27 studies was 0.86 (95% CI, 0.79–0.93), suggesting that pet ownership during childhood leads to an approximately 14% decrease in allergic risk. Although this clinical effect appears small, as the CIs for the meta-analysis did not cross 1, it is statistically significant. Similar results were obtained when evidence of allergen-specific IgE was used to define atopy (n = 20 studies; OR, 0.84; 95% CI, 0.73–0.96). When meta-analyses for dogs (OR, 0.76; 95% CI, 0.65–0.89) and cats (OR, 0.97; 95% CI, 0.87–1.10) were conducted separately, dog ownership appeared to be more protective against the genesis of allergic diseases than cat ownership. Potential explanations for the discordant OR values found for dog and cat ownership include variability in experimental design and/or other idiosyncrasies unique to each study. Alternatively, dog and cat exposures may have distinct immunologic effects on allergic risk.

Livestock Exposure and Allergic Risk

We identified eight studies that compared the prevalence of allergic manifestations in children living on farms with livestock and children without regular livestock exposures. Atopy criteria used to assess for this association were the same as those used for the Figure 1A meta-analysis. The 0.58 OR for clinical manifestations of atopy (95% CI, 0.39–0.87) was significantly reduced for children with regular livestock exposures compared with control children, representing a 42% reduction in allergic manifestations (Fig. 1B). Although it was not statistically different, the OR for developing atopic diseases was lower for children reared with livestock than for children reared with pets, suggesting that farm animals or other associated factors are more protective.

Unpasteurized Milk Consumption and Allergic Risk

Many children reared on farms with livestock have the opportunity to drink unpasteurized milk on a regular or occasional basis. Although we identified only seven studies that considered the influence of unpasteurized milk consumption during childhood on allergic risk, all seven found a protective effect (Fig. 1C). Children drinking unpasteurized milk during the first few years of life had an OR of 0.68 (95% CI, 0.61–0.76) for allergic stigmata, or a 32% reduction in allergic risk compared with children who never drank unpasteurized milk. Although it is not proven, considered in conjunction with previous meta-analyses, this finding suggests that unpasteurized milk consumption may contribute to the protective influence of being reared on a farm with livestock.

Home Endotoxin Exposure and Allergic Risk

In previous studies in which pet ownership was found to protect against the allergic march (Fig. 1A), it was also shown that household pets increased home endotoxin levels. Given the important role that Toll-like receptors (TLRs) play in immune regulation and the reported TLR4 dependence of endotoxin-induced immune responses [1•], this observation has received a great deal of attention from epidemiologists and laboratory-based scientists interested in the origins of allergic diseases. However, in our meta-analysis of 13 pertinent studies, the OR for allergic stigmata was only reduced to 0.90 (95% CI, 0.78–1.00), or 10%, for children living in homes with high rather than low endotoxin levels (Fig. 1D). This relatively weak association suggests that either endotoxin is not an important environmental variable with respect to its influence on allergic risk or that additional microbial products ubiquitous in living environments have an equally important and potentially confounding influence on the genesis of allergic diseases.

Characterizing the Immunologic Activities of House Dust

Previously discussed epidemiologic studies suggest that environmental exposures during the first years of life play an important role in immune homeostasis and in determining allergic risk throughout childhood [1•]. Younger children spend most of their time at home, and accumulating experimental evidence suggests that living environments have a major educational influence on developing immune systems. Nonetheless, the molecular basis for immunomodulation by ambient exposures and understanding of their downstream influence on allergic risk remain highly speculative. By design, most investigations aimed at characterizing how living environments affect host immunity have made *a priori* assumptions about which exposures to pay attention to and which to ignore. As an alternative, we reasoned that the immunologic "ether" associated with homes may be better understood by investigating clinically relevant, sterile, but unpurified environmental samples. Logic suggests that gravity should concentrate immunostimulatory particulates into settled dust, and endotoxin levels previously have been found to be predictive surrogate markers of allergic risk (Fig. 1D). Therefore, our laboratory has begun to charac-

terize the immunostimulatory activities of sterile house dust extracts (HDEs). Studies conducted to date have yielded provocative and reproducible results that are the focus of the following sections of this paper [18••,19••].

HDE-Induced Activation of Dendritic Cells

Dust samples were first collected from the bedrooms of 15 suburban homes in San Diego, California, and then processed by standardized techniques, including suspension in phosphate buffered solution, physical agitation, and sterile filtration [19••]. These HDEs were found to be sterile and nontoxic. In initial experiments, HDEs were shown to activate bone marrow–derived dendritic cells (BMDDCs) in a concentration-dependent manner [19••,20]. Moreover, higher concentrations of most HDEs and optimized concentrations of TLR ligands elicited similar levels of interleukin (IL)-6 production. In contrast, lipopolysaccharide (LPS) (TLR4) and immunostimulatory sequence oligodeoxynucleotide (ISS) (TLR9) induced stronger IL-12p40 responses than any of the HDEs investigated. In a subsequent study, we determined whether a sampling of HDEs elicited the production of bioactive IL-12 (IL-12p70), a heterodimer of IL-12p40 and IL-12p35. However, HDE-induced BMDDC secretion of IL-12p70 was weak compared with responses induced by LPS, ISS, and R848 (TLR7) and similar to the response elicited by Pam 3 Cys (TLR2) [20]. Although HDEs are relatively ineffective at stimulating IL-12p70 production, in unpublished experiments, we recently observed that HDEs potently induce IL-23p19 mRNA synthesis, suggesting that HDEs may preferentially promote the synthesis of bioactive IL-23, a heterodimer of IL-12p40 and IL-23p19, rather than IL-12p70. Purified TLR ligands and HDEs also elicit low levels of BMDDC IL-10 production, whereas IL-4, IL-13, and tumor necrosis factor-α were not detected in any culture supernatants [19••,20].

In additional studies, HDE regulation of BMDDC costimulatory molecule expression was assessed. The BMDDCs stimulated with HDEs displayed increased expression of CD40, CD80, CD86, and major histocompatibility complex class II compared with unstimulated BMDDCs [19••]. Moreover, costimulatory molecule expression levels were similar on BMDDCs activated with HDEs and purified TLR ligands. In unpublished investigations, we further established that like BMDDCs, murine splenocytes and human peripheral blood mononuclear cells were highly responsive to HDEs. Taken together, these observations demonstrate that HDEs can be prepared with standardized methods and that their bioactivities can be readily investigated with traditional laboratory techniques.

Associations Between HDE Endotoxin Levels and Bioactivities

Consistent with other studies, we found that the mean endotoxin content of house dust samples obtained from homes with pets (n = 7) was more than twice that of house dust samples obtained from homes without pets (n = 8) [19••]. In addition, whereas mean IL-6 responses were similar, HDEs from homes with pets elicited IL-12p40 responses that were 60% stronger on average than those of HDEs from pet-free homes. In further analyses, correlations between HDE endotoxin levels and BMDDC cytokine–inducing capacities were assessed [19••]. Considered separately, HDEs from homes with and without pet exposures had correlation coefficients (r values) above 0.5, but they were not statistically significant by Z testing. However, whereas r values were not strengthened, correlations between endotoxin levels and IL-6–inducing (r = 0.523; P = 0.044) and IL-12p40–inducing (r = 0.573; P = 0.024) activities did reach statistical significance when all HDEs were considered together. Although the number of HDEs compared was small, these experimental findings support three major assertions: 1) compared with pet-free homes, HDEs derived from pet exposure homes have increased levels of endotoxin; 2) HDE bioactivities correlate loosely but significantly with their endotoxin content; and 3) endotoxin is unlikely to be the only immunostimulatory molecule contained within HDEs.

TLRs' Role in BMDDC Responsiveness to HDEs

To further evaluate the contribution of TLR4 in mediating responsiveness to HDEs, wild-type (WT) and TLR4 knockout BMDDC responses were compared [19••]. TLR4 knockout BMDDCs demonstrated a marked reduction in HDE-induced (n = 10) cytokine production and costimulatory molecule expression, but residual responsiveness remained. In additional experiments, WT, TLR2 knockout, and TLR9 knockout BMDDCs' responses to HDEs were compared [19••]. Whereas HDE-stimulated TLR2 knockout BMDDCs produced less IL-6 than WT BMDDCs, IL-12p40 production and costimulatory molecule expression were preserved. In contrast, HDE-stimulated TLR9 knockout BMDDCs were found to produce less IL-6 and IL-12p40 than WT BMDDCs. Furthermore, whereas TLR4 knockout BMDDCs displayed a greater deficit, HDE-activated TLR9 knockout BMDDCs expressed lower levels of costimulatory molecules than WT BMDDCs. These observations support the view that in addition to TLR4, TLR2 and TLR9 contribute to HDE-mediated BMDDC responses.

Experimental findings presented thus far suggest that TLR signaling pathways play an important role in mediating HDE-induced BMDDC responses. Nonetheless, these results do not exclude the possibility that HDEs may also activate BMDDCs by TLR-independent pathways. Therefore, as MyD88 plays a critical role in signaling through all TLRs except TLR3 [21,22••], a final series of experiments compared cytokine production and costimulatory molecule upregulation by HDE-activated WT and MyD88

knockout BMDDCs [19••]. In these studies, HDE-stimulated MyD88 knockout BMDDCs produced only small amounts of IL-6 and IL-12p40 and increased costimulatory molecule expression only slightly. These results establish that TLR signaling pathways play a central role in BMDDC activation by HDEs.

HDE Adjuvant Activities

To assess the adjuvant activities of HDEs, mice were intranasally immunized with ovalbumin (OVA) alone or with 21 μL of HDE (100 mg/mL concentration before filtration) on three occasions at weekly intervals [18••]. Additional groups of control mice were intranasally immunized with OVA and Pam 3 Cys, LPS, or ISS, according to the same vaccination schedule. Although adjuvant potential varied, mice intranasally immunized with OVA and HDE had far stronger adaptive responses than mice intranasally immunized with OVA alone, establishing that HDEs have adjuvant activities in the airways. Furthermore, HDEs (n = 10) were consistently found to act as Th2-biasing adjuvants, as they induced strong allergen-specific IgE and Th2-polarized cytokine responses but weak IgG2a and interferon-γ responses. In fact, most HDEs studied were more potent Th2 adjuvants than Pam 3 Cys or low-dose LPS, both of which previously have been described as Th2 adjuvants [18••]. Moreover, the adjuvant activities of HDEs were dependent on MyD88, further suggesting their dependence on signaling through TLRs. In addition to developing Th2-biased adaptive responses, mice immunized with OVA and HDE developed Th2-biased airway hypersensitivities, as reflected in their eosinophil-rich airway inflammatory response and increased bronchial responsiveness to methacholine after intranasal OVA challenge [18••]. These results challenge the commonly held belief that microbial products in general, and TLR ligands in particular, protect against the allergic march by inherently favoring development of Th1-biased immune profiles.

HDE Tolerogenic Activities

Experiments just discussed may be construed to suggest that many, if not all, living environments intrinsically promote allergic asthma development. However, in these studies, mice were airway exposed to the immunostimulatory contents of HDEs at weekly intervals and at levels likely to be in great excess of daily physiologic exposures. In contrast, individuals are thought to inhale air laced with low concentrations of immunostimulatory elements on a semicontinuous basis [23]. Therefore, additional experiments were designed to better model real world exposures. In these investigations, mice received three weekly intranasal OVA immunizations, as in previously described experiments, while low-dose HDE (one seventh the weekly dose, 3 μL) was intranasally delivered daily, beginning 1 week before the first and ending with the last dose of OVA; weekly with OVA (as in the previous experiments); or both [18••].

Daily intranasal HDE delivery had little adjuvant effect on OVA-specific responses. More importantly, daily airway HDE exposures prevented mice concurrently receiving weekly intranasal OVA and HDE (adjuvant dose) from developing Th2-biased adaptive responses and experimental asthma [18••]. Additional studies demonstrated that the Th2 adjuvant and tolerogenic activities of HDEs could be replicated with purified LPS. Further unpublished studies determined whether intranasal daily HDE/weekly OVA delivery induced long-lasting allergen tolerance. In these studies, mice received a series of three weekly intranasal OVA vaccinations alone or with weekly adjuvant doses (21 μL) or daily low doses (3 μL) of HDE, as just described. One month after the last primary OVA immunization, all mice were OVA sensitized by weekly intranasal OVA/adjuvant dose HDE delivery (three doses). Mice receiving intranasal OVA and daily HDE during primary immunization were found to be highly resistant to Th2 sensitization, whereas mice in other primary immunization groups (OVA alone or weekly OVA with HDE) were not.

Recognizing that immunostimulatory molecules are ubiquitous in inspired air but that levels vary widely [23], these experimental results suggest a new paradigm by which ambient exposures may modulate airway immunity and allergic risk during the first years of life. According to this model, basal levels of daily exposure to endotoxin and other immunostimulatory materials present in ambient air are generally not sufficient to provide airway adjuvant activity but rather serve to attenuate innate responsiveness to these molecules. However, episodic exposures to ambient air laced with high concentrations of immunostimulatory molecules can provide sufficient adjuvant activity to induce a breakdown in allergen tolerance if prior immunologic dampening by basal exposures is inadequate. Although far from proven, this model provides an alternative view of how ambient environmental exposures to materials with Th2-adjuvant activities can paradoxically also promote allergen tolerance.

Conclusions

Epidemiologic and laboratory investigations reviewed in this paper strongly suggest that ambient exposures to allergen-nonspecific immunostimulants have the potential to significantly impact allergic risk. Nonetheless, understanding of the molecular variables and mechanisms responsible is far from complete. Studies discussed in the first half of this review demonstrate a correlation between pet, farm, animal, unpasteurized milk, and endotoxin exposures during childhood and a reduced incidence of allergic manifestations. However, as discussed, these epidemiologic trends have been inconsistently reported, and in select studies, associations were relatively weak, nonexistent, or reversed. Moreover, these investigations

provide little insight into the mechanisms by which living environments influence allergic risk.

Laboratory investigations presented in the second half of the paper offer an alternative approach to characterizing how living environments modify host immunity in general and allergic risk in particular. In these studies, TLRs were found to play a central role in sensing and responding to allergen-nonspecific immunostimulatory molecules contained within HDEs and ubiquitous in living environments [19••]. Additional intranasal vaccination experiments revealed that weekly airway exposures to adjuvant doses of HDEs induced Th2-biased airway hypersensitivities to coadministered allergen, whereas daily HDE exposures promoted the development of long-lived allergen tolerance [18••]. The implication of these observations is that the primary immunologic consequence of airway exposures to allergen-nonspecific immunostimulants in living environments is to promote the development of Th2-biased hypersensitivities or allergen tolerance rather than to drive the development of "protective" Th1-biased responses to allergens.

In additional experiments, we found that even the innate airway response to bolus HDE exposure (neutrophilic inflammation and cytokine release) is inhibited by pretreatment of mice with 1 week of daily intranasal, low-dose HDE delivery [18••]. The phenomenon of reduced responsiveness with repetitive exposure has been described previously with LPS tolerance and also can be induced by other TLR ligands [24–26]. Moreover, in unpublished studies, we observed that daily intranasal HDE delivery increases local expression of mRNAs for molecules thought to mediate LPS tolerance (IL-10, STAT3, IRAKM, SHIP) [24,26–28]. These observations may explain why human lungs remain uninflamed despite continuous inhalation of proinflammatory molecules contained in HDEs [29]. Furthermore, they suggest that mechanisms associated with LPS tolerance (innate immunity) also may play an important role in the physiologic development of allergen-specific tolerance by nonatopic infants and toddlers, a focus of ongoing investigations in our laboratory.

If regular and adequate TLR stimulation drives the development of immune and clinical tolerance to ambient allergens by mechanisms associated with LPS tolerance, then exposure levels for individual molecules could prove far less important than the net exposure level for all ambient immunostimulatory molecules in determining a child's allergic risk. This consideration may help to explain why epidemiologic studies have yet to identify a specific molecule for which ambient exposure levels strongly and consistently correlate with relative allergic risk. Another implication of this view is that bioassays of HDE immunostimulatory activity could prove highly predictive of the allergic risk associated with living environments. We are currently testing this hypothesis in ongoing investigations.

Acknowledgments

This work was supported by grants AI61772 and T32RR023254 from the National Institutes of Health.

Disclosures

No potential conflicts of interest relevant to this article were reported.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1.• Horner AA: **Toll-like receptor ligands and atopy: a coin with at least two sides.** *J Allergy Clin Immunol* 2006, **117:**1133–1140.

This paper provides an up-to-date overview of how exposures to purified TLR ligands modify the allergic phenotype.

2. Braman SS: **The global burden of asthma.** *Chest* 2006, **130(Suppl 1):**4S–12S.
3. Keller MB, Lowenstein SR: **Epidemiology of asthma.** *Semin Respir Crit Care Med* 2002, **23:**317–329.
4. Platts-Mills TA, Ward GW Jr, Sporik R, et al.: **Epidemiology of the relationship between exposure to indoor allergens and asthma.** *Int Arch Allergy Appl Immunol* 1991, **94:**339–345.
5. Huss K, Adkinson NF Jr, Eggleston PA, et al.: **House dust mite and cockroach exposure are strong risk factors for positive allergy skin test responses in the Childhood Asthma Management Program.** *J Allergy Clin Immunol* 2001, **107:**48–54.
6. Platts-Mills TA, Woodfolk JA, Erwin EA, Aalberse R: **Mechanisms of tolerance to inhalant allergens: the relevance of a modified Th2 response to allergens from domestic animals.** *Springer Semin Immunopathol* 2004, **25:**271–279.
7. Frew AJ: **Advances in environmental and occupational diseases 2004.** *J Allergy Clin Immunol* 2005, **115:**1197–1202.
8. von Mutius E: **Environmental factors influencing the development and progression of pediatric asthma.** *J Allergy Clin Immunol* 2002, **109(Suppl 6):**S525–S532.
9. Rautava S, Kalliomäki M, Isolauri E: **New therapeutic strategy for combating the increasing burden of allergic disease: Probiotics-A Nutrition, Allergy, Mucosal Immunology and Intestinal Microbiota (NAMI) Research Group report.** *J Allergy Clin Immunol* 2005, **116:**31–37.
10.• Saxon A, Diaz-Sanchez D: **Air pollution and allergy: you are what you breathe.** *Nat Immunol* 2005, **6:**223–226.

Reviews current understanding of how manmade pollutants modify the allergic phenotype.

11.• Perkin MR, Strachan DP: **Which aspects of the farming lifestyle explain the inverse association with childhood allergy?** *J Allergy Clin Immunol* 2006, **117:**1374–1381.

This paper provides an up-to-date overview of farm-associated exposures and their impact on the allergic march.

12. von Mutius E: **The environmental predictors of allergic disease.** *J Allergy Clin Immunol* 2000, **105:**9–19.
13. Tse K, Horner AA: **Defining a role for ambient TLR ligand exposures in the genesis and prevention of allergic diseases.** *Semin Immunopathol* 2008, **30:**53–62.
14. van Strien RT, Engel R, Holst O, et al.: **Microbial exposure of rural school children, as assessed by levels of N-acetyl-muramic acid in mattress dust, and its association with respiratory health.** *J Allergy Clin Immunol* 2004, **113:**860–867.

15. Gehring U, Bischof W, Fahlbusch B, et al.: **House dust endotoxin and allergic sensitization in children.** *Am J Respir Crit Care Med* 2002, **166:**939–944.
16. Roy SR, Schiltz AM, Marotta A, et al.: **Bacterial DNA in house and farm barn dust.** *J Allergy Clin Immunol* 2003, **112:**571–578.
17. Liu AH, Murphy JR: **Hygiene hypothesis: fact or fiction?** *J Allergy Clin Immunol* 2003, **111:**471–478.
18.•• Ng N, Lam D, Paulus P, et al.: **House dust extracts have both Th2 adjuvant and tolerogenic activities.** *J Allergy Clin Immunol* 2006, **117:**1074–1081.

The first paper to describe the Th2 adjuvant and tolerogenic activities of HDEs.

19.•• Boasen J, Chisholm D, Lebet L, et al.: **House dust extracts elicit Toll-like receptor-dependent dendritic cell responses.** *J Allergy Clin Immunol* 2005, **116:**185–191.

The first paper in which the immunostimulatory activities of unpurified but sterile HDEs were characterized.

20. Batzer G, Lam DP, Paulus P, et al.: **Using house dust extracts to understand the immunostimulatory activities of living environments.** *Immunobiology* 2007, **212:**491–498.
21. Oshiumi H, Matsumoto M, Funami K, et al.: **TICAM-1, an adaptor molecule that participates in Toll-like receptor 3-mediated interferon-beta induction.** *Nat Immunol* 2003, **4:**161–167.
22.•• Akira S, Uematsu S, Takeuchi O: **Pathogen recognition and innate immunity.** *Cell* 2006, **124:**783–801.

This cutting-edge review provides a comprehensive overview of cellular receptors that mediate innate responsiveness to bacteria, viruses, and other microbes.

23. Rabinovitch N, Liu AH, Zhang L, et al.: **Importance of the personal endotoxin cloud in school-age children with asthma.** *J Allergy Clin Immunol* 2005, **116:**1053–1057.
24. Sly LM, Rauh MJ, Kalesnikoff J, et al.: **LPS-induced upregulation of SHIP is essential for endotoxin tolerance.** *Immunity* 2004, **21:**227–239.
25. Jacinto R, Hartung T, McCall C, Li L: **Lipopolysaccharide- and lipoteichoic acid-induced tolerance and cross-tolerance: distinct alterations in IL-1 receptor-associated kinase.** *J Immunol* 2002, **168:**6136–6141.
26. Fan H, Cook JA: **Molecular mechanisms of endotoxin tolerance.** *J Endotoxin Res* 2004, **10:**71–84.
27. Kobayashi K, Hernandez LD, Galán JE, et al.: **IRAK-M is a negative regulator of Toll-like receptor signaling.** *Cell* 2002, **110:**191–202.
28. Escoll P, del Fresno C, García L, et al.: **Rapid up-regulation of IRAK-M expression following a second endotoxin challenge in human monocytes and in monocytes isolated from septic patients.** *Biochem Biophys Res Commun* 2003, **311:**465–472.
29. Alexis NE, Eldridge MW, Peden DB: **Effect of inhaled endotoxin on airway and circulating inflammatory cell phagocytosis and CD11b expression in atopic asthmatic subjects.** *J Allergy Clin Immunol* 2003, **112:**353–361.
30. Anyo G, Brunekreef B, de Meer G, et al.: **Early, current and past pet ownership: associations with sensitization, bronchial responsiveness and allergic symptoms in school children.** *Clin Exp Allergy* 2002, **32:**361–366.
31. Arshad SH, Stevens M, Hide DW: **The effect of genetic and environmental factors on the prevalence of allergic disorders at the age of two years.** *Clin Exp Allergy* 1993, **23:**504–511.
32. Karadag B, Ege MJ, Scheynius A, et al.: **Environmental determinants of atopic eczema phenotypes in relation to asthma and atopic sensitization.** *Allergy* 2007, **62:**1387–1393.
33. Kurosaka F, Nakatani Y, Terada T, et al.: **Current cat ownership may be associated with the lower prevalence of atopic dermatitis, allergic rhinitis, and Japanese cedar pollinosis in schoolchildren in Himeji, Japan.** *Pediatr Allergy Immunol* 2006, **17:**22–28.
34. Ludvigsson JF, Mostrom M, Ludvigsson J, Duchen K: **Exclusive breastfeeding and risk of atopic dermatitis in some 8300 infants.** *Pediatr Allergy Immunol* 2005, **16:**201–208.
35. Nafstad P, Magnus P, Gaarder PI, Jaakkola JJ: **Exposure to pets and atopy-related diseases in the first 4 years of life.** *Allergy* 2001, **56:**307–312.
36. Naydenov K, Popov T, Mustakov T, et al.: **The association of pet keeping at home with symptoms in airways, nose and skin among Bulgarian children.** *Pediatr Allergy Immunol* 2008 Feb 11 (Epub ahead of print).
37. Ownby DR, Johnson CC, Peterson EL: **Exposure to dogs and cats in the first year of life and risk of allergic sensitization at 6 to 7 years of age.** *JAMA* 2002, **288:**963–972.
38. Peroni DG, Piacentini GL, Bodini A, et al.: **Prevalence and risk factors for atopic dermatitis in preschool children.** *Br J Dermatol* 2008, **158:**539–543.
39. Tariq SM, Matthews SM, Hakim EA, et al.: **The prevalence of and risk factors for atopy in early childhood: a whole population birth cohort study.** *J Allergy Clin Immunol* 1998, **101:**587–593.
40. Waser M, von Mutius E, Riedler J, et al.: **Exposure to pets, and the association with hay fever, asthma, and atopic sensitization in rural children.** *Allergy* 2005, **60:**177–184.
41. Zirngibl A, Franke K, Gehring U, et al.: **Exposure to pets and atopic dermatitis during the first two years of life. A cohort study.** *Pediatr Allergy Immunol* 2002, **13:**394–401.
42. Frosh AC, Sandhu G, Joyce R, Strachan DP: **Prevalence of rhinitis, pillow type and past and present ownership of furred pets.** *Clin Exp Allergy* 1999, **29:**457–460.
43. Gern JE, Reardon CL, Hoffjan S, et al.: **Effects of dog ownership and genotype on immune development and atopy in infancy.** *J Allergy Clin Immunol* 2004, **113:**307–314.
44. Holscher B, Frye C, Wichmann HE, Heinrich J: **Exposure to pets and allergies in children.** *Pediatr Allergy Immunol* 2002, **13:**334–341.
45. Leynaert B, Neukirch C, Jarvis D, et al.: **Does living on a farm during childhood protect against asthma, allergic rhinitis, and atopy in adulthood?** *Am J Respir Crit Care Med* 2001, **164:**1829–1834.
46. Linneberg A, Nielsen NH, Madsen F, et al.: **Pets in the home and the development of pet allergy in adulthood. The Copenhagen Allergy Study.** *Allergy* 2003, **58:**21–26.
47. Marinho S, Simpson A, Lowe L, et al.: **Rhinoconjunctivitis in 5-year-old children: a population-based birth cohort study.** *Allergy* 2007, **62:**385–393.
48. Phipatanakul W, Celedón JC, Raby BA, et al.: **Endotoxin exposure and eczema in the first year of life.** *Pediatrics* 2004, **114:**13–18.
49. Purvis DJ, Thompson JM, Clark PM, et al.: **Risk factors for atopic dermatitis in New Zealand children at 3.5 years of age.** *Br J Dermatol* 2005, **152:**742–749.
50. Radon K, Windstetter D, Eckart J, et al.: **Farming exposure in childhood, exposure to markers of infections and the development of atopy in rural subjects.** *Clin Exp Allergy* 2004, **34:**1178–1183.
51. Svanes C, Heinrich J, Jarvis D, et al.: **Pet-keeping in childhood and adult asthma and hay fever: European community respiratory health survey.** *J Allergy Clin Immunol* 2003, **112:**289–300.
52. Vargas C, Bustos P, Diaz PV, et al.: **Childhood environment and atopic conditions, with emphasis on asthma in a Chilean agricultural area.** *J Asthma* 2008, **45:**73–78.
53. Wickens K, Lane JM, Fitzharris P, et al.: **Farm residence and exposures and the risk of allergic diseases in New Zealand children.** *Allergy* 2002, **57:**1171–1179.
54. Bråbäck L, Kjellman NI, Sandin A, Björkstén B: **Atopy among schoolchildren in northern and southern Sweden in relation to pet ownership and early life events.** *Pediatr Allergy Immunol* 2001, **12:**4–10.

55. Hagendorens MM, Bridts CH, Lauwers K, et al.: **Perinatal risk factors for sensitization, atopic dermatitis and wheezing during the first year of life (PIPO study).** *Clin Exp Allergy* 2005, **35:**733–740.
56. Illi S, von Mutius E, Lau S, et al.: **The natural course of atopic dermatitis from birth to age 7 years and the association with asthma.** *J Allergy Clin Immunol* 2004, **113:**925–931.
57. Chai SK, Nga NN, Checkoway H, et al.: **Comparison of local risk factors for children's atopic symptoms in Hanoi, Vietnam.** *Allergy* 2004, **59:**637–644.
58. Ege MJ, Frei R, Bieli C, et al.: **Not all farming environments protect against the development of asthma and wheeze in children.** *J Allergy Clin Immunol* 2007, **119:**1140–1147.
59. Radon K, Ehrenstein V, Praml G, Nowak D: **Childhood visits to animal buildings and atopic diseases in adulthood: an age-dependent relationship.** *Am J Ind Med* 2004, **46:**349–356.
60. Riedler J, Braun-Fahrlander C, Eder W, et al.: **Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey.** *Lancet* 2001, **358:**1129–1133.
61. Smit LA, Zuurbier M, Doekes G, et al.: **Hay fever and asthma symptoms in conventional and organic farmers in The Netherlands.** *Occup Environ Med* 2007, **64:**101–107.
62. Von Ehrenstein OS, Von Mutius E, Illi S, et al.: **Reduced risk of hay fever and asthma among children of farmers.** *Clin Exp Allergy* 2000, **30:**187–193.
63. Waser M, Michels KB, Bieli C, et al.: **Inverse association of farm milk consumption with asthma and allergy in rural and suburban populations across Europe.** *Clin Exp Allergy* 2007, **37:**661–670.
64. Bolte G, Bischof W, Borte M, et al.: **Early endotoxin exposure and atopy development in infants: results of a birth cohort study.** *Clin Exp Allergy* 2003, **33:**770–776.
65. Böttcher MF, Björkstén B, Gustafson S, et al.: **Endotoxin levels in Estonian and Swedish house dust and atopy in infancy.** *Clin Exp Allergy* 2003, **33:**295–300.
66. Braun-Fahrländer C, Riedler J, Herz U, et al.: **Environmental exposure to endotoxin and its relation to asthma in school-age children.** *N Engl J Med* 2002, **347:**869–877.
67. Campo P, Kalra HK, Levin L, et al.: **Influence of dog ownership and high endotoxin on wheezing and atopy during infancy.** *J Allergy Clin Immunol* 2006, **118:**1271–1278.
68. Celedón JC, Milton DK, Ramsey CD, et al.: **Exposure to dust mite allergen and endotoxin in early life and asthma and atopy in childhood.** *J Allergy Clin Immunol* 2007, **120:**144–149.
69. Gehring U, Bolte G, Borte M, et al.: **Exposure to endotoxin decreases the risk of atopic eczema in infancy: a cohort study.** *J Allergy Clin Immunol* 2001, **108:**847–854.
70. Gillespie J, Wickens K, Siebers R, et al.: **Endotoxin exposure, wheezing, and rash in infancy in a New Zealand birth cohort.** *J Allergy Clin Immunol* 2006, **118:**1265–1270.
71. Perzanowski MS, Miller RL, Thorne PS, et al.: **Endotoxin in inner-city homes: associations with wheeze and eczema in early childhood.** *J Allergy Clin Immunol* 2006, **117:**1082–1089.
72. Schram-Bijkerk D, Doekes G, Douwes J, et al.: **Bacterial and fungal agents in house dust and wheeze in children: the PARSIFAL study.** *Clin Exp Allergy* 2005, **35:**1272–1278.