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Endotoxin exposure in allergy and asthma: Reconciling a paradox

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Well-established evidence links endotoxin exposure, especially in the workplace, to airways disease. Endotoxin can increase disease severity by acting as a natural adjuvant to augment asthma and atopic inflammation. Recent studies suggest that it can even act on its own, causing a distinct endotoxic form of asthma. Other studies, however, contradict the paradigm that endotoxin's influence is solely a negative one. Epidemiologic associations of environmental endotoxin exposure with allergy and asthma prevention are consistent with *hygiene hypothesis* associations of other microbial exposures or infections with a lower incidence of atopic disease. Currently, microbe-derived products are being developed as potential therapies for allergy and asthma. Thus it is an ideal time to consider endotoxin as a prototype of a natural intervention with microbial components. Nature's ongoing experiment with endotoxin can provide clues for the development of effective and safe microbe-based products for disease treatment and prevention. This article will discuss (1) conventional paradigms in which endotoxin-induced immune modulation by T_H1-type induction leads to mitigation of T_H2-type immune development, allergen sensitization, and atopic inflammation; (2) newer concepts of T_H1-type immune responses that may provide additional asthma-protective effects by preventing airways remodeling; (3) home and environmental features that significantly contribute to endotoxin exposure; (4) different aspects of asthma mediated by endotoxin exposure; and (5) how to understand endotoxin's paradoxical nature of serving as both friend and foe. (J Allergy Clin Immunol 2002;109:379-92.)

Key words: *Allergy, asthma, therapy, prevention, endotoxin, LPS, infection, hygiene, IFN-γ, IL-12, T_H1*

> "Endotoxin and mycoplasma are Nature's darkest secrets. If they are ever solved, Hell itself will open." — Lewis Thomas[1]

Abbreviations used
HSP: Heat shock protein
LAL: Limulus amebocyte lysate
OVA: Ovalbumin
TLR: Toll-like receptor

Over the past century, many immunologists have studied endotoxin and found their experiences to be both scientifically fertile and frustrating. Although studies of endotoxin have enlightened our understanding of the immune response to microbes, each door of knowledge has opened to reveal paradoxes that have challenged our paradigms. In keeping with this legacy, there is mounting evidence that environmental exposure to endotoxin has an ambiguous Jekyll-and-Hyde relationship with allergy and asthma. Somehow, endotoxin exposure aggravates allergy and asthma *and* might have allergy- and asthma-protective effects. Understanding this conundrum requires a brief introduction to endotoxin.

ENDOTOXIN: A PRIMER

Endotoxin, an LPS, comprises most of the outer layer of the outer cell membrane of all gram-negative bacteria (Fig 1). Its potent immune stimulatory capacity is largely attributed to the Lipid A moiety of endotoxin, which is highly conserved across different bacterial species.[1] Very small amounts of endotoxin (ie, picogram amounts of LPS estimated to equal approximately 10 LPS molecules per cell) are immune stimulatory.[2] Endotoxin is also remarkably resilient. For example, destroying endotoxin's immune stimulatory capacity with heat requires prolonged baking at high temperatures (eg, 160°C for 4 hours). Such potency and durability suggests endotoxin's potential to persist as an immune modulator in our environment.

Endotoxin can be measured by using a Limulus amebocyte lysate–based (LAL) bioassay and by means of mass spectrometry. The LAL assay, which measures the biologic activity of endotoxin, is well standardized by the US Food and Drug Administration and widely applied as a sensitive marker of *Escherichia coli* contamination in quality assessment of water, food, and other products. Mass spectrometry can quantify endotoxin biochemically. In a comparison study of house dust endotoxin measure-

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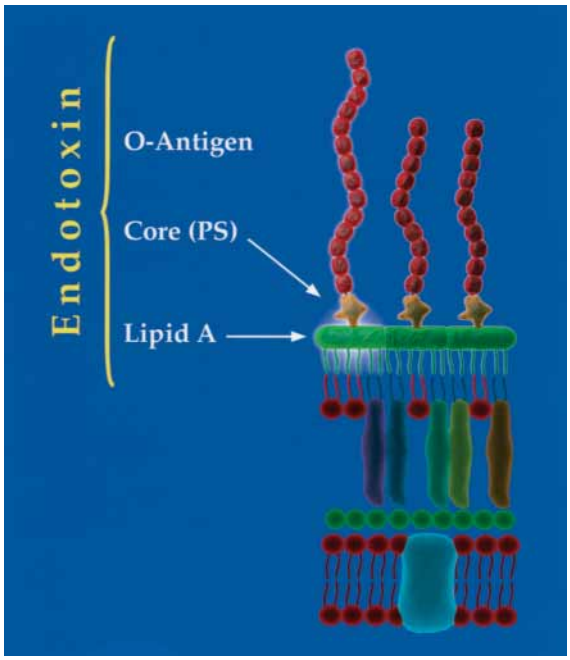

FIG 1. Endotoxin, an LPS, comprises most of the outer layer of the outer cell membrane of all gram-negative bacteria. Its potent immune stimulatory capacity is largely attributed to the Lipid A moiety of endotoxin, which is highly conserved across different bacterial species.[1]

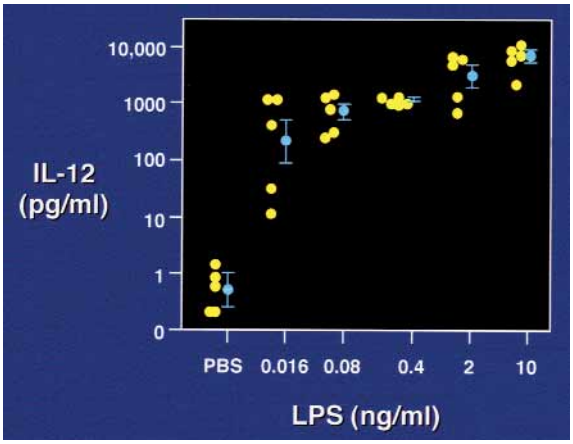

FIG 2. Endotoxin is a potent inducer of IL-12 production. In this example peripheral blood samples from 5 healthy adults were stimulated for 24 hours with LPS, and IL-12 p40 production was measured in cell supernatants (ELISA, Pharmingen). Picogram-to-nanogram amounts of LPS stimulated IL-12 p40 production in a dose-dependent manner ($P < .003$, χ^2 test).

ments with gas chromatography-mass spectrometry (to detect LPS-characteristic 3-hydroxy fatty acids) versus LAL bioassay, these 2 methods had an *r* correlation of 0.59 ($P < .05$) that could be optimized to 0.88 ($P < .001$) by restricting the species of fatty acids analyzed.[3] The commonly used LAL assay underdetects the amount of biochemically measurable endotoxin in dust samples by severalfold but is nevertheless considered to be a sensitive, as well as easier and cheaper, measure of endotoxin.[3,4]

It has long been known that endotoxin can be used as an essential adjuvant in the induction of antigen-specific T-cell memory.[5,6] Although T cells will mount a short-lived proliferative response to protein antigens alone, classic memory immunity (ie, the generation of long-lived memory-effector T cells and a persistent antibody response) is dependent on immunization with adjuvant, a process particularly well demonstrated with LPS.[7,8] Endotoxin is also a potent inducer of IL-12 and IFN-γ, which are key regulators of T_H1-type immune development (Figs 2 and 3).[9,10] Recently, antigen plus LPS–generated memory T cells have been skillfully shown to be IFN-γ–producing effector T cells.[11] LPS strongly influences innate antigen-presenting immune cells, especially dendritic cells, to produce IL-12 and to costimulate T cells to become effector T cells that primarily secrete IFN-γ.[12-16] Moreover, IFN-γ primes innate immune cells to produce greater amounts of IL-12 in response to stimulation,[13,15,17,18] fostering a positive feedback relationship between the innate and adaptive immune compartments for T_H1-type immune development. Consequently, it is tempting to hypothesize that endotoxin exposure, while driving the development of T-cell memory to environmental allergenic proteins, may also steer these memory T cells to produce IFN-γ, thereby inhibiting T_H2 cytokine production (eg, IL-4, IL-5, and IL-13) and preventing atopic immune development and associated disease (Fig 3).

ENDOTOXIN AND OTHER MICROBES: T_H1 INDUCERS CAPABLE OF ATOPY PROTECTION?

The potential of T_H1 inducers like endotoxin and other microbial exposures to mitigate allergy and asthma is consistent with clinical association studies of the *hygiene hypothesis* and is also supported by studies in commonly used rodent models of atopic asthma (Table I).[19-46] Epidemiologic studies have generally found a lower prevalence of allergic rhinitis, asthma, and inhalant allergen sensitization in persons who have experienced significant infections of the respiratory (eg, measles[19] and tuberculosis[20,21]) or gastrointestinal (eg, hepatitis A, *Helicobacter pylori*, *Toxoplasma gondii*,[22] schistosomiasis,[23] and hookworm[24]) tracts. Children raised in rural versus urban areas of developing countries are also less afflicted by allergy and asthma. This is another trend consistent with the notion that improvements in public hygiene lower serious infections while increasing the likelihood of allergy and asthma. Experimental results in murine models of atopic asthma have invariably supported this hypothesis because treatment with living (eg, BCG[47,48] and lactobacillus[49]) or dead (eg, heat-killed listeria[50]) microbes, microbial components (eg, LPS[51] and bacterial CpG DNA[52-55]), or T_H1-type cytokines (eg, IL-12 and IFN-γ[56-65]) all mitigate allergen sensitization and prevent the eosinophilic inflammation and airways hyperresponsiveness that characterize the asthmatic phenotype in these mice.

Dietary and medicinal influences on bacterial colonization of the gastrointestinal tract in young children are

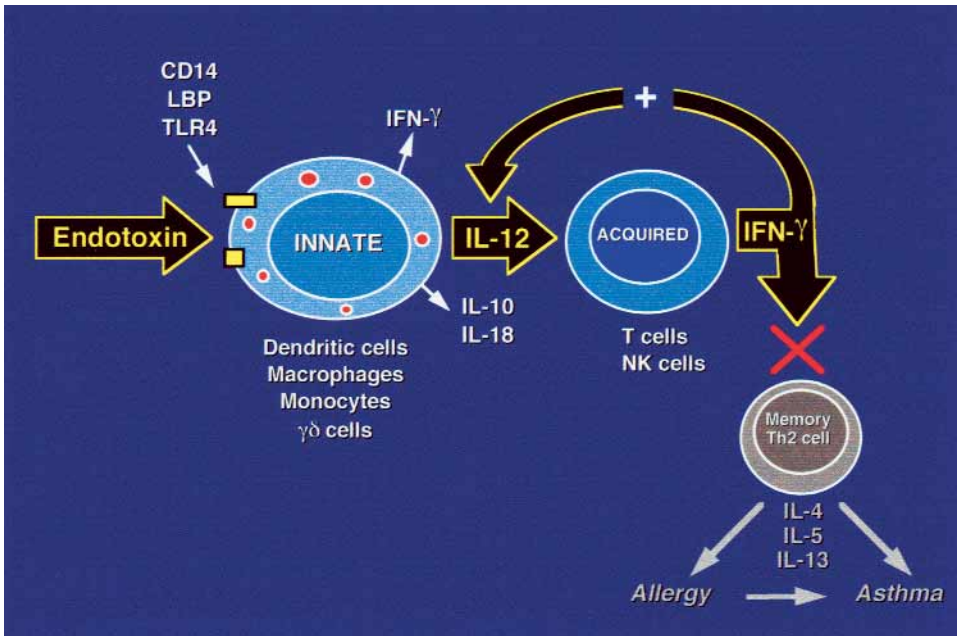

FIG 3. Endotoxin induces a T_H1-type immune response, mitigating T_H2-mediated allergy and asthma. Innate immune cells with recognition receptors for endotoxin (ie, TLR4 and CD14) produce chemokines and cytokines that influence the development of acquired immunity. Importantly, IL-12 production by innate immune cells induces memory-effector T lymphocytes to produce IFN-γ on activation. Both IL-12 and IFN-γ inhibit T_H2 cytokine production (eg, IL-4, IL-5, and IL-13) by T lymphocytes. IFN-γ also augments IL-12 production by stimulated innate immune cells, generating a positive feedback loop between the acquired and innate immune compartments.

hypothesized to shape T_H1 versus T_H2 immune development. Significant differences in gastrointestinal colonization have been found in infants who later have atopy in contrast to those who remain nonallergic (more clostridia and *Staphylococcus aureus*; less enterococci, bifidobacteria, and bacteroides).[25] Antibiotic use in childhood has also been associated with a higher prevalence of allergy and asthma.[26,27] In a cohort of children attending Rudolf Steiner schools, prolonged breast-feeding, lack of antibiotic use, and ingestion of fermented vegetables (containing lactobacillus) were features of the anthroposophical lifestyle of children that, when combined, were associated with a lower risk of atopy.[28] Mouse model investigations substantiate the potential relevance of intestinal microflora in the promotion of T_H1 versus T_H2 immune responses. Young, 3-week-old mice given a 1-week course of kanamycin that sterilized their gastrointestinal tracts had (1) higher serum IgE levels, (2) increased IL-4 and reduced IFN-γ production from stimulated splenocytes, and (3) reduced IL-12 production from unstimulated splenic dendritic cells.[66] Older, 1-year-old mice, however, did not demonstrate this T_H2-deviated response to treatment with kanamycin. This research has led to clinical trials of probiotic dietary supplementation. In a randomized, controlled intervention study, daily oral supplementation with *Lactobacillus rham-*

TABLE I. Microbial exposures associated with less allergy and asthma: Clinical studies

Infections
Respiratory tract: measles,[19] tuberculosis[20,20a]
Gastrointestinal tract: hepatitis A, *Helicobacter pylori*, *Toxoplasma gondii*,[22] schistosomiasis,[23] hookworm[24]
Common colds[41]
Early gastrointestinal tract colonization[25]
Less antibiotic use[26-28]
Anthroposophical lifestyle (Rudolf Steiner schools)
Less antibiotic use[26,28]
Eating fermented, lactobacillus-containing vegetables[28]
Farming lifestyle[29-33]
Animal contact[31]
Stables exposure[34]
Drinking unpasteurized farm milk[34]
Endotoxin[21,35]
Metropolitan lifestyle
Endotoxin[42]
Common colds[41]
Early day care[35a,36]
Larger family size[26,36-40]
Animal exposure
Rural homes[19,46]
Farms[31,34]
Pet keeping[40,43-45]

nosus near the end of pregnancy through the first 6 months of infancy was associated with a lower prevalence of atopic dermatitis at 2 years of age.[67] Although 6 months of probiotic treatment did not significantly reduce the incidence of other manifestations of atopy, such randomized controlled intervention studies in young children are a bold step toward understanding the relevance of microbial exposures in the gastrointestinal tract to immune development and atopic disease.

Farming environments are also strongly associated with a lower prevalence of childhood allergic rhinitis, asthma, inhalant allergen sensitization, and airways hyperresponsiveness.[29-33] A particularly intriguing feature of these modern farming communities (ie, Bavaria, Switzerland, Austria, Finland, and Quebec, Canada) is that the public health of these children is believed to be generally excellent. For example, the immunization rate of farmers' children in the European communities was 95%.[34] In these atopy- and asthma-protective locales, we can use endotoxin as a measure of ongoing microbial exposure distinct from severe infections and their associated risks. House dust endotoxin content, as a proxy for endotoxin exposure, is much higher in rural homes and farm homes than in nonfarm metropolitan homes.[21,35] In rural farming communities a strong atopy-protective association has been found with exposure to farm stables and drinking farm milk (ie, unpasteurized milk), especially in early childhood.[34] Indeed, farm barns have significantly higher dust endotoxin levels than farm and rural homes. Furthermore, farm home endotoxin levels correlate well with their associated barns, suggesting a related source of endotoxin for these locales.[35] Unpasteurized farm milk has higher endotoxin levels than does pasteurized milk, presumably because of greater gram-negative bacterial growth (E. von Mutius, personal communication). In this way, endotoxin seems to serve as a marker of microbial exposure in early childhood, especially in these farming communities.

In modern metropolitan communities, public hygiene measures are well implemented, and microbial exposures typical for children raised in farming communities seem markedly reduced. One might presume that an effect of microbial exposures on reducing the likelihood of allergy and asthma might not be found in these locales. Surprisingly, clinical clues suggest that a protective effect from microbes in these clean metropolitan environments still occurs. For example, 2 longitudinal prospective cohort studies (Children's Respiratory Study, Tucson, Ariz, and Multicentre Allergy Study, Berlin, Germany) have found a protective association with the common respiratory colds of childhood. In the Tucson study, a lower risk of symptomatic wheezing in later childhood was associated with exposure to younger children in early childhood, either by being born into a family with 2 or more older siblings or by entry into day care in the first 6 months of life. The presumption is that frequent exposure to younger children results in an increased number of common viral infections.[36] This is consistent with numerous studies that have reported a lower risk of hay fever, asthma, and allergen sensitization in children born into larger families and in children with greater numbers of older siblings.[26,37-40] In the Multicentre Allergy Study, a higher number of reported common colds in the first 3 years of life was associated with a lower risk of asthma and airways hyperresponsiveness (an important objective measure of asthma) at age 7 years.[41]

House dust endotoxin exposure in modern metropolitan homes, although generally much lower than those in farm and rural homes, can still be significant.[42] In fact, house dust endotoxin levels vary widely in metropolitan homes (approximately 100-fold), suggesting a wide variation in endotoxin exposure for children living in these locales.[42,68,69] Allergen-sensitized infants were found to have lower levels of house dust endotoxin than their nonsensitized counterparts.[42] In contrast, higher house dust endotoxin levels correlated with increased proportions of IFN-γ–producing T_H cells in the peripheral blood of these infants, supporting the hypothesis that microbial exposures promote T_H1-type immune development.[42] Furthermore, infants with higher endotoxin levels found in the mattress dust of mothers' bedding were significantly less likely to have atopic dermatitis in the first 6 months of life.[70] Therefore if frequent and benign exposures to endotoxin in early life (ie, in house dust, mothers' mattress dust, unpasteurized farm milk, and stables) truly influence immune development to prevent atopy, allergic disease, and asthma, then the benefit from microbial exposures can be separated from the harm of infections.

FACTORS INFLUENCING CHILDHOOD ENDOTOXIN EXPOSURE

Only recently has endotoxin exposure of children in a nonoccupational setting reached the published literature. Endotoxin content in household dust has been the main test used thus far to assess environmental endotoxin exposure in children. The presence of animals is associated with higher levels of house dust and airborne endotoxin in metropolitan homes. This has been consistently observed in homes with dogs but also in those with other pets or pests that are probably colonized with gram-negative bacteria.[69,71,72] Even though the relationship between dust endotoxin levels and actual exposure as a result of inhalation has not been clarified, a study using personal monitors for air sampling of asthmatic children also found significantly higher respirable endotoxin exposure in children with pets.[73]

In farm homes, house dust endotoxin levels correlate with associated barn dust endotoxin levels, raising the likelihood that barn animals outside of the home are serving as a source of endotoxin transmission to the homes.[35] Recent farm studies have found the strongest negative associations with inhalant allergen sensitization to be frequent animal exposure,[31] early childhood exposure to stables, and the consumption of farm milk (ie, unpasteurized milk).[34] This implies that much of the endotoxin (and possibly associated microbe) exposure for farm children occurs outside of the home environment.

Others have similarly reported a lower likelihood of allergen sensitization or asthma in children with pets in early childhood.[40,43-45,74] Even studies in rural areas have associated the presence of animals (eg, pigs) in the home with a lower likelihood of allergy.[19,46] Indeed, when comparing allergen sensitization, a recent study found a marked difference in allergen sensitization with cat versus mite allergen exposure. High levels of cat allergen exposure (ie, with cat ownership) were associated with a lower incidence of allergen sensitization. In contrast, high levels of mite allergen exposure were associated with an increasing likelihood of sensitization.[75] Thus it is appealing to speculate that the common thread of an atopy-protective influence of animal exposure in all of these settings may be endotoxin and other related microbial exposures that come with it.

Other home features influence home endotoxin levels. There are, however, a limited number of published investigations on this subject, some of which are contradictory. Central air conditioning has been associated with lower house dust endotoxin levels in metropolitan homes.[71] This might be due to temperature and humidity regulation by whole-home air conditioning, as well as exclusion of seasonal (ie, warm weather) increases in outdoor endotoxin levels by closing up the home. (Seasonal increases in ambient endotoxin levels in warm summer months suggest greater endotoxin exposure for children playing outdoors at these times.[68]) Yet although some studies have noted seasonal differences in indoor endotoxin levels,[76,77] others have not.[68,71] Similarly, there are studies that positively associate home humidity with indoor airborne endotoxin levels,[68] particularly dehumidifier use with lower levels of airborne endotoxin,[69] and studies that find no association between humidifiers or home dampness and dust endotoxin.[71] We can anticipate that some of these discrepancies between studies of home and lifestyle influences on endotoxin exposure may be attributed to unknown locale-specific differences. Other potential sources and correlates of endotoxin in homes include tobacco smoke,[78] air pollution particulate matter,[79,80] number and young age of children in home,[37] and general tidiness. To date, no significant association of these exposures with house dust or airborne endotoxin levels have been found.[69,71]

Finally, it is intriguing to note that house dust endotoxin levels of medical and public health professionals and their friends are lower than the levels in homes of low-income families living in inner-city neighborhoods.[71] Nevertheless, endotoxin levels in the inner-city homes are still markedly lower than levels found in rural and farm homes.[35,42,68] This highlights the likelihood that other currently unidentified home, lifestyle, and locale factors will significantly alter home endotoxin exposure. Personal hygiene measures, such as fastidiousness of bathing, laundering, and floor cleanliness, are prime endotoxin-reducing suspects. More rigorous studies that assess potential home endotoxin effectors by altering single parameters would help to clarify the reported observations.

ENDOTOXIN'S POTENTIAL FOR ASTHMA PREVENTION BY ADDITIONAL T_H1-MEDIATED PROCESSES

Pathologic hallmarks of asthma, in addition to allergen sensitization and atopic inflammation, include (1) fibrotic changes that occur in the tissues surrounding the airway lumen, (2) smooth muscle hypertrophy-hyperplasia, (3) mucous gland hypertrophy-hyperplasia, and (4) damaged respiratory epithelium. Hypothetically, these remodeling abnormalities result from aberrant repair processes after airways injury and inflammation. Indeed, mite allergen sensitization and exposure by inhalation in rhesus monkeys demonstrates the capacity of an allergen-driven process to induce allergen sensitization, eosinophilic inflammation, airways hyperresponsiveness, and the complete spectrum of airway remodeling features of asthma (ie, mucous gland hypertrophy and hyperplasia, basement membrane thickening, and epithelial exfoliation).[81] Recent studies on the influence of IFN-γ and IL-4 on these airways tissues have significantly broadened the scope of potential influence of these T_H1/T_H2 cytokines on asthma pathogenesis and airway healing. Because almost all cell types in the body express IFN-γ receptors, a predominant T_H1-type immune response might divert pathologic remodeling processes in asthma, as well as the development of atopy (Fig 4).

Perhaps the strongest evidence of additional asthma-protective benefits from T_H1-type induction exists for the role of fibroblasts in asthma pathogenesis. Thickening of the reticular basement membrane, which occurs early in the development of asthma, does not occur in all inflammatory conditions of the airways (eg, chronic bronchitis).[82] Furthermore, a substantive increase in myofibroblasts seen in the late phase of allergen-challenged asthma may be a link to the increase in airway smooth muscle that typifies asthma.[83] If this fibrotic process results from airways injury and inflammation, then how is repair guided to include or exclude fibrosis?

Intriguingly, T_H2 cytokines (IL-4 and IL-13) induce fibroblasts to proliferate and produce collagen in vitro.[84-86] Therefore, IFN-γ's downregulatory influence on IL-4 production would be expected to provide a milieu for airway repair without fibrosis. IFN-γ also directly inhibits the proliferation of lung fibroblasts, their differentiation into myofibroblasts, and collagen synthesis.[87-90] In addition, IFN-γ inhibits fibroblast activity by interfering with essential intracellular activation pathways triggered by transforming growth factor β,[91-93] the master on-switch of lung fibroblast proliferation, differentiation, and collagen synthesis.[94,95] Furthermore, IFN-γ inhibits transforming growth factor β expression and production by human fibroblasts.[96] This has been of particular interest in the management of lung fibrotic conditions (eg, idiopathic pulmonary fibrosis), in which various forms of IFN are being used in therapeutic trials, with some success.[97]

The potential for IFN-γ immune responses to augment other aspects of airway repair after injury is less clear but still considerable. Murine models of asthma have recent-

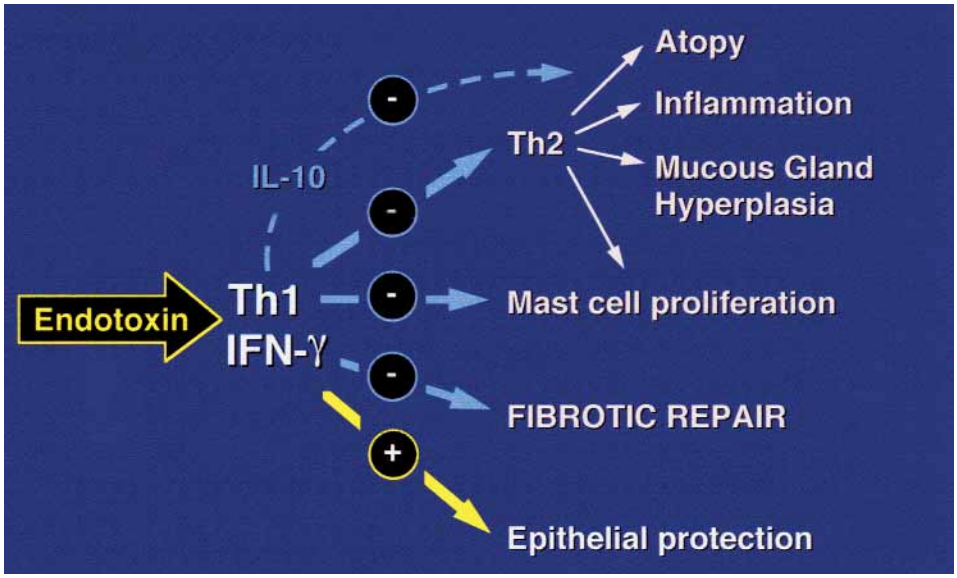

FIG 4. Endotoxin-driven T_H1-type immune development may mitigate the hallmark pathologic features of asthma by (1) inhibiting atopic inflammation, (2) inhibiting mucous gland hypertrophy and hyperplasia, (3) inhibiting fibroblast proliferation, collagen synthesis, and differentiation to myofibroblasts, (4) inhibiting mast cell proliferation and increases in airways tissues, and (5) inducing epithelium-protective mechanisms (eg, defensins and collectins production, intracellular mechanisms that inhibit viral replication). Endotoxin also induces IL-10 production, which may have important immune regulatory and anti-inflammatory actions in asthma. Therefore, a *modified* T_H1 immune response, combining IL-10 with IFN-γ and IL-12, might have a powerful allergy-protective and asthma-protective effect.

ly revealed that T_H2-type cytokines (ie, IL-4, IL-9, and IL-13) are strongly linked to mucous gland hypertrophy, hyperplasia, and hypersecretion[98-102] and that IFN-γ–producing T cells are essential to the regulation and prevention of this pathologic process.[98,103] Greater numbers of mast cells are typically found in asthmatic airways,[104,105] especially in the late-phase asthmatic response to allergen.[106,107] Although the in vivo mechanisms leading to mast cell accumulation in the airways are unclear, IFN-γ directly inhibits the proliferation of human mast cells in vitro.[108,109]

IFN-γ may also protect the airways by inhibiting viral replication in epithelial cells through several well-defined cell molecular mechanisms. In fact, in human subjects inoculated with rhinovirus, less rhinovirus shedding was associated with increased in vitro IFN-γ production before infection.[110] Because viral replication in airway epithelial cells is strongly linked to epithelial proinflammatory cytokine production and damage in response to viral infections, a vigorous IFN-γ response to viruses may protect the epithelium by containing virus-mediated damage and inflammation. Indeed, sustained expression of IL-12 in mouse airways with a vaccinia virus gene vector not only prevented the development of allergic asthma but also induced a robust antiviral cytotoxic T-cell response and greatly reduced viral shedding.[65]

Hypothetically, a T-cell repertoire shaped by environmental immune stimuli to produce IFN-γ in response to antigen-driven inflammation (eg, by viruses or allergens) may promote tissue healing without fibrosis and other remodeling abnormalities. In Brown Norway rats that have an asthma phenotype after Sendai virus infection, nebulized IFN-γ administered from 2 to 7 days after infection does not reduce viral shedding or leukocytes in airway lavage fluid but does greatly reduce bronchiolar inflammation, fibrosis, and airflow obstruction.[111] A clinical study of severe infantile bronchiolitis supports these potential benefits from IFN-γ. Hospitalized bronchiolitic infants who did not have asthma at age 2 years had higher IFN-γ production in response to IL-2 stimulation in vitro, both during and 5 months after the bronchiolitis episode.[112] Moreover, these infants' IFN-γ production correlated with higher airflow and less airways hyperresponsiveness at age 2 years. Heightened IFN-γ immune responsiveness in infancy is therefore associated with a lack of persistent airways impairment and disease after viral bronchiolitis, suggesting nonpathogenic repair from airways injury.

IFN-γ and LPS can induce airway epithelial cells and macrophages to produce defensins[113,114] and collectins (ie, Surfactant Protein A[115]), endogenous antibiotics that protect the airways from microbial infections. Thus endotoxin induces a number of biologic responses that protect airway epithelium, both through direct stimulatory mechanisms and through T_H1-type induction.

There are several important caveats to the potential IFN-γ–mediated protective effects of microbes. First,

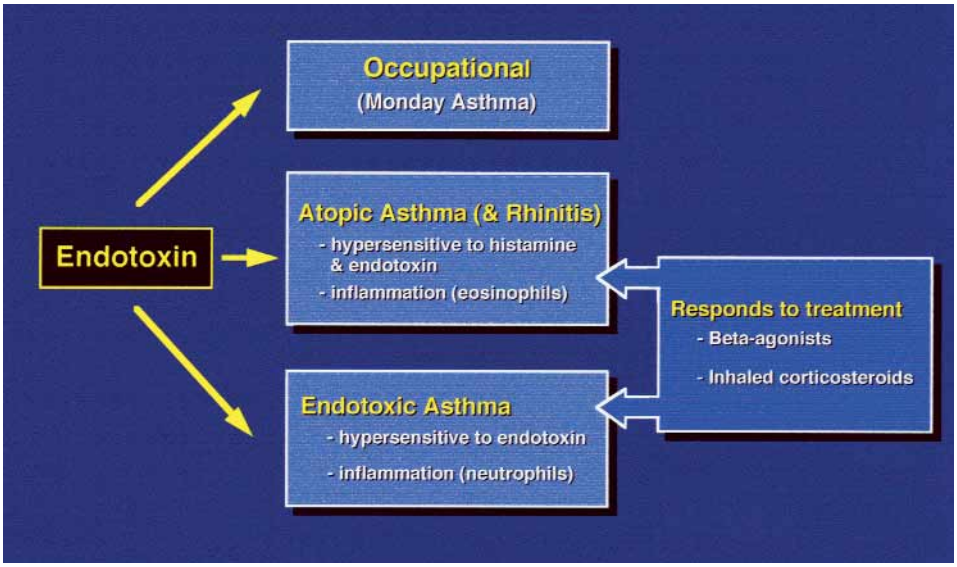

FIG 5. Endotoxin-mediated asthma has taken the form of several overlapping clinical presentations, whereby (1) high levels of endotoxin exposure in occupational settings induce Monday asthma, (2) endotoxin augments atopic inflammation and induces symptoms in patients with asthma, and (3) subjects with endotoxic asthma demonstrate airways and immune hyperresponsiveness to endotoxin. Both conventional and endotoxic asthma respond favorably to conventional asthma therapy (ie, β-agonists[119a] and inhaled corticosteroids[120]).

microbial stimulation (including LPS) induces IL-10 production, which may have important immune regulatory and anti-inflammatory actions in asthma. In some murine asthma model studies, IL-10 mitigates airways inflammation.[116,117] IL-10 can also differentially regulate stimulated B cells to produce IgG_4 instead of IgE.[118] Considering these links between IL-10 and allergy and asthma development, a *modified* T_H1 immune response to microbes, consisting of IL-10, IFN-γ, and IL-12, may be the most winning of combinations.

Second, although IFN-γ–mediated immune responses may have multiple beneficial effects on the development of asthma, it is important to consider the different autoimmune conditions that are associated with self-specific, IFN-γ–producing T cells (eg, multiple sclerosis, type 1 diabetes, autoimmune thyroiditis, and Crohn's disease). A discussion of the distinguishing features of IFN-γ immune responses that are protective versus pathogenic is beyond the scope of this article, but a clear understanding of these distinctions would help to alleviate potential concerns of T_H1-inducing interventions.

ENDOTOXIC ASTHMA: THE OTHER SIDE OF THE COIN

After the rationale that endotoxin exposure may be asthma protective, it may seem illogical to consider evidence that endotoxin causes asthma. Nevertheless, more than a century of investigation into the *Monday asthma* of cotton workers (ie, byssinosis) has revealed an essential role for endotoxin exposure in occupational asthma (Fig 5). A recent review article elucidates the role of endotoxin exposure in occupational asthma.[121] In summary, endotoxin exposure induces varying degrees of airflow obstruction and neutrophil inflammation in most nonasthmatic subjects.[119] In an occupational setting, a characteristic pattern of cough and chest tightness on Monday (or the first day of the work week) is usually followed by improvement in symptoms during the subsequent days of the work week; however, a day or two off of work is followed by the return of symptoms on the ensuing Monday.[122,123] Although long believed to result from cotton dust exposure, some byssinosis outbreaks were, surprisingly, associated with relatively low levels of respirable dust. Eventually, challenge studies with different samples of cotton dust demonstrated that the endotoxin content of the cotton dust, and not the dust exposure itself, correlated with induced airflow obstruction.[124,125] Since then, endotoxin exposure has been associated with respiratory symptoms and disease in a long list of workplace settings (eg, livestock handling, lab animal handling, grain and vegetable agriculture, sawmills, waste management, fiberglass manufacturing, and sick building syndrome). Finally, the effects of workplace endotoxin exposure can go from Monday respiratory symptoms into persistent symptoms throughout the week, and eventually pulmonary debilitation from chronic obstructive pulmonary disease, fibrosis, or both.[122,123]

Asthmatic subjects are hypersensitive to endotoxin exposure, typically reacting with a combined early and

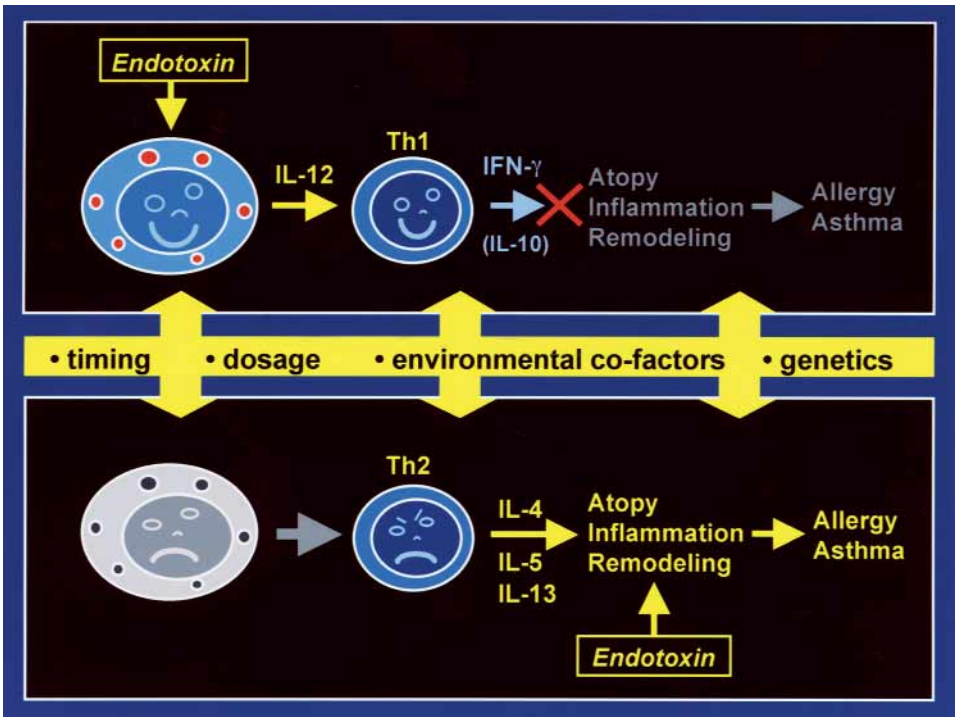

FIG 6. How do we reconcile endotoxin's paradoxical nature of being both friend and foe to allergy and asthma? The key parameters of timing, dosage, environmental cofactors, and genetics influence whether endotoxin can be optimized to provide benefit while minimizing harm.

persistent late-phase response.[126] Low levels of endotoxin exposure significantly augment the inflammatory response to allergen exposure in sensitized subjects with asthma,[119,127] in subjects with allergic rhinitis,[128] and in skin test wheal-and-flare responses to allergen.[129] In metropolitan homes, higher house dust endotoxin levels have been associated with increased asthma symptoms, medication (ie, prednisone) use, and degree of airflow obstruction in children and adults with asthma.[72,77,130,131] Higher house dust endotoxin levels are also associated with more wheezing symptoms in the first year of life.[69a,70] Possible explanations for this association of endotoxin exposure with increased asthma symptoms at any age include an adjuvant-like effect of endotoxin exposure on airways inflammation, increased susceptibility to viral respiratory tract infections caused by endotoxin exposure, and respiratory manifestations in endotoxin-sensitive children.

A recent study builds on this role of endotoxin-mediated asthma and allergy by demonstrating airways hyperreactivity to inhaled endotoxin in subjects with neither inhalant allergen sensitization nor airways hyperresponsiveness to histamine, the cardinal features of the common atopic asthma phenotype.[132] If we consider that the bronchoconstrictor effect of endotoxin exposure can be well controlled with pretreatment with conventional β-agonist bronchodilators (salbutamol and salmeterol)[119a] and inhaled corticosteroids,[120] then we can conceive of endotoxic asthmatic patients who are hypersensitive to endotoxin and responsive to conventional asthma therapy but without allergies or airways hyperresponsiveness to histamine.

Thus, endotoxin-mediated asthma takes the form of several clinical presentations, whereby (1) high levels of endotoxin exposure, typically in certain workplaces, induce Monday asthma; (2) endotoxin augments airways inflammation and induces symptoms in patients with asthma; and (3) people without classic atopic asthma but who are hypersensitive to endotoxin manifest recurrent asthma symptoms with chronic natural exposure (eg, live on a farm; Fig 5). These distinctions are not intended to imply discrete types of endotoxin-mediated asthma; on the contrary, they are likely to overlap. Therapeutically, both atopic and endotoxic asthmatic subjects derive benefit from conventional treatment with β-agonist bronchodilators and inhaled corticosteroids, indicating mechanistic similarities between these asthma types.

ENDOTOXIN: RECONCILING A PARADOX

If endotoxin exposure can be both harmful and beneficial in the context of allergy and asthma, can benefit be separated from potential harm? Studies on the immune response to endotoxin hint at the importance of timing, dosage, environmental cofactors, and genetics for optimizing benefit from microbial immunomodulation while minimizing adverse outcomes (Fig 6).

Timing

Rodent models for asthma have consistently shown that microbial or engineered T_H1-type immune interventions mitigate the atopic asthmatic phenotype when administered early. One recent study with endotoxin has been especially informative.[51] In a rat model of atopic asthma, rats are sensitized with allergen (ie, ovalbumin [OVA]) by means of intraperitoneal injection and then challenged with nebulized OVA 11 days later. LPS was administered as a single inhaled dose either the day before OVA sensitization or up to 1 day before OVA nebulization. Early LPS administration (ie, from 1 day before to 4 days after OVA sensitization) reduced OVA-specific IgE levels, increased OVA-specific IgG levels, and prevented lung inflammation and eosinophilia, airways edema, and airways hyperresponsiveness. In marked contrast, LPS exposure on day 6 to day 10 led to significantly increased airways inflammation and edema over that seen in LPS-untreated but OVA-sensitized rats.

In this atopic asthma model, a dichotomous effect of endotoxin exposure on atopic inflammation and airways hyperresponsiveness can be well demonstrated: early exposure prevents disease, and later exposure augments it. The clinical studies reviewed above report similar benefits of early microbial exposures: (1) higher endotoxin levels in nonatopic versus atopic infants' homes[42,70]; (2) more viral infections in early childhood associated with decreased risk of asthma and airways hyperresponsiveness at age 7 years[41]; and (3) farm stables and farm milk exposure in the first year of life associated with remarkably less allergen sensitization, hay fever, and asthma.[34] One might hypothesize from this that microbe-derived immune modulatory interventions might be most effective and safest when initiated before allergen sensitization and established atopic inflammation in the lungs.

Dose

Endotoxin dosimetry (ie, concentration × respiratory ventilation × duration) associated with occupational asthma is believed to be much greater than that expected in homes (Table II).[4,69,73,124,125,133,134] Airborne sampling of endotoxin content in homes has been rarely reported, but the measured levels so far are quite low.[68,69,73] The immune response to endotoxin is significantly different with low-dose versus high-dose endotoxin exposure. A tolerogenic effect of high-dose LPS exposure, observed in models of endotoxin-induced septic shock and in in vitro studies, is suspected to underlie the Monday asthma effect of occupational endotoxin exposure. In contrast, low-level LPS exposure (ie, picogram per milliliter concentrations) in vitro *primes* monocyte and neutrophil immune responsiveness to stimuli.[2,135,136] Indeed, low-level LPS exposure preferentially primes macrophages to release cytokines (ie, TNF-α and IL-12), whereas greater LPS exposure (eg, 5 ng/mL) primes toxic radical production (ie, nitric oxide metabolite).[137,138] Therefore T_H1-promoting immune responses may occur with lower and more frequent endotoxin doses, avoiding the inflammatory or toxic immune responses seen at higher doses.

TABLE II. Examples of personal airborne endotoxin exposure in different settings

Locale	Endotoxin levels (ng/m³)* Mean	Range
Cotton mill	—	70-5620[125]
	—	6-780[124]
Swine confinement facility	1200	900-1400[4]
Poultry slaughterhouse	400	20-1500[133]
Sawmill	7.6	0.7-62[134]
Homes (Boston)	0.77*	0.01-30.2[69]
Homes of asthmatic children (Denver)	1.9	0.17-5.6[73]

*Samples were obtained with personal monitors for air sampling. The LAL assay was used to measure endotoxin levels; however, there were significant differences in the specific equipment used and endotoxin determination protocols. For homes in Boston, airborne samples were obtained from stationary monitors in family rooms.

Environmental cofactors

There are immune stimulatory microbial components that could influence immune development through natural exposure other than endotoxin. As an example, we have measured one such T_H1-inducing microbial component, heat shock protein (HSP), in dust samples from different locales. Using an assay that recognizes the highly conserved portion of the common HSP-60, we found that dust HSP-60 levels correlate with endotoxin.[139] Of particular interest, HSP-60 levels are much higher in barn dust than in dust samples obtained from homes (either metropolitan, rural, or farm homes). Additionally, in metropolitan German homes, house dust β-(1→3)-glucan (an immune stimulatory cell-wall component of fungi, yeasts, and plants) was found to correlate with house dust endotoxin levels.[140] Thus, the correlations between dust endotoxin and HSP or β-(1→3)-glucan exemplify endotoxin's potential to serve as a surrogate marker for exposures to various microbial components.

Combined microbial component exposure may have an enhanced immune modulatory effect in atopy and asthma prevention. For example, bacterial DNA differs from mammalian DNA on the basis of its immune stimulatory, unmethylated CpG sequences. There are different receptors for CpG DNA and LPS, Toll-like receptor (TLR) 9 and TLR4, respectively. Perhaps this is why combined LPS and CpG DNA are more potent immune stimuli than either alone.[141,142] However, some combined exposures might be harmful. For example, endotoxin exposure enhances ozone-induced mucous cell metaplasia in airway epithelia in a rat model.[143,144] In a murine model of occupational asthma, mice subjected to subchronic corn dust extract inhalation (4 h/d and 5 d/wk for 8 weeks) had airways inflammation and persistent subepithelial fibrosis.[145] In TLR4 mutant mice unable to respond to endotoxin, subepithelial fibrosis also developed, but without airways inflammation or persistent fibrotic changes. This suggests that adverse outcomes

may result when chronic endotoxin exposure is combined with other dust components. The ability of combination microbe-derived interventions to potently increase immune modulatory effects, although interesting, should be considered carefully because of the potential for adverse outcomes.

Genetics

It stands to reason that genetic variation in the immune response to endotoxin influences the benefit, harm, or both resulting from endotoxin exposure. In healthy subjects a demonstrated delineation between LPS responders and LPS nonresponders to inhaled endotoxin was associated with airways hyperresponsiveness to histamine.[146] However, healthy subjects with allergen sensitization exhibit a blunted systemic response to 50 μg of inhaled LPS compared with that of nonatopic healthy control subjects (ie, less of a rise in body temperature and peripheral blood neutrophils, C-reactive protein, and LPS-binding protein).[147] This alludes to the possibility that impaired responses to endotoxin might be a risk factor for the development of atopy.[147]

Specific genetic investigations have reported the influence of polymorphisms in the receptor-enhancer proteins for endotoxin. A TT polymorphism in the promoter region for CD14 was associated with higher levels of soluble CD14 in peripheral blood samples and, in allergen-sensitized subjects, lower serum IgE levels and sensitization to fewer allergens.[148,149] Indeed, CD14 in the airways, both soluble and membrane bound, was associated with the magnitude of the inflammatory response (ie, neutrophils) to inhaled LPS.[150] TLR4 mutations, seen in a small subpopulation (ie, 3% to 6% of several studied cohorts), grossly impair airways and immune responsiveness to endotoxin.[151] It will be interesting to learn whether this endotoxin-insensitive population will have a different prevalence of allergy, asthma, or other diseases (eg, gram-negative sepsis or septic shock).

CONCLUSION

Our understanding of the biologic response to endotoxin provides an extensive framework for thinking about the treatment and prevention of allergy and asthma with immune modulatory intervention. Indeed, early clinical studies with various T_H1-type immune modulators to treat atopic diseases (CpG DNA,[152] *Mycobacterium vaccae*,[153] and lactobacillus[67]) and even modified endotoxin (Lipid A)[154] have recently published promising results that herald more studies to come. In addition, allergen-specific immunotherapy has been shown to promote T_H1-type immune responses in the skin,[155] nasal mucosa,[156] and peripheral blood,[157] correlating well with clinical improvement. Endotoxin's potential to mitigate allergen sensitization and atopic inflammation by promoting T_H1-type immunity is a conventional paradigm, but endotoxin may also model processes by which T_H1-type immune responses protect the airways from the hallmark remodeling changes of asthma: inhibiting fibrotic repair in response to injury, inhibiting mucous gland hypertrophy-hyperplasia-hypersecretion, and protecting airway epithelium from damage.

Rigorous studies are still needed to clarify the findings of nature's ongoing experiment with endotoxin exposure. Certainly, the associations between endotoxin exposure and a lower prevalence of allergy and asthma do not yet demonstrate a cause-and-effect relationship. It is currently unclear whether endotoxin is actually responsible for this atopy- and asthma-protective effect. Perhaps endotoxin is a marker for other microbial exposures or environmental or lifestyle factors that are actually preventing disease onset. Prospective studies in different locales will better determine whether endotoxin exposure has an atopy-protective role, an asthma-protective role, or both. Future studies will also discern those at risk from those protected from endotoxic forms of asthma and will clarify which home and lifestyle variables significantly influence endotoxin exposure. We need to identify biomarkers of response to endotoxin exposure and genetic polymorphisms and mutations that affect endotoxin sensitivity in order to strengthen endotoxin's links to disease and health. With microbe-derived immune modulatory therapies already in clinical trials, investigators eagerly seeking to optimize therapeutic efficacy and safety with these products can expect to find some guidance with endotoxin. Already, the key parameters of timing, dosage, environmental cofactors, and genetics have revealed their potential to be optimized to provide effective and safe microbe-derived interventions for allergies and asthma.

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