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The role of endotoxin and its receptors in allergic disease

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Abstract

Objective—To summarize the existing literature on the association of endotoxin with respiratory diseases and allergic sensitization and to review the potentially modifying effects of endotoxin receptor polymorphisms.

Data Sources—English-language articles were identified from the MEDLINE and PubMed databases using combinations of the following search terms: *endotoxin*, *toll-like receptor*, *polymorphisms*, *atopy*, *asthma*, and *allergy*. Other sources included experts in the field and the bibliographies of pertinent articles.

Study Selection—Relevant articles were selected based on the authors' expert opinion.

Results—Cross-sectional studies, particularly those of children raised in rural European communities, suggest that early endotoxin exposure may protect against the development of allergic sensitization and atopic asthma. However, endotoxin exposure may also contribute to other nonatopic respiratory disorders and may exacerbate disease in individuals with preexisting asthma. Paradoxically, among individuals exposed to high levels of endotoxin, carriers of a functional mutation in toll-like receptor 4, which reduces cellular responsiveness to endotoxin, may be at lower risk of developing allergic sensitization.

Conclusions—The effect of endotoxin exposure on allergic sensitization and asthma appears to be influenced by the timing of exposure, the presence or absence of preexisting disease, and polymorphisms in the genes that encode endotoxin receptors. Further studies are needed to define the window period for this effect, as well as the underlying immunologic mechanism.

INTRODUCTION

For many years, the prevalence of asthma and other allergic disorders has risen worldwide.¹ For example, the prevalence of self-reported asthma in the United States increased 75.2% between 1980 and 1994.² The rapidity of the increase suggests that the underlying cause is a changing exposure rather than changing genetics. In 1989, Strachan³ demonstrated that children from large families were at lower risk for hay fever and eczema. He speculated that these findings could be explained if infections in early childhood, in this case passed on from

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“unhygienic” siblings, prevented the subsequent development of allergic disease. This theory, known as the “hygiene hypothesis,” has been proffered as an explanation for the rising prevalence of allergic disorders in that diminished exposure to early childhood infections has resulted in a population predisposed to allergies. A number of studies, including our own cohort study, support an inverse association between early infectious exposures and the development of allergic diseases.^{3–9} Recent data suggest that environmental exposure to endotoxin, even in the absence of infection, may protect against the development of allergic diseases.^{10,11} However, because endotoxin is a potent stimulator of the innate immune response, the inflammation that it produces can also have deleterious effects.^{12–17}

Endotoxin comprises the outer lipopolysaccharide (LPS) component of gram-negative bacteria cell walls. Richard Pfeiffer (1858–1945) was the first to use the term *endotoxin* when he discovered that the toxic properties of *Vibrio cholerae* were present even after the bacteria had undergone lysis.¹⁸ He proposed that this heat-stable toxin was a component of the bacterial cell itself. Later, the Italian pathologist Eugenio Centanni (1863–1948) found that the fever-producing and toxic properties of endotoxin were inseparable, and he dubbed this toxin “pyrotoxina.”¹⁸

Currently, it is difficult to compare the level of endotoxin exposure among available studies, because the methods of expressing these levels in dust vary. For example, some authors have reported results as endotoxin units (EU) per volume of fluid used to extract the dust sample, others have reported EU per gram of dust, and still others have reported EU per square meter of surface vacuumed. In this review, we mention the values provided in the reports and do not attempt to convert them to a standard expression. Another problem when trying to compare units is the difficulty in standardizing measurements among laboratories.¹⁹ In addition to the problems of standardizing analytic methods, there are many questions about the effects of different methods of collecting, storing, sieving, and extracting dust samples before analysis.

Limitations aside, we review existing epidemiologic evidence for an association between endotoxin exposure and allergic conditions, such as atopy and asthma. We also discuss the receptors involved in endotoxin recognition and the effect that polymorphisms in the genes encoding these receptors may have on allergic disease. Source material for this review included English-language articles identified from the MEDLINE and PubMed databases using combinations of the following search terms: *endotoxin*, *toll-like receptor*, *polymorphisms*, *atopy*, *asthma*, and *allergy*. Articles were also identified through experts in the field and the bibliographies of pertinent studies. We selected articles based on their relevance to the aforementioned topics.

ENDOTOXIN: THE GOOD

Evidence for a protective association between early endotoxin exposure and allergic disease and asthma comes from a number of independent observations. In particular, children raised on farms have been shown to be at lower risk for both allergies and allergic diseases.^{18,20–23} As part of the Swiss Study on Childhood Allergy and Respiratory symptoms with Respect to air Pollution (SCARPOL), Braun-Fahrlander and colleagues²⁰ studied a group of 1,620 children ages 6 to 15 years from 3 rural communities in Switzerland. They found that the children of farmers were less likely to report current wheezing (5.2% vs 9.5%), sneeze during pollen season (2.6% vs 8.6%), and hay fever (7.2% vs 13.3%) when compared with children whose parents were not farmers. Even after adjusting for age, sex, parental education, family history of asthma, family history of hay fever or eczema, number of siblings, maternal smoking, pet ownership, indoor humidity, and heating fuels, farmers’ children were significantly less likely to demonstrate an elevated level of allergen-specific IgE (ie, seroatopy) to indoor (odds ratio [OR], 0.15; 95% confidence interval [CI], 0.04–0.57) and outdoor antigens (OR, 0.38;

95% CI, 0.16–0.87) when compared with the other children. Studying a population of 2,283 children in Salzburg, Austria, Riedler et al²⁴ also showed that children living on farms were less likely to have had hay fever (3.1% vs 10.3%, $P < .001$) or asthma (1.1% vs 3.9%, $P = .02$) when compared with children not on farms. Of the 1,006 children who underwent skin prick tests (SPTs) for atopy, 18.8% of children living on farms had a positive SPT result compared with 32.7% of children not living on farms ($P = .001$). Of all the potential explanatory variables tested, including living conditions, infections, diet, and pet exposure, only regular contact with livestock and poultry appeared to modify the relationship between living on a farm and allergic sensitization, suggesting that part of the protective effect of living on a farm was mediated through contact with farm animals. von Ehrenstein et al²¹ showed that among children of farming parents, the protective effect of livestock exposure was dose dependent. The prevalence of atopic diseases among children with rare, occasional, and frequent livestock contact was 22.9%, 18.8%, and 13.6%, respectively (test for trend, $P < .005$).

In a search for the protective factor in livestock exposure, von Mutius et al²⁵ measured endotoxin levels in 84 farming and nonfarming households with children aged 1 to 14 years in Bavaria and Switzerland. Dust endotoxin levels were found to be highest in stables (geometric mean [GM], 649 EU/mg). Endotoxin levels were also increased in children's mattresses from farming families (GM = 49,479 EU/m²) compared with nonfarming families with livestock contact (GM = 23,340 EU/m²) and nonfarming families without livestock contact (GM = 9,383 EU/m²). A similar population of 2,618 grade school children in rural Austria, Germany, and Switzerland participated in the Allergy and Endotoxin (ALEX) Study. In this cross-sectional survey, Riedler et al²⁶ showed that exposure to stables and farm milk in the first year of life was protectively associated with asthma (OR, 0.14; 95% CI, 0.04–0.48), wheezing in the past year (OR, 0.17; 95% CI, 0.07–0.45), hay fever (OR, 0.20; 95% CI, 0.08–0.50), and seroatopy (OR, 0.32; 95% CI, 0.17–0.62) compared with children with no such exposure in their first year. This suggested that the protective factor mediated its effect early in life.

To address the potential relationship between early endotoxin exposure and allergic sensitization, Gereda et al²⁷ collected dust samples from the homes of 61 infants aged 9 to 24 months. These infants were all considered to be at high risk of developing asthma, because each had 3 prior episodes of physician-documented wheezing. Dust samples were collected by vacuuming the living room floor, kitchen floor, bedroom floor, and child's bed. Atopy was evaluated by skin testing for 5 aeroallergens and 3 food allergens. Significantly lower levels of endotoxin were found in the homes of sensitized infants (GM = 468 EU/mL, dust samples diluted in saline to a concentration of 5 mg/mL) compared with non-sensitized infants (GM = 1,035 EU/mL). This same relationship was found when separately analyzed for inhalant and food allergens. In a larger cross-sectional study, Gehring et al¹¹ randomly selected 740 children aged 5 to 14 years from Saxony-Anhalt, Germany, who had participated in 1 of 2 prior surveys. Endotoxin levels were measured from dust collected from living room floors (expressed as EU per square meter of living room floor); data were available for 444 children. This study found a statistically significant inverse association between endotoxin levels and having an elevated IgE level to 2 or more allergens. The association between endotoxin levels and sensitization was even stronger when the analysis was limited to children who had lived in the same house since birth, again suggesting the importance of early exposure. In recent data from the ALEX Study, Braun-Fahrlander et al¹⁰ showed that endotoxin levels have a protective effect in both farming and nonfarming households. In this study, mattress endotoxin levels were measured for 812 children. Compared with children in the lowest quartile of mattress endotoxin exposure, children in the highest quartile were less likely to have had the following: hay fever (OR, 0.53; 95% CI, 0.35–0.81), sneezing and itchy eyes in the past year (OR, 0.50; 95% CI, 0.34–0.72), seroatopy (OR, 0.76; 95% CI, 0.58–0.98), atopic asthma (OR, 0.48; 95% CI, 0.28–0.81), and atopic wheeze (OR, 0.62; 95% CI, 0.39–0.99). Similar results were seen in the subgroup

analysis of children from nonfarming households. Thus, from cross-sectional studies, it appears as though early exposure to endotoxin may directly act to prevent the development of childhood allergies and allergic diseases or it may serve as a marker for other protective exposures.

ENDOTOXIN: THE BAD

Inhalation of endotoxin by healthy patients has been shown to result in symptoms of chest tightness; a rise in body temperature and markers of inflammation, such as blood C-reactive protein; neutrophilic sequestration in the lung and increases in sputum monocytes, neutrophils, and lymphocytes; a decrease in pulmonary function; and an increase in bronchial reactivity.^{12,13} Given its ability to directly elicit an inflammatory response in the lung, it is not surprising that studies have found a positive association between endotoxin exposure and nonatopic asthma and wheezing.^{10,28}

In the ALEX Study, although there was a consistent negative association between endotoxin exposure and atopic asthma and wheeze, there was also a statistically significant positive association between endotoxin exposure and nonatopic wheeze among nonfarming children.¹⁰ A Boston-area study also found the level of family room dust endotoxin to be positively associated with the risk of wheezing in the first year of life.²⁹

In a study of 1,614 adult Norwegian farmers, Eduard et al²⁸ found that cattle farmers (OR, 1.8; 95% CI, 1.1–2.8) and swine farmers (OR, 1.6; 95% CI, 1.0–2.5) were more likely to have asthma when compared with crop farmers. Long-term exposure to elevated levels of dust endotoxin has also been implicated in a number of occupational pulmonary diseases, such as byssinosis in cotton and flax mill workers,^{14,15} grain dust–induced lung disease in grain handlers,¹⁶ and swine worker's disease.¹⁷

ENDOTOXIN: THE UGLY (OR AT LEAST CONFUSING)

The effect of endotoxin exposure on allergic diseases may depend, in part, on the timing of exposure. Tulic et al³⁰ showed that rats who received LPS 1 day before and up to 4 days after sensitization with ovalbumin had reduced ovalbumin-specific IgE production and bronchoalveolar fluid cell infiltration following challenge when compared with ovalbumin-sensitized rats not exposed to LPS. Exposure to LPS before sensitization also abrogated allergen-induced airway hyperresponsiveness. In contrast, animals exposed to LPS 6 or more days after ovalbumin sensitization had a greater inflammatory response following allergen challenge when compared with sensitized rats not exposed to LPS.

In humans, early exposure to higher levels of environmental endotoxin may protect against allergic sensitization, but later exposure may exacerbate preexisting asthma. In a cross-sectional study by Michel et al,³¹ the level of indoor endotoxin was inversely correlated with pulmonary function and positively associated with the daily need for oral and inhaled corticosteroids, inhaled β -agonists, and xanthines among adults sensitized and exposed to high levels of house dust mite allergen. Asthma-related impairment and clinical severity of asthma were also positively associated with the level of endotoxin exposure. Rizzo et al³² have shown levels of house dust endotoxin to be associated with the severity of childhood asthma. Individuals with asthma appear to have a heightened response to the pulmonary effects of endotoxin,^{33,34} which may relate to allergen-induced secretion of LPS-binding proteins into the bronchoalveolar space.³⁵ Conversely, endotoxin exposure appears to increase allergen responsiveness. Boehlecke et al³⁶ showed that among asthmatic patients sensitive to dust mites, preexposure to endotoxin more than halved the concentration of *Dermatophagoides farinae* extract required to cause a 20% or more decrease in forced expiratory volume in 1 second. Therefore, among sensitized individuals, endotoxin exposure may potentiate the response to allergen and vice versa.

ENDOTOXIN AND ITS RECEPTORS

Further evidence for a relationship between endotoxin and allergy has come from genetic studies. Linkage analysis has mapped the gene or genes that control total serum IgE to chromosome 5q31 to 33.^{37–39} This region has also been linked to asthma⁴⁰ and bronchial hyperresponsiveness.⁴¹ Candidate genes in this region include interleukin 3 (*IL-3*), *IL-4*, *IL-5*, *IL-9*, *IL-12B*, and *IL-13*; however, the loci most closely associated with total serum IgE was *D5S399/D5S393*, whose nearest gene is *CD14*.⁴² *CD14* is now known to be an important cofactor in binding endotoxin and initiating the innate immune response.⁴³

The innate immune system is capable of recognizing and responding to structural motifs on viruses and bacteria called pathogen-associated molecular patterns (PAMPs). These PAMPs include but are not limited to endotoxin, peptidoglycans, lipoproteins, lipoteichoic acid, lipoarabinomannan, flagellin, and unmethylated CpG oligonucleotides.^{44,45} A group of highly conserved receptors, known as toll-like receptors, mediate the host response through their recognition of specific PAMPs. Toll-like receptors are similar in that they have a leucine-rich extracellular portion, as well as similar cytoplasmic domains. The latter is homologous to the signaling region of the IL-1 receptor and is therefore dubbed the toll-IL-1R (TIR) domain.

The critical role of toll-like receptor 4 (TLR-4) in the signaling pathway of endotoxin was clarified in 1998 when it was discovered that both C3H/HeJ and C57BL/10ScCr mice, which are insensitive to LPS, carried mutations in TLR-4.⁴⁶ Toll-like receptor 2 (TLR-2) may also be able to mediate a response to LPS.^{47–49} Dziarski et al⁵⁰ was able to show that in the presence of coreceptor MD-2 both TLR-2 and TLR-4 were able to respond to purified endotoxin. TLR-2 is also able to recognize a wide variety of PAMPs found on gram-positive bacteria, mycobacteria, spirochetes, and mycoplasmas.^{51,52}

In addition to MD-2, CD14 also appears to be important in mounting a response to endotoxin. After gaining systemic access, LPS is bound by the circulating LPS-binding protein,^{43,53} which recognizes the lipid A component.⁵⁴ Wright et al⁴³ showed that antibodies to CD14 could both block the binding of erythrocytes coated with LPS and LPS-binding protein to macrophages and inhibit tumor necrosis factor α production in LPS-stimulated human blood. CD14 exists on mononuclear phagocytes as a glycosylphosphatidyl inositol-anchored membrane protein, mCD14, and because it does not have a cytoplasmic region, it is incapable of intracellular signaling by itself.⁵⁵ CD14 is also excreted by monocytes and exists in a free, soluble form, sCD14.⁵⁶ Soluble CD14 is capable of precipitating a response to LPS in cells that lack membrane-bound CD14, such as endothelial cells, epithelial cells, and dendritic cells.^{56,57} Work by da Silva Correia et al⁵⁸ has shown that CD14, TLR-4, and MD-2 must form a complex to effectively bind LPS and that sCD14 could substitute for mCD14 in delivering LPS to TLR-4 and MD-2.

Once bound, endotoxin triggers a series of cellular events, including the production of inflammatory cytokines,⁵⁹ the maturation of antigen-presenting cells,⁵⁷ and the up-regulation of receptors, such as those for IL-12.⁶⁰ Most of these events are triggered through the intracytoplasmic TIR domain of the TLR. For example, the production of inflammatory cytokines IL-1, IL-6, and tumor necrosis factor α is mediated through a series of accessory proteins, including MyD88, and the eventual activation of transcription factor NF- κ B.⁵⁹

Lauener et al⁶¹ analyzed *CD14*, *TLR-2*, and *TLR-4* gene expression in blood taken from 96 participants of the ALEX Study (25 farmers' children and 71 controls). They found the expression of *CD14* and *TLR-2* to be significantly higher in farmers' children compared with nonfarmers' children. *TLR-4* was slightly lower in farmers' children but not significantly so.

GENE-ENVIRONMENT INTERACTIONS

If endotoxin exposure affects allergy risk, one would expect functional mutations in the genes that encode endotoxin receptors to modify the effect. Although a number of studies have looked at the relationship between polymorphisms in LPS receptors and asthma or allergies, few have actually accounted for endotoxin exposure (Table 1). Therefore, it is not surprising that many of the studies of endotoxin receptor polymorphisms have had conflicting or negative results.

CD14

The proximity of the *CD14* gene to loci linked to total serum IgE,^{37–39} asthma,⁴⁰ and bronchial hyperresponsiveness⁴¹ made it an obvious first place to look for polymorphisms associated with these outcomes. Vercelli et al⁶² looked at the correlation between polymorphisms at –1359, –1145, and –159 in the promoter region of *CD14* and serum levels of sCD14 and total IgE in 265 children aged 11 years, who were enrolled in the Tucson Children's Respiratory Study. The authors noted that homozygosity for T at –1359 appeared most strongly related to IgE levels, and children with the genotype –1359TT/–1145AA/–159CC had the highest IgE levels and the lowest sCD14 concentrations. Baldini et al⁶³ also reported results from the Tucson Children's Respiratory Study in which children 11 years of age were screened for the C-to-T transition at base pair –159. Four hundred eighty-one children were genotyped, and the frequencies of CC, CT, and TT genotypes were 29.4%, 49.4%, and 21.3%, respectively. A sample of individuals with the TT genotype was found to have significantly higher serum levels of sCD14 when compared with those of the CC genotype. However, associations between these genotypic differences and outcomes were only found in subgroup analysis. For example, only among non-Hispanic whites with positive skin test results was the TT genotype associated with lower total IgE levels and fewer positive skin test results when compared with the CT and CC genotypes. In addition, the level of interferon- γ and IL-4 secreted from stimulated peripheral blood mononuclear cells did not differ with respect to these genotypic variations. However, the authors showed that sCD14 levels were positively and negatively related to the interferon- γ and IL-4 responses, respectively.

Gao et al⁴² also looked at the single nucleotide polymorphism at –159 among 300 British and 200 Japanese individuals. Their findings differed somewhat from those of Baldini et al in that the association between total IgE levels and –159 T/C variation was only seen among British patients with a negative radioallergosorbent test result. Among these individuals, the TT and TC genotypes had the lowest IgE levels when compared with CC. Koppleman et al⁶⁴ also examined the association of the –159 promoter polymorphism with atopy. Their study population was selected from a family study of the genetics of asthma and consisted of 159 asthma probands and 158 spouses. Among asthma probands, 32.1% were CC homozygotes, 47.8% were CT heterozygotes, and 20.1% were TT homozygotes at the –159 base pair. Spouses had a different genotypic distribution with 19.6% CC, 53.8% CT, and 26.6% TT. Among the asthmatic patients 79.9% had atopy according to skin prick testing when compared with 29.1% of spouses. Among individuals with positive skin test results, CC homozygotes had significantly higher IgE levels and number of positive skin test results when compared with individuals with the CT and TT genotype. The authors concluded that this study confirmed the association between the *CD14*/–159 polymorphism and IgE levels among individuals with positive skin test results. However, based on their study sample, asthma diagnosis may have confounded this association. Woo et al⁶⁵ examined whether the –159 C-to-T polymorphism was associated with either food allergy or asthma. Their sample consisted of 175 adult asthmatic patients (128 with atopic asthma and 47 with nonatopic asthma), 77 patients with a food allergy, and 61 nonatopic, nonallergic adults. Nonatopic asthmatic patients and patients with food allergy were more likely to have a T allele at –159 when compared with controls (OR, 2.0; 95% CI, 1.1–2.8; OR, 1.7; 95% CI, 1.1–2.8; respectively).

Two recent, large German studies did not find an association between the *CD14 C-159T* genotype and serum IgE levels, asthma, atopic dermatitis, or allergic rhinitis.^{66,67} However, another recent study suggests that some of these relationships may be age dependent. O'Donnell and colleagues⁶⁸ followed up a cohort of 718 children aged 8 to 25 years in New South Wales, Australia. Three hundred five individuals were genotyped for the *CD14 -159* single nucleotide polymorphism. Compared with the individuals possessing either the CT or TT genotypes, patients with the CC genotype were more likely to have atopy at the ages of 8, 12, 14, and 16 years; had a greater number of positive skin prick test results at the ages of 12 and 16 years; had higher total serum IgE level at the age of 18 years; and had a higher prevalence of bronchial hyperresponsiveness at the age of 8 years. Significant differences were not seen at the age of 25 years.

In summary, the *-159 C-to-T* promoter polymorphism is associated with increased levels of sCD14. This single nucleotide polymorphism may also influence the prevalence of atopy at different ages, as well as the degree of atopy among those already sensitized. However, because none of these studies measured endotoxin exposure, it is unclear whether the *CD14* promoter polymorphism modifies endotoxin's effect on the expression of atopy or asthma.

TOLL-LIKE RECEPTOR 4

Arbour et al⁶⁹ screened the coding region of *TLR-4* in 83 persons and identified 2 cosegregating mutations in 10 individuals (1 individual was homozygous). The first mutation, an A → G substitution at nucleotide 896, resulted in an aspartic acid to glycine change at amino acid 299 (D299G), and the second, a C → T substitution at nucleotide 1196, resulted in a threonine to isoleucine change at amino acid 399 (T399I). These polymorphisms appeared to result in functional changes, because persons carrying the mutant alleles were significantly less likely to experience a decrease in forced expiratory volume in 1 second following LPS inhalation.⁶⁹ Cells transfected with either of the *TLR-4* mutant alleles in vitro were less responsive to LPS as evidenced by decreased NF-κB activity.⁶⁹ Michel et al⁷⁰ also found that individuals heterozygous for either the +896 or +1196 polymorphism had a lower systemic response to LPS inhalation challenge, as evidenced by lower plasma levels of C-reactive protein and a smaller increase in the peripheral white blood cell count, when compared with individuals homozygous for the common allele. Agnese et al⁷¹ studied these polymorphisms in a group of 77 patients admitted to the intensive care unit for sepsis. Fourteen patients were found to carry the mutations, and all were heterozygous at both alleles. Gram-negative sepsis was significantly more prevalent in those with polymorphisms (79% vs 11%, $P = .004$), suggesting that these individuals were less able to mount a successful immune response to LPS.

Raby et al⁷² looked for linkage between traits for asthma and atopy and 4 polymorphisms on *TLR-4* in 2 family-based cohorts: the Childhood Asthma Management Program (589 families) and a Quebec cohort (167 families). In the Quebec cohort, a weak association was noted between an A → G nucleotide polymorphism at loci -6142 and serum eosinophil levels ($P = .04$); however, there was no evidence of transmission of asthma, airway hyperresponsiveness to methacholine, total serum IgE, or skin prick reactivity with any of the polymorphisms tested. Similarly, Yang et al⁷³ found no association between having the *D299G TLR-4* allele and asthma; however, the *D299G* polymorphism was associated with worse atopy severity scores among the oldest siblings with asthma. Unfortunately, because both of these studies did not measure environmental endotoxin levels, they could not account for the potentially confounding effect of endotoxin exposure.

Werner et al⁷⁴ studied 334 persons aged 20 to 44 years who were participating in a follow-up to the European Community Respiratory Health Survey at the German centers of Hamburg and Erfurt. Participants were tested for the *D299G* and *T399I TLR-4* mutations, and endotoxin

exposure was estimated from dust samples taken from the participants' living rooms. Among adults with wild-type *TLR-4*, higher levels of dust endotoxin were associated with a greater likelihood of asthma and recent wheezing. Conversely, among mutant allele carriers, higher levels of endotoxin exposure appeared protective for bronchial reactivity.

TOLL-LIKE RECEPTOR 2

Functional mutations have also been described for *TLR-2*.^{75–77} Lorenz et al⁷⁵ have described a polymorphism that results in an arginine to glutamine substitution at amino acid 753 (R753Q) in the highly conserved C-terminus of *TLR-2*. Among individuals with septic shock, this polymorphism was found more frequently in persons with gram-positive sepsis, suggesting that this mutation confers susceptibility to gram-positive infections.⁷⁵ Kang et al⁷⁶ discovered a C → T nucleotide polymorphism at base pair 2029, which results in an arginine to tryptophan substitution at amino acid 677 (R677W). Monocytes taken from carriers of the *TLR-2* R677W polymorphism have been shown to produce significantly lower amounts of IL-12 in response to stimulation with either *Mycobacterium leprae* or LPS when compared with monocytes taken from individuals homozygous for wild-type *TLR-2*.⁷⁷

In a small case-control study, Ahmad-Nejad et al⁷⁸ compared the frequency of *TLR-2* and *TLR-4* polymorphisms in adult patients with mild to severe atopic dermatitis with healthy, age-matched control patients. Eighteen (24%) of 77 patients with atopic dermatitis (AD) were found to have either the *TLR-2* R753Q polymorphism or the *TLR-4* D299G/T399I polymorphisms when compared with 2 (5%) of the 39 control patients ($P = .01$). Among patients with AD, individuals with the *TLR-2* R753Q polymorphism had significantly higher total IgE levels when compared with patients with wild-type *TLR-2* and *TLR-4*. The former also had significantly higher levels of total IgE, IgE against staphylococcal enterotoxins, and IgE against the dust mite antigen, Der p, when compared with individuals who possessed *TLR-4* D299G/T399I polymorphisms. However, in a Japanese study of 32 children with asthma and their families, Noguchi and colleagues⁷⁹ did not find an association between polymorphisms in *TLR2* and either total IgE levels or asthma transmission.

A recent report from Eder et al⁸⁰ suggests that polymorphisms in the genes that encode *TLR-2* and *TLR-4* can modify the relationship between farm and endotoxin exposure and allergic and respiratory outcomes. Six hundred nine participants of the ALEX Study were genotyped for polymorphisms in the *TLR-2* and *TLR-4* genes. Among farmers' children, individuals heterozygous or homozygous for an A → T nucleotide polymorphism at base pair –16,934 of the *TLR-2* gene were less likely to have had an asthma diagnosis, report current asthma symptoms, demonstrate atopy by serologic testing, or report current hay fever symptoms when compared with AA homozygous individuals. The association was not seen among nonfarmers' children or in groups stratified by high or low levels of mattress endotoxin. Among children exposed to high levels of mattress endotoxin, being heterozygous or homozygous for the previously described *TLR-4* polymorphism at nucleotide 896 (resulting in a D229G amino acid change in the receptor) appeared protective for seroatopy. A similar, nonsignificant protective relationship was seen for farmers' children; however, no such relationship was seen among nonfarmers' children or those with low endotoxin exposure. These findings underscore the importance of gene and environment interaction in asthma and allergy development.

BEYOND THE TOLL: LINKS TO ALLERGIC DISEASE AND T-CELL DIFFERENTIATION

Allergic diseases are typically characterized by predominance of T_H2 cytokines, such as IL-4 and IL-5, which promote IgE production and eosinophilia.⁸¹ However, the process by which microbes and their products, such as endotoxin, influence expression of this phenotype remains

unknown. Some propose that early exposures result in a skewing of T-cell differentiation toward the T_H1 phenotype and away from T_H2.⁸² Evidence for this mutual exclusivity comes from laboratory studies, which show that T_H1 cytokines, such as interferon- γ , are capable of suppressing IL-4 and IL-5 production,⁸³ and observational studies, which show that house dust endotoxin levels are positively associated with interferon- γ production by CD4⁺ T cells but not IL-4, IL-5, or IL-13.²⁷ Critics of this binary mechanism point out that the incidence of both T_H2 diseases, such as asthma, allergic rhinitis, and atopic dermatitis, and T_H1 diseases, such as type 1 diabetes mellitus, multiple sclerosis, and Crohn disease, has increased, suggesting a more general loss of T-cell suppression.⁸⁴ Although endotoxin exposure can result in IL-12 production and the subsequent expression of interferon- γ ,⁸⁵ a T_H1 cytokine, TLR-4 signaling has also been shown to be important for optimal T_H2 development.⁸⁶

In a recent study by Tulic et al,⁸⁷ explanted nasal mucosa from 22 children (mean age, 3.4 years) and 17 adults (mean age, 38.3 years) was stimulated with allergen and/or LPS. In mucosa taken from children with atopy, stimulation with both allergen and LPS abrogated T_H2 cytokine expression and increased T_H1 cytokine production when compared with allergen stimulation alone. However, T_H2 cytokine expression was restored when these cells were also incubated with neutralizing antibody against IL-10, IL-12, or interferon- γ , suggesting that the effect of LPS was mediated in part through the production of these cytokines. Stimulation of cells with both allergen and LPS also resulted in an increase in the number of T-cells expressing TLR-4 receptors, an increase in the number of TLR-4–positive cells that contain IL-10, and an increase in the number of cells that express both CD4 and CD25 when compared with allergen stimulation alone. The effect of LPS stimulation on *IL-10* transcription and TLR-4 expression appeared diminished in adults when compared with children. Together these findings suggest that LPS may down-regulate allergic response through T_H1 skewing and/or the expansion of regulatory T cells, but that these effects are most prominent in children, whose developing immune system is still susceptible.

To date, 2 cross-sectional studies suggest that in situations of high environmental endotoxin exposure, functional mutations in the *TLR-4* gene may protect against bronchial reactivity⁷⁴ and atopy.⁸⁰ The protective effect of *TLR-4* function mutations on airway responsiveness may reflect reduced localized inflammation in the lung.⁶⁹ However, the mechanism by which high endotoxin exposure and *TLR-4* functional mutations may interact to lower the risk of atopy has yet to be established. Perhaps, as has been shown by Pasare et al,⁸⁸ stimulation of TLR-4 receptors on dendritic cells can remove the suppressive effect of CD4⁺CD25⁺ regulatory T-cells on effector T cells; therefore, ineffective TLR-4 signaling may result in unopposed inhibition of both T_H1 and T_H2 expression by regulatory T-cells.

CONCLUSIONS

A number of cross-sectional studies have demonstrated a protective relationship between early endotoxin exposure and atopic conditions. However, the host response to environmental endotoxin appears to depend, in part, on the time of exposure, the preexistence of pulmonary disease such as asthma, and genetic differences in the receptors involved in endotoxin recognition. Paradoxically, a mutation that is thought to diminish responsiveness to LPS also appears to decrease the risk for atopy among individuals highly exposed to endotoxin. The mechanism for this gene-environment interaction has yet to be elucidated. Still needed are prospective studies to clarify the window period in which endotoxin may exert a protective effect, as well as its influence on the developing immune system.

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Summary of Studies Examining the Relationship Between Polymorphisms in Genes Encoding Endotoxin Receptors and Atopic Diseases

Table 1

Gene	Polymorphism	Source	Study population	Endotoxin exposure assessed	Findings
<i>CD14</i>	Cytosine (C) to thymine (T) transition at position -159	Baldini et al ⁶³	481 US children	No	Among non-Hispanic white children with a positive SPT result, children with CC or CT genotype had higher total IgE levels and number of positive SPT results when compared with TT individuals Among British patients with negative RAST results, IgE levels were higher in individuals with CC compared with those with CT or TT
		Gao et al ⁴²	300 UK individuals 200 Japanese individuals	No	Among SPT-positive individuals, persons with CC had significantly higher IgE levels and number of positive SPT results when compared with those with CT or TT Compared with controls, patients with either nonatopic asthma or food allergy were more likely to be TT homozygous
		Koppelman et al ⁶⁴	159 asthma probands and 158 spouses from the Netherlands	No	Total serum IgE levels were not significantly different among individuals with CC, CT, or TT
		Woo et al ⁶⁵	175 adults with asthma, 77 persons with food allergy, and 61 controls from the United States	No	Genotype was not associated with serum IgE levels or the number of positive SPT results
		Sengler et al ⁶⁶	872 German children	No	Compared with CT and TT genotypes, persons with CC were more likely to have atopy, higher serum IgE levels, and BHR at various times between 8 and 18 years of age; differences were not seen at age 25 years
		Kabesch et al ⁶⁷	2048 German children and 888 adults	No	Association between SNP at position -6142 and serum eosinophil levels in the Canadian cohort alone
		O'Donnell et al ⁶⁸	305 Australian individuals	No	Among D299G/T399I carriers, moderate levels of endotoxin exposure were associated with a lower likelihood of BHR when compared with low levels of exposure
Toll-like receptor 4 (<i>TLR-4</i>)	Five SNPs, including D299G and T399I	Raby et al ⁷²	589 US families and 167 Candian families	No	Among children exposed to high levels of endotoxin, carriers of D299G were less likely to have atopy when compared with noncarriers
	D299G and T399I	Werner et al ⁷⁴	334 persons ages 20 to 44 years from Germany	Yes	Among first affected siblings, D299G carrier status was associated with a higher mean atopy score when compared with noncarriers; asthma transmission was not associated with D299G
	Five SNPs, including D299G	Eder et al ⁸⁰	609 school-aged children from Austria and Germany	Yes	Among children living on farms, carriers of an adenine to thymine SNP at position -16934 of TLR-2 were less likely to have asthma, atopy, or hay fever when compared with AA individuals
	D299G	Yang et al ⁷³	336 families with ≥ 2 siblings with asthma and 179 controls from the United Kingdom	No	Individuals with AD were more likely to possess either the TLR-2 R753Q or the TLR-4 D299G/T399I polymorphisms when compared with controls
Toll-like receptor 2 (<i>TLR-2</i>)	Three SNPs in TLR-2	Eder et al ⁸⁰	609 school-aged children from Austria and Germany	Yes	Polymorphisms in TLR-2 did not transmit with the asthma phenotype and were not associated with total serum IgE levels; genotypic distributions were not significantly different between individuals with asthma and controls
	R753Q (as well as D299G and T399I in TLR-4)	Ahmad-Nejad et al ⁷⁸	78 adult patients with AD and 39 controls from Germany	No	
	Four polymorphisms in TLR2	Noguchi et al ⁷⁹	137 families with 32 asthma probands, 133 patients with child-onset asthma, and 190 controls from Japan	No	

Abbreviations: AD, atopic dermatitis; BHR, bronchial hyperresponsiveness; D299G, a SNP resulting in the replacement of aspartic acid by glycine at amino acid 299 of translated TLR-4; R753Q, a SNP resulting in the replacement of arginine by glutamine at amino acid 753 of translated TLR-2; RAST, radioallergosorbent test; SNP, single nucleotide polymorphism; SPT, skin prick test; and T399I, a SNP resulting in the replacement of threonine by isoleucine at position 399 of translated TLR-4.