

The Hygiene Hypothesis Revisited

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The hygiene hypothesis was proposed by Strachan [1] in 1989 as a possible explanation for the rise in the prevalence of hay fever and other allergic diseases in industrialized countries. In an epidemiologic study of British children, he observed an inverse association between hay fever and number of siblings. Interestingly, this effect was stronger relative to the number of older siblings. These observations led to the proposal that societal changes over the past few decades, such as the decline in family size, improved sanitation, and higher standards of personal cleanliness, has led to reduced exposure to childhood infections, which in turn has altered immunologic development to favor an increase in allergic diseases (Fig. 1). This provocative hypothesis prompted investigators to search for evidence that environmental exposures and genetic factors interact in childhood either to promote or protect against the development of atopic diseases. This article discusses information that has been gathered over the past 15 years to test the hygiene hypothesis. Remarkably, since this theory was originally proposed, knowledge of innate immune mechanisms has blossomed, and this has led to new epidemiologic studies, and a number of new experimental approaches, to define how microbial expo-

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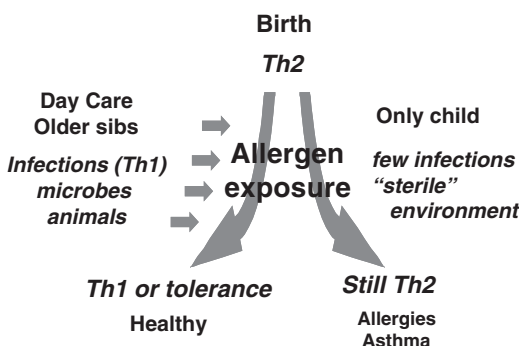


Fig. 1. The hygiene hypothesis.

surements might modify immune development and the incidence of allergic diseases in childhood.

How does contact with other children affect immune development and atopy?

The inverse association between sibship size and hay fever noted by Strachan [1] has since been well substantiated in the literature. In this regard, exposure to older siblings has been shown to exert a stronger protective effect than younger siblings. Furthermore, large family size has also been related to a reduction in the number of positive skin tests [2].

Daycare centers provide another significant source of exposure to childhood infectious diseases, and as such, several studies have evaluated the role that the daycare environment may play in the development of atopic diseases. A number of studies found that daycare attendance was associated with increased respiratory infections in early childhood, including episodic wheezing before the age of 3 [3,4]. There is now evidence to indicate, however, that early entry into daycare may have beneficial effects on the risk of allergy and asthma later on in childhood. For example, Kramer et al [5] demonstrated that entrance into daycare at an early age was associated with a decreased prevalence of atopy in school children. Furthermore, Ball et al [6] found that entry into daycare in the first 6 months of life was associated with a tendency toward increased virus-induced wheezing in the short run, but a reduced risk of asthma between 6 and 13 years of age. These studies underscore the concept that the timing of exposures may be important relative to the long-term risk of allergy and asthma.

In addition to the age of entry into daycare, family history is another important factor to consider. In a study of 453 6-year-old children in Boston with a parental history of atopy, Celedon et al [7] found an inverse association between daycare exposure in the first year of life and eczema. In children without a

maternal history of asthma, daycare attendance was inversely associated with asthma and recurrent wheezing at age 6. Because there was no protective effect of daycare on asthma or recurrent wheezing among children with a maternal history of asthma, these findings suggest that there are additional factors, possibly genetically determined, that influence the relationship between daycare attendance and the risk of wheezing.

Do infections promote or inhibit allergy and asthma?

The implications of the original hygiene hypothesis were that the decrease in family size led to a reduction in the number of childhood infections, which adversely affected immune development to increase the prevalence of atopic diseases. Numerous cross-sectional and longitudinal studies have been performed to determine the associations between family size, childhood infections, and atopic diseases (Table 1). Despite these studies, the question of the influence of childhood infections on the development of atopic disease remains controversial. Some studies have failed to demonstrate an association, positive or negative, between childhood infections and atopic diseases [8,9]. In a retrospective longitudinal study of United Kingdom children, McKeever et al [9] demonstrated that neither personal infections nor sibling infections influenced the development of allergic diseases. Other studies suggest that infections might actually promote atopy. Bodner et al [10] were able to confirm the sibling effect on atopic diseases; however, with the exception of a weakly protective effect of measles infection after the age of 3 on the development of asthma, this effect could not be explained by exposure to childhood infections. In fact, their data actually showed positive correlations between some atopic manifestations and certain childhood infections, and a cumulative effect of illnesses before the age of 3 and an increased risk for asthma, hay fever, and eczema.

Several studies suggest that certain infectious diseases might protect against the development of atopic diseases. Using positive tuberculin skin tests as a marker of *Mycobacterium tuberculosis* infection, Shirakawa et al [11] concluded that *M tuberculosis* infection protects against the development of atopic disease because positive tuberculin skin tests were inversely associated with atopy; total IgE; asthma; and production of the Th2 cytokines interleukin (IL)-4, IL-10, and IL-13. Moreover, positive tuberculin skin tests correlated with expression of the Th1 cytokine interferon (IFN)- γ . In a study of adults in Guinea-Bissau, Shaheen et al [12] demonstrated that a history of natural measles infection was associated with a reduction in the risk of atopic sensitization; however, in a larger cross-sectional study of the Finnish population, Paunio et al [13] demonstrated a positive association between measles infection and atopic diseases including eczema, allergic rhinitis, and asthma.

Several cross-sectional studies performed by Matricardi et al [14–16] have demonstrated a protective effect of hepatitis A infection on the development of atopy, asthma, and allergic rhinitis in both Italy and the United States. Inverse

Table 1
Selected studies of infections and the development of atopic diseases

Author, year	Type of study	Study population	Infectious etiology infection	Results
Bodner et al 1998 [10]	Cross-sectional	2111 Aberdeen school children, ages 10–14 years	Common childhood infections	Rubella was associated with an increased risk of eczema. Measles after age 3 was protective of asthma. Pertussis after age 3 was associated with increased risk of eczema. Varicella after age 3 was associated with increased risk of asthma. An increasing cumulative number of common childhood infections were associated with an increased risk of asthma, hay fever, and eczema.
Farooqi and Hopkins 1998 [8]	Longitudinal (retro)	1934 United Kingdom subjects from Oxfordshire family practice birth cohort up to age 12	Common childhood infections	No association between childhood infections and the development of atopic diseases.
Illi et al 2001 [19]	Longitudinal (prosp)	1314 German children in birth to age 7 cohort	Clinical colds, LRIs	≥ 2 clinically defined colds in first year of life was associated with decreased asthma and wheeze at age 7 and decreased atopy in the first 3 years. ≥ 4 lower respiratory tract infections in first 3 years was associated with increased wheeze at age 7.
Kosunen et al 2002 [18]	Cross-sectional (population-based)	645 Finnish residents, ages 15–54 years	<i>H pylori</i>	<i>H pylori</i> was inversely associated with atopy
Matricardi et al 1997 [14]	Cross-sectional	1659 Italian men, ages 17–24 years	Hepatitis A	Hepatitis A was inversely associated with atopy and allergic rhinitis or asthma.

Matricardi et al 2000 [15]	Cross-sectional (retro, case-controlled)	480 Italian men, ages 17–24	Multiple bacterial and viral pathogens	<i>Toxoplasma gondii</i> and Hep A were inversely associated with atopy. Cumulative exposure to orofecal and food-borne pathogens was inversely associated with atopy, allergic rhinitis, and allergic asthma.
Matricardi et al 2002 [16]	Cross-sectional	33,994 US residents in public registry, ages 1–90 years	Hep A, HSV-1, <i>T gondii</i> Hep B and C, HSV-2	Hep A, <i>T gondii</i> , HSV-1 were inversely associated with hay fever and asthma. Hep A was inversely associated with atopy.
McKeever et al 2002 [9]	Longitudinal (retro)	29,238 UK children in historical birth cohort from General Practice Research Database, ages 0–11 years	Common childhood infections	No association between common childhood infections and atopic diseases.
Paunio et al 2000 [13]	Cross-sectional (population-based)	547,910 Finnish residents, ages 14 months–19 years	Measles	Measles infection was positively associated with eczema, allergic rhinitis, and asthma
Shaheen et al 1996 [12]	Longitudinal (retro)	262 adults in Guinea-Bissau originally surveyed in first 6 years of life	Measles	Measles infection was associated with decreased risk of atopy.
Shirakawa et al 1997 [11]	Cross-sectional	867 children in Honsu, Japan, ages 12–13	<i>M tuberculosis</i>	<i>M tuberculosis</i> was inversely associated with atopy, asthma, allergic rhinitis, eczema.
Sigurs et al 2005 [22]	Longitudinal (case-controlled)	47 hospitalized, 93 control subjects followed from infancy to age 13	RSV (requiring hospitalization)	RSV infection in infancy requiring hospitalization was associated with increased risk of asthma, atopy, and allergic rhinoconjunctivitis up to age 13.
Stein et al 1999 [20]	Longitudinal (population-based)	1246 children in the Tucson Children's Respiratory Study prospective birth cohort through age 13	RSV	RSV was associated with increased risk of wheezing in ages 6–11, but the risk declines with age. RSV was not associated with atopy.

associations between both herpes simplex virus-1 and toxoplasmosis and atopic manifestations have also been demonstrated [16]. Recent work by McIntire et al [17] has suggested that a genetic polymorphism in TIM-1, the receptor for the hepatitis A virus, may confer protection against atopic diseases among those infected with hepatitis A, perhaps by altering the Th2 phenotype. In addition, cumulative exposures to the food-borne and orofecal infections hepatitis A, *Helicobacter pylori*, and toxoplasmosis, which are considered markers of poor hygiene, are inversely associated with atopy and atopic diseases [15,18]. These findings suggest that gastrointestinal infections may play a more central role in protection from atopy than infections transmitted by other routes [15].

Several longitudinal studies have been performed to determine the role that respiratory viruses may play in the development of recurrent wheezing, asthma, atopy, and allergic rhinitis. Illi et al [19] demonstrated that episodes of rhinitis, as a marker for clinical colds, were inversely related to asthma, wheezing, and allergic sensitization; however, lower respiratory tract infections increased the risk of wheezing in childhood. Similar findings have been reported from the Tucson Children's Respiratory Study, a prospective birth cohort study. Respiratory syncytial virus (RSV) infections in the first 3 years of life were associated with an increased risk of wheezing between the ages of 6 and 11, although this effect decreased with time. There was no association between RSV infection and the development of allergic sensitization or serum IgE levels [20]. In contrast, RSV infection severe enough to require hospitalization in infancy has been shown to increase the risk of allergic sensitization, allergic rhinoconjunctivitis, and allergic asthma by age 13 in a case-controlled longitudinal study [21,22]. Causality cannot be established in these epidemiologic studies, however, and it is unclear whether severe RSV infections promote atopic diseases or whether there is an underlying factor, perhaps genetically determined, that predisposes to both atopy and severe RSV infections.

Growing up on a farm

Several studies in Europe and North America demonstrate that children growing up on a farm have a lower prevalence of allergic sensitization, hay fever, and asthma [23]. In addition, children raised by farming parents have a lower risk of atopic sensitization than their counterparts in the same community who were not raised on a farm [24,25]. This protective effect seems to persist into adulthood [26]. Finally, living on a farm during the first 5 years of life, but not after, seems to reduce the risk of subsequent asthma [25]. Collectively, these findings uniformly suggest that living on a farm during early childhood protects against atopy, and begins to provide clues as to which elements of this lifestyle confer protection. There is evidence that specific protective factors on the farm could include exposure to endotoxin, contact with animals, and diets rich in dairy products or fermented beverages [27].

Endotoxin

It has been suggested that increased exposure to endotoxin, a component of the cell wall of gram-negative bacteria, on farms or in homes with animals could modify immune responses to reduce the prevalence of atopic diseases. Farmhouses are indeed associated with increased endotoxin exposure, but levels of this microbial product are increased not only in stables but also in house dust and mattresses of children raised in farming families [28]. In a study of German children between the ages of 5 and 10, Gehring et al [29] found an inverse association between endotoxin exposure and sensitization to one or more allergens. In contrast, exposure to endotoxin did not modify the risk of asthma, hay fever, or eczema. In the United States, Gereda et al [30] demonstrated that endotoxin levels were significantly lower in the homes of infants with positive skin prick tests. In addition, they reported that endotoxin levels correlated with higher proportions of IFN- γ -producing CD4⁺ T cells. More recently, Braun-Fahrlander et al [31] demonstrated an inverse association between endotoxin levels in the mattresses of children and the occurrence of atopy, hay fever, and atopic asthma in school-age children. They also suggested possible immune mechanisms associated with such exposure by identifying an inverse relationship between endotoxin levels and lipopolysaccharide (LPS)-stimulated peripheral blood mononuclear cell (PBMC) production of cytokines associated with the innate immune system: tumor necrosis factor- α , IFN- γ , IL-10, and IL-12 [31]. This finding suggests down-regulation of the immune system on exposure to higher levels of endotoxin.

Two studies have reported that endotoxin exposure may also reduce the risk of developing atopic dermatitis. In a high-risk prospective birth cohort of children with a parental history of asthma or allergies, Phipatanakul et al [32] found that endotoxin levels in the home at 2 to 3 months of age were inversely associated with eczema in the first year of life. Similar findings were reported by Gehring et al [33], who found that endotoxin exposure in the first 6 months of life was inversely related to eczema. Although these findings suggest the possibility that endotoxin alters immune development, they are not the only exposures that are unique to the farming lifestyle and may simply be a marker for increased exposure to other microbial products. For example, microbial DNA and peptidoglycan, a component of gram-positive bacterial cell walls, have also been linked to a reduction in atopy [34,35].

Pet exposure

Conventional wisdom long held that the best way to prevent allergic sensitization to domestic pets was to avoid them. Recent findings have altered the thinking regarding the use and effects of pet allergen avoidance in early childhood, because several recent studies have demonstrated that early contact with pets may protect against the development of allergic diseases. The influence

of the timing of such exposures on the developing immune system has not been clearly defined, but inverse associations with pet ownership in the first year of life have been demonstrated for allergic rhinitis and asthma [36] and allergic sensitization and atopic dermatitis [37]. Ownby et al [38] demonstrated that the presence of two or more dogs or cats in the home in the first year of life is associated with a decreased risk of atopic sensitization at 6 to 7 years of age. The fact that this association did not hold true for just one pet suggests that pet exposure may alter immune development in a dose-dependent manner. The relationship between exposure to cat and sensitization may resemble a bell-shaped curve, in which individuals with the lowest and highest exposure to cat allergen have the lowest prevalence of sensitization [39]. Another interesting observation is that the beneficial effects of exposure to high levels of cat allergen seem to be specific for the induction of tolerance to cats, whereas exposure to dogs and farm animals seems to have broad effects to reduce allergy in general. These findings suggest that there may be unique mechanisms of protection afforded by exposure to cats compared with other animals, and there is evidence that this may be related to induction of IgG rather than IgE antibodies by high-level allergen exposure [40].

Parasitic infections, bowel flora, and allergy

The gastrointestinal tract is an active immunologic organ. The huge surface area of the mucosal surface together with the exposure of this organ to large concentrations of foreign proteins and microbial components throughout life suggest that it is likely to have a major influence on early immune development and potentially the development of atopic diseases. Differences in the commensal gut flora of atopic versus nonatopic children have been demonstrated. In a prospective study of infants in Estonia and Sweden, countries with low and high prevalence of allergic diseases, respectively, Bjorksten et al [41,42] identified differences in bowel flora among atopic infants before the manifestations of atopic disease were present. Children who had atopic disease at 2 years of age had lower counts of enterococci, bifidobacteria, and *Bacteroides* and higher counts of clostridia, coliforms, and *Staphylococcus aureus* at various time points in their first year of life when compared with nonatopic children. These findings have generated interest in intervention studies involving *Lactobacillus*. This bacteria, administered orally to mothers prenatally and to their high-risk infants in their first 6 months of life, has been shown to reduce the risk of atopic dermatitis in the first 4 years of life; however, there was no association with allergic sensitization at age 4 [43]. These studies suggest that exposure to certain microbes in the gut might provide stimulation that alters the development of the immune system away from or toward an atopic phenotype.

In developing countries with a high burden of parasitic infections, the prevalence of atopic diseases is much lower than industrialized nations. For example,

infection with *Schistosoma* was found to be inversely related to sensitization to house dust mite in Gabonese schoolchildren. In addition, this parasitic infection correlated with enhanced helminth-induced IL-10 production, which in turn was inversely associated with allergic sensitization [44]. These findings suggest that the anti-inflammatory properties of IL-10 induced by helminth infection may attenuate the allergic response or promote tolerance [44]. Similar inverse relationships with reduced skin prick sensitivity have been demonstrated for other helminth infections including ascariasis, trichuriasis, and hookworm [45]. Finally, a Brazilian study found that schistosomiasis was associated with milder asthma in terms of symptoms, medication use, and abnormal spirometry; although infection did not reduce the prevalence of asthma [46].

Vaccinations and antibiotics

Because exposure to childhood infections was at the heart of the hygiene hypothesis, attention turned to the role that childhood vaccinations might play in this hypothesis. Do vaccinations prevent or attenuate childhood infections that are necessary for immune maturation? Do vaccinations enhance Th2 responses and thereby promote atopy? These questions have been extensively studied in recent years, and were the subject of a comprehensive review. There is conclusive evidence that childhood vaccinations have dramatically reduced, and in some cases eradicated, a number of childhood infectious diseases associated with significant morbidity and mortality. In addition, there is no evidence that vaccines in common use today increase the risk of atopy or allergic diseases [47].

Another cornerstone in the management of infectious disease in childhood is the use of antibiotics, and the types of antibiotics have continuously changed and usage has undoubtedly increased over time. These trends may be most pronounced in Western countries with high rates of allergy and asthma. A number of studies have demonstrated a positive relationship between antibiotic use in the first few years of life and an increased risk of asthma, hay fever, and atopic dermatitis [8,48–50]. A recent study of an historical birth cohort from the West Midlands General Practice Research Database demonstrated a dose-dependent relationship between antibiotic use and the risk of developing asthma, eczema, and hay fever; however, after adjusting for physician consulting behavior, only the increased risk of asthma remained significant [9]. The authors argue that this could be explained by the fact that children with asthma might receive more antibiotics because they are likely to have more respiratory infections. This relationship was also tested in a prospective study of a birth cohort of children with atopic parents. In this study, antibiotic use in the first year of life did not increase the risk of atopic disorders at the age of 5 [51]. The question of antibiotic use and the development of allergic diseases remains controversial.

Mechanisms

Epidemiologic studies relating exposure to other children, infectious diseases, and microbial products led to the concept that suggested that environmentally induced alterations in immune development acted to reduce rates of allergic diseases. Over the past 15 years, there has been considerable progress in understanding developmental changes in the immune system, and in particular, two important new concepts have evolved with major implications related to the hygiene hypothesis. The first is that innate immune responses to certain microbial products can greatly affect the adaptive immune responses. Secondly, a subset of T cells, termed “T regulatory cells,” play an important role in the control of inflammatory responses, and potentially the development of tolerance versus allergy after exposure to a foreign protein.

Innate and adaptive immune responses

Over the past 15 years, a family of receptors that initiate immune responses to microbial products has been identified in mammalian cells. These receptors, named for their structural similarity to toll receptors in fruit flies, and related proteins constitute an essential component of the innate immune system [52]. When activated, these pathways influence and direct the development of adaptive immune responses to pathogens and to allergens. Many common bacteria, fungi, viruses, and protozoans have repetitive structural motifs known as “pathogen-associated molecular patterns,” which bind to specific toll-like receptors ([TLRs] Table 2). For example, LPS binds primarily to TLR4, which forms complexes with CD14 and a variety of intracellular signaling molecules. The net result of LPS binding to TLR4 on a variety of cells is the generation of cytokines, including IL-10, IL-12, and IFN- γ . These cytokines have the potential to initiate immune responses to microbes, and in the context of allergy, these types of responses may inhibit the process of allergic sensitization. Paradoxically, LPS and other innate stimuli can amplify allergen-induced effects on Th2 cytokines if sensitization has already occurred.

T regulatory cells and allergen tolerance

There is convincing evidence that allergy and asthma in childhood are associated with immune dysregulation leading to the overproduction of IgE; eosinophilic inflammation; and ultimately, airway dysfunction and wheezing. The concept that allergic diseases and asthma are exclusively caused by Th2 skewing is incomplete, however, and a new paradigm for the regulation of inflammatory responses has been proposed. There is experimental evidence, primarily in animal models but also in a few human studies, that specific subsets of T and NKT cells seem to regulate inflammation through the production of anti-inflammatory cytokines (IL-10, transforming-growth factor- β) [53]. Although information regarding the development of these cells in humans is just begin-

Table 2
Toll-like receptor ligands

TLR	Ligands	Organism or origin
TLR 1	Triacyl lipopeptides	Bacteria, mycobacteria
	Soluble factors	<i>Neisseria meningitides</i>
TLR 2	Atypical lipopolysaccharide	Leptospira or porphyromonas
	Lipoproteins or lipopeptides	Multiple pathogens
	Peptidoglycan	Gram-positive bacteria
	Lipoarabinomannan	Mycobacteria
	Mannosylated phosphatidylinositol	Mycobacteria
	Glycolipids	Treponema
	Glycophosphatidylinositol anchors	Trypanosomes
	Lipoteichoic acid	Gram-positive bacteria
	Porins	Neisseria
	Heat shock proteins	Host
	Zymosan	Fungi
	A phenol-soluble modulin	<i>Staphylococcus epidermidis</i>
	Yeast cell walls	Yeast
TLR 3	Double-stranded RNA	Viruses
TLR 4	Lipopolysaccharide	Gram-negative bacteria
	Taxol	Plants
	Fusion protein	RSV
	Lipoteichoic acid	
	Fungal components	<i>Aspergillus fumigatus</i> , <i>Cryptococcus neoformans</i>
	Heat shock proteins	Chlamydia, host
	Oligosaccharides of hyaluronic acid	Host
	Fibrinogen or fibronectin	Host
	Polysaccharide fragments of heparan sulfate	Host
TLR 5	Flagellin	Bacteria
TLR 6	Zymosan	
	Mycoplasmal macrophage-activating lipopeptide 2	Mycoplasma
	Lipoproteins	<i>M tuberculosis</i> , <i>Borrelia burgdorferi</i> , <i>Staphylococcus epidermidis</i>
TLR 7	Diacyl lipopeptides	Mycoplasma
	Viral RNA	Viruses
TLR 8	Imidazoquinoline R848	
TLR 9	CpG-DNA	Bacteria, protozoans
TLR 10	Under investigation	

Abbreviations: RSV, respiratory syncytial virus; TLR, toll-like receptor.

Adapted from Takeda K, Kaisho T, Akira S. Toll-like receptors [review]. *Annu Rev Immunol* 2003; 21:335–76; Dalpke A, Heeg K. Signal integration following toll-like receptor triggering. *Crit Rev Immunol* 2002;22:217–50.

ning to accumulate, it seems as if T_R cells are functionally immature and continue to differentiate during early infancy. Collectively, these findings suggest that immature (reduced) T_R cell function during infancy is associated with an increased risk for recurrent wheezing, allergies, and asthma. Of interest relevant to the hygiene hypothesis is that pathogen-associated molecular patterns can

activate specific subsets of dendritic cells that can drive the development of T_R cells [53]. This observation has important implications for respiratory allergy, as has been demonstrated by Akbari et al [54] in a murine model of respiratory allergy, in which dendritic cells were shown to influence the development of T_R cells in mice. After allergen challenge, pulmonary dendritic cells secreted IL-10, and adoptive transfer of these cells stimulated the development of $CD4^+$ T_R cells that also produced high levels of IL-10 [54]. Furthermore, dendritic cells from IL-10(+/+), but not IL-10(−/−), mice induced antigen-specific tolerance in the recipient mice. These results suggest the possibility that stimuli, such as the microbial product LPS, which induces dendritic cell IL-10 production, could promote the development of specific T_R cells on exposure to allergens. There are currently a number of clinical studies to evaluate the relationship between microbial exposure, the development of T_R cells, and the incidence of atopic disease in children.

Genetics

Recent studies have shown that an individual's response to environmental and microbial exposures is partially dependent on genetic polymorphisms that alter immune responses to environmental stimuli. Interestingly, many of the genetic factors linked to allergic outcomes are genes involved in responses to LPS (Fig. 2) and other microbial products. For example, Baldini et al [55] identified polymorphisms in the gene for CD14, a high-affinity receptor for LPS, in subjects enrolled in the Tucson Children's Respiratory Study prospective birth cohort. A C-to-T transition in the promoter region of the gene (CD14/-159) was identified, and TT homozygotes had higher levels of soluble CD14 (sCD14) than the CC or CT genotypes. This polymorphism is associated with specific alterations in adaptive immune responses. For example, sCD14 levels positively correlated with IFN- γ production and negatively correlated with IL-4 production by PBMCs stimulated with concanavalin A and phorbol myristate. In addition, the TT genotype was associated with lower levels of serum IgE in skin prick-positive children than the CC or CT genotypes [55]. In addition, Koppelman et al [56] demonstrated an increase in hay fever, allergic rhinitis symptoms, positive skin tests, and serum IgE in adults with the CD14/-159 CC genotype versus the CT and TT genotypes. Finally, in the COAST prospective birth cohort study, children with the CD14/-159TT genotype were less likely to develop atopic dermatitis in infancy if there was a dog in the home [37]. These findings strongly implicate a mechanism involving CD14 in determining the risk for atopic disease.

Three polymorphisms in caspase recruitment domain-containing protein 15 (CARD15), an intracellular LPS receptor that promotes apoptosis, have been associated with increased risk of allergic rhinitis, atopic dermatitis, or allergic sensitization; however, no associations have been made between these polymorphisms and asthma [57]. This protein is of great interest because it has been identified as an intracellular receptor for some forms of endotoxin. Finally,

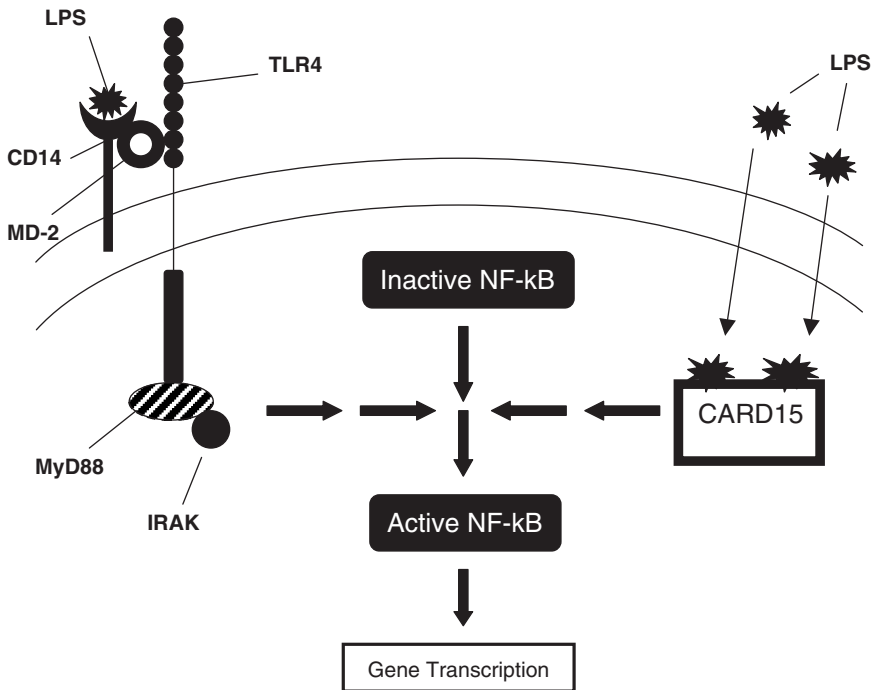


Fig. 2. The role of CD14, TLR4, and CARD15 in the innate immune response to LPS.

polymorphisms in the gene for TLR4, an endotoxin receptor expressed on the surface of cells, have been associated with a decreased response to LPS stimulation in humans [58]. The Asp299Gly TLR4 polymorphism is associated with reduced production of IL-12 and IL-10 by LPS-stimulated PBMCs and a fourfold increase in the risk of asthma in school-age children [59].

Summary and clinical implications

The hygiene hypothesis originated from epidemiologic observations relating exposure to other children to a reduced risk of allergy. Knowledge regarding other environmental factors associated with this protection has blossomed, and has led to a number of testable hypotheses related to mechanisms associated with modification of personal risk for allergy and asthma. Concurrent with this activity has been an expansion in knowledge about innate immune responses, T regulatory mechanisms, immune development in early childhood, and the genetics that affect each of these processes. Studies are now in progress in a number of laboratories worldwide to incorporate these new advances in immunology into models linking environmental exposures to the induction of tolerance. Although treatments based on these principles have not yet reached the community al-

lergic, as this work progresses one can look forward to new strategies for the treatment and prevention of allergies and asthma.

References

- [1] Strachan DP. Hay-fever, hygiene, and household size. *BMJ* 1989;299:1259–60.
- [2] von Mutius E, Martinez FD, Fritzsche C, et al. Skin test reactivity and number of siblings. *BMJ* 1994;308:692–5.
- [3] Nystad W, Skrandal A, Magnus P. Day care attendance, recurrent respiratory tract infections and asthma. *Int J Epidemiol* 1999;28:882–7.
- [4] Marbury MC, Maldonado G, Waller L. Lower respiratory illness, recurrent wheezing, and day care attendance. *Am J Respir Crit Care Med* 1997;155:156–61.
- [5] Kramer U, Heinrich J, Wjst M, et al. Age of entry to day nursery and allergy in later childhood. *Lancet* 1999;353:450–4.
- [6] Ball TM, Castro-Rodriguez JA, Griffith KA, et al. Siblings, day-care attendance, and the risk of asthma and wheezing during childhood. *N Engl J Med* 2000;343:538–43.
- [7] Celedon JC, Wright RJ, Litonjua AA, et al. Day care attendance in early life, maternal history of asthma, and asthma at the age of 6 years. *Am J Respir Crit Care Med* 2003;167:1239–43.
- [8] Farooqi IS, Hopkin JM. Early childhood infection and atopic disorder. *Thorax* 1998;53:927–32.
- [9] McKeever TM, Lewis SA, Smith C, et al. Early exposure to infections and antibiotics and the incidence of allergic disease: a birth cohort study with the West Midlands General Practice Research Database. *J Allergy Clin Immunol* 2002;109:43–50.
- [10] Bodner C, Godden D, Seaton A. Family size, childhood infections and atopic diseases. *Thorax* 1998;53:28–32.
- [11] Shirakawa T, Enomoto T, Shimazu S, et al. The inverse association between tuberculin responses and atopic disorder. *Science* 1997;275:77–9.
- [12] Shaheen SO, Aaby P, Hall AJ, et al. Measles and atopy in Guinea-Bissau. *Lancet* 1996;347:1792–6.
- [13] Paunio M, Heinonen OP, Virtanen M, et al. Measles history and atopic diseases: a population-based cross-sectional study. *JAMA* 2000;283:343–6.
- [14] Matricardi PM, Rosmini F, Ferrigno L, et al. Cross sectional retrospective study of prevalence of atopy among Italian military students with antibodies against hepatitis A virus. *BMJ* 1997;314:999–1003.
- [15] Matricardi PM, Rosmini F, Riondino S, et al. Exposure to foodborne and orofecal microbes versus airborne viruses in relation to atopy and allergic asthma: epidemiological study. *BMJ* 2000;320:412–7.
- [16] Matricardi PM, Rosmini F, Panetta V, et al. Hay fever and asthma in relation to markers of infection in the United States. *J Allergy Clin Immunol* 2002;110:381–7.
- [17] McIntire JJ, Umetsu SE, Macaubas C, et al. Immunology: hepatitis A virus link to atopic disease. *Nature* 2003;425:576.
- [18] Kosunen TU, Hook-Nikanne J, Salomaa A, et al. Increase of allergen-specific immunoglobulin E antibodies from 1973 to 1994 in a Finnish population and a possible relationship to *Helicobacter pylori* infections. *Clin Exp Allergy* 2002;32:373–8.
- [19] Illi S, von Mutius E, Lau S, et al. Early childhood infectious diseases and the development of asthma up to school age: a birth cohort study [see comment]. *BMJ* 2001;322:390–5.
- [20] Stein RT, Sherrill D, Morgan WJ, et al. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. *Lancet* 1999;354:541–5.
- [21] Sigurs N, Bjarnason R, Sigurbergsson F, et al. Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. *Am J Respir Crit Care Med* 2000;161:1501–7.
- [22] Sigurs N, Gustafsson PM, Bjarnason R, et al. Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. *Am J Respir Crit Care Med* 2005;171:137–41.

- [23] Riedler J, Eder W, Oberfeld G, et al. Austrian children living on a farm have less hay fever, asthma and allergic sensitization. *Clin Exp Allergy* 2000;30:194–200.
- [24] Braun-Fahrlander C, Gassner M, Grize L, et al. Prevalence of hay fever and allergic sensitization in farmer's children and their peers living in the same rural community. *Clin Exp Allergy* 1999;29:28–34.
- [25] Adler A, Tager I, Quintero DR. Decreased prevalence of asthma among farm-reared children compared with those who are rural but not farm-reared. *J Allergy Clin Immunol* 2005;115:67–73.
- [26] 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;164:1829–34.
- [27] von Mutius E. Environmental factors influencing the development and progression of pediatric asthma. *J Allergy Clin Immunol* 2002;109(6 Suppl):S525–32.
- [28] von Mutius E, Braun-Fahrlander C, Schierl R, et al. Exposure to endotoxin or other bacterial components might protect against the development of atopy. *Clin Exp Allergy* 2000;30:1230–4.
- [29] Gehring U, Bischof W, Fahlbusch B, et al. House dust endotoxin and allergic sensitization in children. *Am J Respir Crit Care Med* 2002;166:939–44.
- [30] Gereda JE, Leung DYM, Thatayatikom A, et al. Relation between house-dust endotoxin exposure, type 1 T cell development, and allergen sensitisation in infants at high risk of asthma. *Lancet* 2000;355:1680–3.
- [31] Braun-Fahrlander 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;347:869–77.
- [32] Phipatanakul W, Celedon JC, Raby BA, et al. Endotoxin exposure and eczema in the first year of life. *Pediatrics* 2004;114:13–8.
- [33] 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;108:847–54.
- [34] Roy SR, Schiltz AM, Marotta A, et al. Bacterial DNA in house and farm barn dust. *J Allergy Clin Immunol* 2003;112:571–8.
- [35] 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;113:860–7.
- [36] Hesselmar B, Aberg N, Aberg B, et al. Does early exposure to cat or dog protect against later allergy development? *Clin Exp Allergy* 1999;29:611–7.
- [37] Gern JE, Reardon CL, Hoffman S, et al. Effects of dog ownership and genotype on immune development and atopy in infancy. *J Allergy Clin Immunol* 2004;113:307–14.
- [38] 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;288:963–72.
- [39] Custovic A, Hallam CL, Simpson BM, et al. Decreased prevalence of sensitization to cats with high exposure to cat allergen. *J Allergy Clin Immunol* 2001;108:537–9.
- [40] Platts-Mills TAE, Vaughan JW, Blumenthal K, et al. Serum IgG and IgG4 antibodies to Fel d 1 among children exposed to 20 microg Fel d 1 at home: relevance of a nonallergic modified Th2 response. *Int Arch Allergy Immunol* 2001;124:126–9.
- [41] Bjorksten B, Sepp E, Julge K, et al. Allergy development and the intestinal microflora during the first year of life. *J Allergy Clin Immunol* 2001;108:516–20.
- [42] Bjorksten B, Naaber P, Sepp E, et al. The intestinal microflora in allergic Estonian and Swedish 2-year-old children. *Clin Exp Allergy* 1999;29:342–6.
- [43] Kalliomaki M, Salminen S, Arvilommi H, et al. Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* 2001;357:1076–9.
- [44] van den Biggelaar AHJ, van Ree R, Rodrigues LC, et al. Decreased atopy in children infected with *Schistosoma haematobium*: a role for parasite-induced interleukin-10. *Lancet* 2000;356:1723–7.
- [45] Cooper PJ, Chico ME, Rodrigues LC, et al. Reduced risk of atopy among school-age children infected with geohelminth parasites in a rural area of the tropics. *J Allergy Clin Immunol* 2003;111:995–1000.

- [46] Medeiros M, Figueiredo JP, Almeida MC, et al. Schistosoma mansoni infection is associated with a reduced course of asthma. *J Allergy Clin Immunol* 2003;111:947–51.
- [47] Koppen S, de Groot R, Neijens HJ, et al. No epidemiological evidence for infant vaccinations to cause allergic disease. *Vaccine* 2004;22:3375–85.
- [48] Wickens K, Pearce N, Crane J, et al. Antibiotic use in early childhood and the development of asthma. *Clin Exp Allergy* 1999;29:766–71.
- [49] von Mutius E, Illi S, Hirsch T, et al. Frequency of infections and risk of asthma, atopy and airway hyperresponsiveness in children. *Eur Respir J* 1999;14:4–11.
- [50] Droste JH, Wieringa MH, Weyler JJ, et al. Does the use of antibiotics in early childhood increase the risk of asthma and allergic disease? *Clin Exp Allergy* 2000;30:1547–53.
- [51] Celedon JC, Litonjua AA, Ryan L, et al. Lack of association between antibiotic use in the first year of life and asthma, allergic rhinitis, or eczema at age 5 years. *Am J Respir Crit Care Med* 2002;166:72–5.
- [52] Takeda K, Kaisho T, Akira S. Toll-like receptors [review]. *Annu Rev Immunol* 2003;21:335–76.
- [53] Mills KH. Regulatory T cells: friend or foe in immunity to infection? *Nat Rev Immunol* 2004;4:841–55.
- [54] Akbari O, DeKruyff RH, Umetsu DT. Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure to antigen. *Nat Immunol* 2001;2:725–31.
- [55] Baldini M, Lohman IC, Halonen M, et al. A polymorphism* in the 5' flanking region of the CD14 gene is associated with circulating soluble CD14 levels and with total serum immunoglobulin E. *Am J Respir Cell Mol Biol* 1999;20:976–83.
- [56] Koppelman GH, Reijmerink NE, Stine OC, et al. Association of a promoter polymorphism of the CD14 gene and atopy. *Am J Respir Crit Care Med* 2001;163:965–9.
- [57] Kabesch M, Peters W, Carr D, et al. Association between polymorphisms in caspase recruitment domain containing protein 15 and allergy in two German populations. *J Allergy Clin Immunol* 2003;111:813–7.
- [58] Arbour NC, Lorenz E, Schutte BC, et al. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet* 2000;25:187–91.
- [59] Bottcher MF, Hmani-Aifa M, Lindstrom A, et al. A TLR4 polymorphism is associated with asthma and reduced lipopolysaccharide-induced interleukin-12(p70) responses in Swedish children. *J Allergy Clin Immunol* 2004;114:561–7.