Why Old McDonald had a farm but no allergies: genes, environments, and the hygiene hypothesis

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ALLERGIES, A MAJOR URBAN MALICE

Asthma, atopic dermatitis, and atopic rhinoconjunctivitis are three distinct conditions, which are characterized by the production of specific immunoglobulin E (IgE) against common, ubiquitous allergens such as house dust mites, pollen, or animal dander. Once sensitized, re-exposure to allergen induces an inflammatory reaction of the affected organ: the bronchial system, the nasal and ocular mucosa, or the skin, respectively. At present, atopic diseases are the most common chronic illnesses in children living in the industrialized world.

In general, asthma and atopy rates are higher in affluent, Western countries with a high degree of industrialization than in developing countries with a large, rural population. In a worldwide study in approximately 500,000 school-aged children from 56 countries, the highest prevalence of asthma was found in the United Kingdom, New Zealand, and Australia, and most developing countries had comparatively low prevalence rates.

Within European countries, a West–East gradient in the prevalence of childhood asthma is present, which became apparent in studies shortly after the fall of the communist East. In the Baltic area of Northern Europe, Swedish children had a higher prevalence of atopic sensitization and asthma than did those from Poland and Estonia [1, 2]. Likewise, the prevalence of asthma, allergic rhinitis, and atopic sensitization was significantly higher in West Germany than in the East [3], a genetically homogeneous study population that had lived under very different economic and environmental circumstances for 40 years. In the years since unification, however, atopic sensitization as well as atopic rhinitis have increased significantly in East German children, almost to levels found in Western Germany, and levels of asthma were still low [4]. As most children in the communist system grew up in day-care centers from an early age, an intriguing explanation for these findings was that the early exposures to infections and microbial matter in day-care centers may have influenced the susceptibility to atopic diseases.

THE BRIGHT SIDE OF FARMING LIFE

An inverse association between infections and the development of asthma was observed in a number of Eastern European countries [2, 5], and a study from Southern Italy showed that military recruits who were sero-positive for hepatitis A, *Toxoplasma gondii*, or *Helicobacter pylori* had a significantly lower prevalence of atopic sensitization to common allergens and a lower prevalence of asthma than their peers who did not have antibodies against these pathogens [6]. A dose-response relationship was observed: The more oro-fecal infections these recruits had suffered, the lower the prevalence of asthma. Thus, it seemed as if priming of the immune system by exposure to pathogens kept it from developing allergies.

However, exposure to microbes occurs in most cases without signs of infection. Viable and nonviable parts of microorganisms are found in varying concentrations almost everywhere. These microbial substances are recognized by the innate-immune system inducing immunological responses in the absence of overt infections. Subtle and persistent environmental exposure to microbial products may be crucial during the maturation of a child's immune response, causing the early development of tolerance to naturally occurring components of the environment such as pollen and animal dander.

Growing up on a traditional farm seems to confer such protection against the development of asthma and atopy [7–9]. However, this protective effect is only found in children growing up in farming families, not in those living in rural communities without contact with farming. Living conditions on farms differ in many respects from those of other families: more pets, larger family size, heating with wood and coal, less maternal smoking, more dampness, and different dietary habits. However, none of these factors could adequately explain the strong inverse association among atopy, asthma, and growing up on a farm. Therefore, it was suggested that the exposure to certain immune-modulating factors specific for farm life may prevent the occurrence of these conditions. Frequent contact with livestock seems to be associated with the protective effect of farm life (**Fig. 1**). A dose-response relationship between exposure to farm animals and the prevalence of atopic disease was reported among farmers' children in Bavaria [8]. This protective effect was not limited to children growing up on a farm, but frequent contact with farm animals by children who did not live on a farm was also reported to confer significant protection against the development of atopic sensitization [10].

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Received October 9, 2003; revised December 1, 2003; accepted December 2, 2003; doi: 10.1189/jlb.1003468.

Fig. 1. Gene environment interaction at work in farming families. Children exposed to stable environment from early on are protected against the development of asthma and allergy. Copyright 2004 Peter Mosimann. Used with permission.

THE COMPLEXITIES OF ENVIRONMENTAL EXPOSURES

A potential explanation for this protective effect of farming may be the exposure to bacterial products of animal origin such as endotoxin and lipopolysaccharide (LPS) in stables and farmhouses [9]. However, in the development of atopic diseases, the role of LPS is considered controversial [11]. Protection against, as well as enhancement of, atopic sensitization has been observed [12]. The effect may depend on the timing of exposure in relation to allergen sensitization and immunological age of the individual. Animal models have shown that LPS administration before allergen exposure was protective against sensitization, and exposure to LPS after sensitization caused exacerbation of the allergic T helper cell type 2 (Th2) response. Even more intriguing, exposure of mice to inhalation of LPS-free and highly purified ovalbumin did not result in allergic sensitization [13]. Innate-immune pathways also seem to be involved in the modulation of allergic responses, as the lack of Toll-like receptor 4 (TLR4) in knock-out mice results in an inability to sensitize animals to allergens [14]. Thus, low levels of inhaled LPS and intact signaling through TLR4 are necessary to mount Th2 responses to inhaled antigens in a mouse model of allergic sensitization. Conversely, stimulation of the innate-immune system before allergic sensitization seems to diminish the allergic response and may be one possible explanation for how the protective effect of exposure to farming life is conferred at the molecular level.

INNATE GOES ADAPTIVE: TWO SIDES OF STORY

When microbial compounds such as LPS come in contact with the human body, they are first recognized by the constitutively expressed receptors of the innate-immune system. The innate-immune system is an evolutionarily old, conserved defense system [15, 16]. Engagement of its receptors elicits an inflammatory response and production of antimicrobial substances. Molecules specifically expressed by pathogens such as LPS have been termed pathogen-associated molecular patterns (PAMP) [17]. The cellular receptors of the innate-immune system recognizing these PAMP are termed pattern recognition receptors (PRR). Examples of PRR are CD14 [18] or the members of the family of TLRs [19, 20]. For TLRs, a variety of different ligands has been identified, which specifically activate shared downstream signaling pathways. Ligands for TLRs include bacterial components such as peptidoglycans (TLR2), LPS (TLR4), unmethylated CpG motifs of bacterial origin (TLR9), as well as double-stranded, viral DNA (TLR3). A complex intracellular signal-transduction cascade consisting of different, partially redundant pathways leads to the activation of downstream effector mechanisms aimed at amplifying immunological host defense (for a review, see ref. [20]).

In addition to cell-surface receptors, intracellular PAMP receptors such as the nucleotide-binding oligomerization domain (NOD)1/apoptosis-activating factor-1 (APAF1) gene family have also been discovered (renamed CARD for Caspase

recruitment domain). CARD15 molecules, which are primarily expressed in monocytes, activate nuclear factor-κB (NF-κB) on stimulation with LPS, thereby initiating apoptosis and other immunoregulatory effects. It is intriguing that it has been shown that LPS from different bacterial sources has different effects on CARD15-dependent NF-κB activation [21].

The innate-immune system and the adaptive-immune system do not act independently of each other; rather, they are intertwined in many ways [22]. Activation of an innate-immune response is a major prerequisite for activation of an adaptive-immune response [23], and TLRs provide critical links between innate and adaptive immunity [24, 25].

GENE ENVIRONMENT CROSS-TALK

The struggle of the human organism with microbial matter provides a thriving impetus for evolution. Similarities have been detected between receptor molecules for PAMP, even reaching as far back as between plants and mammals. Thus, one could argue that this defense system is one of the greatest success stories in the history of eukaryotic evolution. Its components have been shaped and evolved by millions of years of exposure to a microbial-dominated environment. Thus, the innate-immune system at its present state could be regarded as a gene environment interaction in progress, in which the immune system is trying to adapt to current challenges of life. Comparing its function in farm and nonfarm environments allows identification of how recent changes in lifestyle have affected the immune system and how these changes may have contributed to the increasing incidence of immune deviations in recent decades such as allergies and autoimmune diseases.

Farm populations may provide a very good human model for studying the effects of extensive microbial exposure on the innate- and adaptive-immune systems. In contrast to animal studies, in this human model, the microbial exposure, which cannot be controlled as in laboratory conditions, has to be monitored thoroughly to assess the effect of exposure on immune interactions. Thus, LPS was measured as a marker for microbial exposure in mattresses of farmhouses. This revealed an inverse correlation between the amount of LPS exposure in the home of farmers' children and the prevalence of hay fever, atopic sensitization, and atopic asthma [9]. It seems that relatively low levels of LPS exposure at an early age have a profound, immunological effect protecting against atopic diseases.

In analogy with animal study data, it has been speculated that in a setting of high exposure to microbial matter, effects on the adaptive-immune system, such as enhancement of Th1-like responses by microbial compounds or modifications of Th2-like responses, are mediated by the activation of the innate-immune system. However, the Th1–Th2 paradigm may not be sufficient to explain the protective effect of growing up on a farm: The production of Th1 and Th2 leukocyte-derived cytokines [tumor necrosis factor α, interferon-γ, interleukin (IL)-10, and IL-12] in the serum of these children was inversely related to the amount of exposure to LPS [9]. In vitro studies have shown that exposure of cells to LPS leads to an up-regulation of the innate-immune receptors CD14 and TLR2. A comparison of the basal, nonstimulated mRNA expression of CD14, TLR2, and TLR4 genes in peripheral blood mononuclear cells from children of farmers exposed to high levels of microbial substances to samples from children of nonfarmers was analyzed, and it was found that the expression of CD14 as well as TLR2 was elevated, and no difference was observed in TLR4 expression [26]. These findings were confirmed in part in another farm population from Southern Germany (unpublished). However, it is not clear at this point if timing or a combination of different stimuli is important to confer the protective effects of growing up on a farm.

Although environmental factors play an important role in the development of allergies, the genetic makeup of an individual also contributes significantly to the capability of the host to interact with the environment. Human genes coding for components of the innate-immune response against microbial matter play a key role in the interaction with the environment. Mutations and polymorphisms in these genes may result in profound, functional changes and lead to the development of various diseases. Ultimately, the interaction between environmental exposure and genetic makeup determines the potential repertoire of host responses on an individual level. Previous studies have indicated that single nucleotide polymorphisms (SNPs) in innate-immune genes such as the CD14 receptor [27, 28], TLRs [29], and intracellular LPS receptors may be associated with the development and severity of atopic diseases and airway hyper-reactivity [30].

In CD14, a promoter polymorphism was identified, which leads to a 20% increase in CD14 gene expression after endotoxin stimulation [31]. The increased CD14 expression was seen on the level of mRNA as well as soluble CD14 [27]. An inverse association among the CD14 polymorphisms, soluble CD14, and decreased serum IgE levels was observed in some populations but not in others [28, 32]. It was proposed that environmental differences may have contributed to the discrepant results [33]. An "endotoxin switch" has been suggested by Vercelli [33], where the CD14 promoter polymorphism changes the threshold at which environmental endotoxin stimulation leads to a Th2-immune response. According to this hypothesis, the CD14 promoter polymorphism would not influence the development of atopy at very low or very high concentrations (farmers' populations) of environmental endotoxin. However, the translation of endotoxin stimulation into an IgE-related response may be complex and may involve regulation through timing of stimulation, molecular interaction with other immune pathways, and external signals apart from endotoxin. Thus, the relevance of the CD14 promoter polymorphisms may depend on the presence or absence of other, so far unknown, exposures or genetic cofactors present in different populations.

For TLR4, it has been shown that two common, cosegregating, missense mutations in the extracellular domain of the receptor confer hyporesponsiveness to inhaled LPS [29]. In a population of adults in which exposure levels to LPS were measured, these polymorphisms were associated with a modified response to endotoxin. In a population-based study, the presence of high endotoxin levels measured in the homes of study subjects correlated with more asthma in individuals without TLR4 polymorphisms. Carriers of TLR4 polymor-

phisms, conversely, tended to have a lower risk to develop asthma [34].

Functional polymorphisms have also been identified in the intracellular LPS receptor family NOD1/APAF1. Recent reports have shown that three SNPs in the CARD15 gene, which impair the ability to mount a NF-κB response to LPS, were not only associated with the development of the inflammatory bowl disease Morbus Crohn [21] but also with the development of atopic diseases [30]. It is tempting to hypothesize that CARD15 polymorphisms influence the development of atopic diseases by changes in the capability to mount an adequate NF-κB response following bacterial challenges. It has also been shown that CARD15 enhances apoptosis through caspase-9 [35]. In this context, it is important that mechanisms involved in the regulation of the survival and apoptosis of inflammatory cells may play an important role in the persistent inflammatory process characterizing asthma and atopy. The shared genetic background between atopy and Crohn's disease supports the notion that malfunctional recognition of microbial components contributes to an excessive response of both types of immune responses. The prevalence of atopic and autoimmune diseases such as Crohn's disease has increased concomitantly over the past several decades in Western societies [36]. This may challenge the paradigm of a dichotomy, contrasting allergic illnesses as the prototype of a Th2-like immune response to autoimmune disorders such as Crohn's disease as the antagonistic, Th1-driven disease.

It may be speculated that the impairment of recognition of microbial compounds as a result of polymorphisms reduces the general capability of the innate-immune system to interact with bacterial matter early in life and to develop a robust regulatory T cell (Tr) reservoir [37]. Repeated, early stimulation of the Tr cell system as well as innate-immunity feedback loops may suppress unwanted and exaggerated responses against pathogens. If such early stimulation is absent, or this interaction is impaired by genetic predisposition, autoimmune as well as atopic diseases may emerge [37]. Thereby, the protective effect against the development of atopic diseases that is conferred by early contact with microbial matter may be diminished [7].

Although previous studies have focused on the assessment of the effects of environmental exposure or genetic changes within innate-immunity genes, future studies have to address interactions between genes and environment. The initial results from genetic studies in farming and nonfarming populations indicate that genetic variations in innate-immune genes such as CD14 or CARD15 may have different effects depending on environmental conditions: Polymorphisms, which lower the potential to mount a strong immune response when encountering pathogens, may be advantageous in a setting of high bacterial exposure by avoiding a persistent, excessive activation of the immune system. However, in a setting of low levels of microbial exposure, carriers of the same polymorphisms may not be able to react adequately to microbial challenges and consequently, will develop a predominant Th2-like-immune reaction.

Future studies investigating gene environment interactions have to be well designed. Ideally, a large birth cohort of farmers and nonfarmers would be required, in which prenatal and early exposures to microbial matter during early childhood are measured, and genetic data, gene expression patterns over time, and disease outcomes are assessed. Thus, genetic as well as timing effects could be addressed, and functional pathways involved in protection against the development of allergies could be more definitively identified.

BACK TO THE ROOTS: WHAT WE CAN LEARN FROM OLD McDONALD

Farm studies have shown that environmental exposure to microbial matter is protective against the development of atopic diseases, confirming previous observations that early infections are associated with a decreased prevalence of atopy. These effects seem to be conferred by an increased activation of innate-immunity pathways, as indicated by an increased expression of PAMP receptors. Which of the components found in farming environments is responsible for the activation of the innate-immune system facilitating protection is not yet clear. Timing of exposure and interaction with allergens are critical for disease development. Furthermore, the ability to mount an adequate immune response through innate-immune pathways may be altered by genetic variations in immunoregulatory genes. To dissect the role of the innate-immune system in the development of atopic diseases will be a challenging endeavor of the near future. In the end, early intervention strategies to prevent allergies may be achieved on the basis of immune modulation. To reach this goal, a combination of functional studies to dissect innate-immune pathways gene by gene and gene environment interaction studies in highly informative populations will be needed.

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