

Invited Review

The Hygiene Hypothesis of Atopic Disease—An Extended Version

Samuli Rautava, Olli Ruuskanen, Arthur Ouwehand, Seppo Salminen, and Erika Isolauri

ABSTRACT

The hygiene hypothesis of atopic disease suggests that environmental changes in the industrialized world have led to reduced microbial contact at an early age and thus resulted in the growing epidemic of atopic eczema, allergic rhinoconjunctivitis, and asthma. The epidemiological findings have been combined with the Th1/Th2 paradigm of immune responsiveness to provide a coherent theory. Recent advances in epidemiology and immunology demonstrate, however, that the hygiene hypothesis may need to be extended in three respects.

First, the importance of infections in causing immune deviance may be outweighed by other sources of microbial stimulation, perhaps most importantly by the indigenous intestinal microbiota. Second, immunomodulatory and suppressive immune responses complement the Th1/Th2 paradigm. Third, in addition to protection against atopy, protection against infectious, inflammatory, and autoimmune diseases may also depend upon healthy host-microbe interactions implicated in the hygiene hypothesis. *JPGN* 38:378–388, 2004. © 2004 Lippincott Williams & Wilkins

THE HYGIENE HYPOTHESIS

The hygiene hypothesis was first proposed by Strachan in 1989 in part to explain the increasing prevalence of atopic conditions (1). The idea originated from epidemiological observations suggesting an inverse correlation between family size and the prevalence of allergic rhinitis. This observation led to a more general hypothesis that infections in early childhood acquired from older siblings might confer protection against the development of atopic diseases such as atopic eczema, allergic rhinoconjunctivitis, and asthma. Subsequent research into the association between childhood infections and atopic sensitization or atopic disease have offered conflicting results. Indeed, our understanding of the timing, the mechanism, and the specific infections that might carry anti-allergenic potential are by no means satisfactory (2,3).

The T helper (Th)1/Th2 paradigm of adaptive immune responses (4–6) provided the initial immunological backbone for the hygiene hypothesis. On the basis of cytokine production patterns, T cell responses may be divided into counter-regulatory Th1 and Th2 subtypes. Th2 responder phenotype is associated with atopic sensitization and atopic disease. Indeed, inflammation of the Th2 type

appears to be active in the initial stage of the pathogenesis of atopic eczema (7,8), allergic rhinoconjunctivitis (9,10), and asthma (11,12).

A link between the Th1/Th2 paradigm and the risk of developing allergic disease was made upon demonstration that initial atopic sensitization may even take place in utero when a Th2 polarized immune environment is thought to prevail (2). In healthy infants, the Th2 skewed immune system of the newborn is redirected and matures during the first year of life. In infants who are to develop atopic disease, however, Th2 responsiveness is further augmented (Fig. 1) (13). Th1 type response, typically elicited against intracellular microbial pathogens, counter-balances Th2 type immunity. Hence, the hygiene hypothesis may be combined with the Th1/Th2 paradigm to argue that reduced contact with microbes and diminished burden of infectious disease at an early age lead to weakened immunological drive in the Th1 direction resulting in overactivity of Th2 responsiveness.

Accumulating evidence indicates that the Th1/Th2 paradigm, albeit a useful initial frame of reference for studying the pathogenesis of human disease, is not adequate for understanding the mechanisms involved in host immune defense and tolerance and the development of autoimmune and atopic disease. The need to revise the Th1/Th2 paradigm has become very apparent as the role of the innate immune system in determining adaptive immune responses and the complexity of T cell sub-

Address correspondence and reprint requests to Samuli Rautava, Department of Paediatrics, Turku University Central Hospital, Kiinamyllynkatu 4-8, 20520 Turku, Finland (e-mail: samrau@utu.fi).

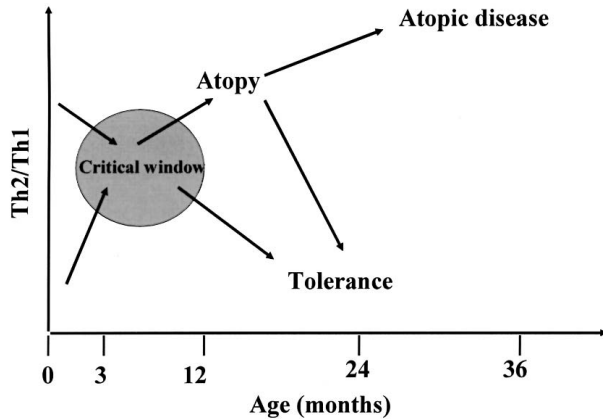


FIG. 1. Development of immune responses in healthy and atopic infants.

classes have begun to unravel. Indeed, the central role of fetal Th2 skewed immunity in protection against maternal rejection has been challenged and the role of the innate immunity has come to the fore (14). There is growing appreciation that novel regulatory T cell classes may be essential in the acquisition and maintenance of mucosal and systemic tolerance (15) and, hence in the pathogenesis of atopic disease. Consequently, as the immunological basis of the hygiene hypothesis is scrutinized, a critical review of the epidemiological support of the original hygiene hypothesis with regard to the type of infections involved and the immunological effects is needed.

CANDIDATE MICROBES AND INFECTIONS

Several microbes and infections have been suggested to be involved in causing the immune deviance proposed by the hygiene hypothesis. For instance, mycobacterial infections are potent inducers of Th1 responsiveness. It is therefore rational to assume that exposure to mycobacteria or to the BCG (*Bacillus Calmette-Guerin*) vaccination might be among the microbial stimuli that down-regulate atopic type immunity. Indeed, strong tuberculin responses in vaccinated children appear to be associated with reduced risk of sensitization and atopic disease (16). In a recent study, however, there was no evidence that BCG vaccination reduced atopic sensitization, and protection against atopic disease was limited to those at high genetic risk (17). If this hypothesis were true, one would expect to see differences in the prevalence of atopic disease between countries such as Finland, where practically all infants are vaccinated with BCG and countries such as neighboring Sweden where BCG vaccination is not common. No such data has been provided. It has recently been argued that the negative tuberculin responses seen in atopic individuals might in fact be the result of atopic type responsiveness *per se* (2). Thus, a causal relationship between vaccination and atopy is by

no means clear. Measles infection is often mentioned among the candidate infections involved in the hygiene hypothesis because of the observation linking measles infection to reduced sensitization (18). Further studies have been unable to corroborate this initial report (19–21). Indeed, one alternative explanation for the phenomenon is that infants and children with an atopic predisposition display heightened mortality from measles (22).

Common viral and bacterial infections of the upper and lower respiratory tract, although heterogeneous with regard to the type and magnitude of immune responses they elicit, have been linked to the development of asthma (23,24). On the other hand, there are studies to suggest that respiratory tract infections may be protective against the development of atopic eczema (3) and asthma (25,26). Orofacial acquired infections including hepatitis A, toxoplasmosis, and *Helicobacter pylori* have also been implicated in the hygiene hypothesis of allergy (27,28).

Paradoxically, helminthic infections, which naturally provoke a strong Th2 response (29), appear to be associated with a lower prevalence of sensitization and atopic disease (30,31). This phenomenon thus seems to contradict the Th1/Th2 immune deviance interpretation of the hygiene hypothesis. It has been suggested that chronic parasitic infection may actually result in an anti-inflammatory response mediated by transforming growth-factor beta (TGF- β) and interleukin 10 (IL-10) designed to restrict tissue damage produced by continuous antigenic challenge (32). This phenomenon closely resembles the acquisition and maintenance of tolerance to environmental allergens (15) which will be discussed below.

WHAT ARE THE RELEVANT INFECTIONS?

An important criterion for any infection proposed to reduce the risk of allergic disease is that it be prevalent in developed countries where atopic disease is prevalent. Moreover, since the first expression of the atopic immune responder type frequently occurs within the first months of life, the infection should be one that occurs early in infancy.

Knowledge about the frequency and type of infections in early childhood may be extracted from several large prospective studies (33). The most common infections of young children are viral respiratory tract infections. Most children experience between two to eight infections annually during their first 5 years of life. In the Tecumseh family study (34), which took place in the 1960s and 1970s, 1000 individuals were followed for 10 years in two phases. Information about infections was collected by weekly questionnaires, specimen collection from symptomatic patients and regular serologic screening for identification of infections. The mean incidence of respiratory illness was 6.1/year in the first year of life, after

which the frequency decreased to 3.4/year between 5 to 9 years. Episodes of infectious disease were more frequent in boys than in girls up to 3 years of age.

A large number of viruses can cause infections in young children. Many studies have shown that that rhinovirus and respiratory syncytial virus (RSV) are the most frequently detected agents causing respiratory infections and also the most important infections associated with acute otitis media (35). Using viral culture, the Tecumseh family study identified rhinoviruses as the most prevalent isolate in all age groups including those less than 5 years of age (34). Using more advanced virologic techniques, Vesa et al. (36) identified rhinoviruses in 580 of 2005 nasopharyngeal aspirates (63% of virus-positive cases) from children less than 2 years of age with acute upper respiratory tract infection. Among the remainder of positive cases, 15% were RSV, 7% were influenza A virus, 7% were parainfluenza-3 virus, and 6% were adenoviruses. RSV is the most common cause of lower respiratory tract infections including bronchiolitis and pneumonia. A family study in Houston, Texas found an infection rate of 69% during the first year of life and 83% during the second year of life (37). Recent studies using polymerase chain reaction techniques (PCR) have provided novel data. For example, enteroviruses may play a more prominent role in the etiology of upper respiratory infections than earlier assumed, with up to 25 to 35% of the children being PCR-positive for enteroviruses (38). It is of note that enterovirus infections often occur in young infants and may persist for months in the gastrointestinal tract.

In addition to respiratory infections, children experience one to two gastrointestinal infections per year. The major role of rotavirus in the etiology of childhood diarrhea is well established. Recent studies have shown that noroviruses and sappoviruses (subgroups of caliciviruses) are also common causative agents of acute gastroenteritis in children (39).

An important question in the hygiene hypothesis is whether one specific infection or a series of infections can permanently change the immune responder type and whether there is an age range during which the infection must occur to produce the change. Children experience respiratory virus infections during the first months of life making these infections good candidates as modulators of cell-mediated immunity. In a recent study in Finland, 329 children were monitored for respiratory infections from age 2 months to 2 years (36). Eighty-five percent of the children completed the follow-up. During the study, 87% of the children experienced one or more respiratory infections. 24% of the children experienced 4 to 13 episodes (median, 8 episodes), whereas the rest of the children had 0 to 8 episodes (median, 3 episodes). The median age at the first infection was 146 days in children with recurrent respiratory infections, compared to 217 days in those with less frequent episodes. This finding may be interpreted to demonstrate that predisposition to

recurrent infections becomes manifest at an early age or, conversely, that early infections have a significant impact on subsequent immune competence. The occurrence of respiratory infections peaks between 6 and 12 months. The peak incidence of RSV bronchiolitis and pneumonia occurs between age 2 and 6 months. Children who develop RSV bronchiolitis in early life are at increased risk of developing asthma-like symptoms later in life (40). Clinical and experimental studies suggest a key role of cell-mediated immunity in RSV infection (41). It is of interest that age at the first RSV infection appears to determine the outcome of reinfection in a murine model. Neonatal priming produces more severe infection (including T helper 2 responses and eosinophils) during reinfection, whereas delayed priming produces enhanced interferon- γ production and less severe disease (42). Still, the causal link between RSV and atopy remains elusive, and it is unclear whether RSV infection leads to atopy or whether infants who are to develop atopy have more severe manifestations during RSV infection (43).

Recently, rhinoviruses have also been suggested to play a role in the development of asthma (44). As in the studies of RSV, the data are less persuasive for their possible role in the development of atopy. Important evidence that viral infections indeed have a long-term impact on the development of the atopic responder phenotype was provided by the report by McIntire et al. According to this report, hepatitis A virus seropositivity protects individuals carrying a particular variant of the hepatitis A virus receptor gene from atopy (45). The same gene is expressed by T cells during the development of Th2 responses and regulates cytokine production (46) suggesting that the protective effect may be the result of direct immunomodulation upon contact with the hepatitis A virus.

These studies strongly suggest that infections do play a significant role in directing the immunological development in early life. It also appears that infections may both protect and predispose the child to atopic disease. Moreover, the impact of infections on the development of atopic responder phenotype and atopic disease appears to be age-dependent (3), and the critical period for microbes to exert their protective effect is during the first months of life.

THE HYGIENE HYPOTHESIS—NEED OF EXTENSION?

The hygiene hypothesis of allergic disease implies that recent advances in infection control and changes of lifestyle in western societies have reduced the extent of microbial exposure. If the hygiene hypothesis holds true, infectious disease in early childhood may prevent allergic disease, and with reduction in the prevalence of infection an increase in atopy ensues. However, it appears that such a development has not taken place. Instead

not only has the risk of developing allergic disease increased, but also the burden of infectious disease continues to present a challenge confronting clinicians and scientists.

In a similar manner, according to the cross-regulatory properties of Th1 and Th2 cells, one would hypothesize that together with the rising prevalence of atopic diseases there would be a decline in the prevalence of autoimmune diseases, which are considered to arise from excessive Th1 type immunity (47). This simplistic assumption has been challenged epidemiologically (48), clinically (49), and immunologically (50). Indeed, atopic diseases, including atopic eczema, allergic rhinoconjunctivitis, and asthma, together with chronic inflammatory bowel diseases and diabetes are conditions of rising importance in industrialized countries worldwide.

A unifying hypothesis may be that there is an absence of inflammation of either type in normal subjects (Fig. 2). Importantly, it has become evident that the establishment and maintenance of a disease-free state on the mucosal surfaces, and thereby also systemically, is in large part the result of active anti-inflammatory processes that counter-regulate both arms of polarized T responsiveness, the lack of which may result in Th1 mediated autoimmune disease or Th2 type atopic disease.

These anti-inflammatory, tolerogenic responses are mediated by a coordinated interaction between the innate and adaptive immune systems. Recent advances in understanding the mechanisms of oral tolerance have un-

derscored the importance of regulatory T cells in orchestrating tolerogenic responses. The nomenclature regarding these novel mucosa-derived T cell subsets, often referred to as T regulatory (Tr1) cells or Th3 cells, is not yet uniformly established (51). Current data are coherent in indicating that TGF- β and IL-10 are the most important cytokines mediating the decisive regulatory effect (15). TGF- β has been demonstrated to have a suppressive effect on both Th1 and Th2 cells (52,53), and indeed, TGF- β has been implicated in protection against Th1 type autoimmune disease (54) as well as Th2 mediated atopic disease (55). In particular, the number of TGF- β producing T cells is reduced in children with food allergy, a manifestation of a primary failure to establish oral tolerance (56). Tolerance induced in the intestinal mucosa is mediated at other sites and target organs by Th3 cells, which are distributed systemically and produce TGF- β upon reactivation (57). In addition to its immunomodulatory effects on T cell responses, TGF- β has a profound effect on B cells (58) and contributes to the maintenance of mucosal barrier function by inducing IgA antibody production (59,60).

TGF- β and IL-10 appear to function in a synergistic fashion (Fig. 2) with TGF- β favoring the production of IL-10 (61). IL-10 is considered central in promoting tolerance towards the indigenous intestinal microbiota (62). Consequently, failure to elicit appropriate IL-10 responses in the intestine may have a role in the pathogenesis of inflammatory bowel disease. In addition to the intestine, predominance of IL-10 may be considered the hallmark of the healthy respiratory tract (63).

The intimate interplay between the innate and adaptive immune systems is demonstrated by the fact that upon contact with microbial antigens, intestinal macrophages and monocytes produce TGF- β (64), which in turn may lead to the development of TGF- β -secreting T cells (57). Furthermore, TGF- β alters the function of antigen-presenting cells (65). In parallel, IL-10 has a profound impact on the Th1/Th2/Th3 driving capacity of dendritic cells (66,67) and appears to favor the development of suppressor T cell subsets (68).

These findings taken together suggest that host defense mechanisms in the gut, primed to assimilate potentially harmful challenges, have declined in the Western societies during the past decades. The gut barrier consists of chemical, physical, and immunological components—the function of which is to restrict mucosal colonization by pathogens, to prevent foreign antigens and pathogens from excessively penetrating the mucosa, and to regulate the antigen-specific immune responses (69) thereby creating the anti-inflammatory tone of the intestinal milieu. In infancy, the main components of the gut barrier are immature, and hence, the intestinal surface is relatively permeable, which may partly explain the susceptibility of infants to atopic sensitization. Ingested dietary and microbial antigens are degraded by digestive enzymes and by the gut microbiota, and it has

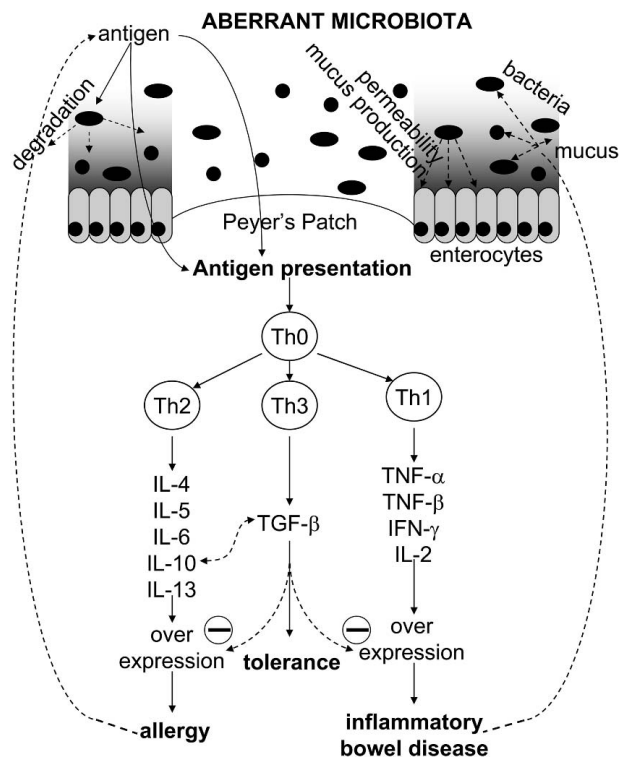


FIG. 2. The gut microbiota: the immunomodulatory potential.

been demonstrated that oral tolerance is not achieved without intraluminal degradation of antigenic structures (70). Furthermore, the intestinal microbiota have a profound impact on the development of IgA antibody responses (71), which constitute an important part of the immunological barrier involved in pathogen and allergen exclusion in the gut lumen.

On this basis a new gatekeeper hypothesis may be proposed to extend the original hygiene hypothesis of allergy and provide a better explanation of why infectious, allergic and autoimmune diseases continue to present a formidable burden in Western societies. Elements of the new hypothesis suggest that individuals in affluent societies with excellent hygienic conditions fail to mount vigorous immune and anti-inflammatory responses to antigens and allergens, a phenomenon recently characterized as “lazy immune system” (72). Furthermore, it suggests that constant stimulation of the intestinal immune system by the indigenous intestinal microbiota, the complex composition and function of which we are only beginning to grasp, may be more important to the establishment of tolerance than occasional intercurrent infections.

GUT MICROBIOTA—THE THREE ERAS OF RESEARCH

The indigenous intestinal microbiota develop over time (Fig. 3) and are defined by genetic factors, the character of the surrounding environment, diet, and disease. As a result, every individual has unique microbiota (73). Thus, the human intestinal microbiota as a defined entity does not exist, but is instead a dynamic mixture of microbes.

The establishment of the gut microbiota, a process commencing immediately after birth, provides an early and massive source of microbial stimuli, and consequently, may be a good candidate “infection” in the extended hygiene hypothesis (i.e., protection against atopic and inflammatory diseases). It is of note that the impact of the indigenous microbiota on the development of the

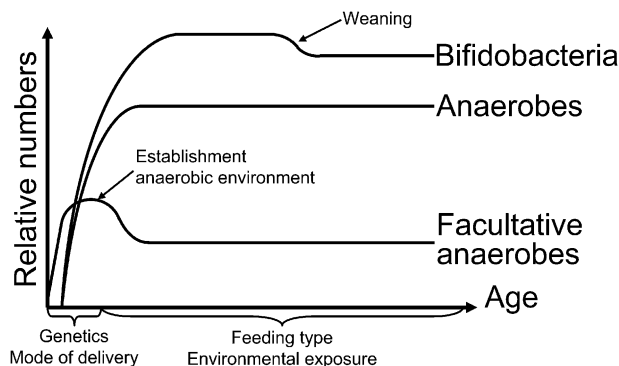


FIG. 3. The stepwise compositional development of the gut microbiota.

infant’s immune system may not be dependent on specific microbial strains, as disturbances in establishing normal gut microbiota appear to result in maturational delay (74). It may be argued that continuing stimulation by the dynamic microbiota as a whole might override the importance of any individual strain or infection.

The step-wise process of establishing indigenous microbiota begins with intestinal colonization by facultative anaerobics such as the enterobacteria, coliforms, and lactobacilli first followed quickly by bifidobacteria and lactic acid bacteria (Fig. 3). Disturbance in this succession has been linked to the risk of developing infectious, inflammatory, and allergic diseases later in life. This realization has stimulated research to elucidate the composition and function of the intestinal microbiota. These efforts have been faced with considerable methodological difficulties the gradual overcoming of which has improved our understanding stage by stage.

First Stage of Gut Microbiota Characterization: Culture Technique

Our understanding of the intestinal microbiota of the human is largely based on culture-based assessment pioneered by Japanese researchers (75,76) in which fecal microorganisms were plated on selective media designed to recover and identify the numerically important microbial groups. The methods used until now have often been only semi-selective and the identification of different microbial species and strains has been dependent on phenotypic characteristics and unique metabolic activities of the microbes. There are several bacteria, however, which are not culturable and cannot be isolated or identified by these traditional methods. Another drawback is that freshly voided fecal samples are required as gut bacteria react differently to conditions outside the gut and to storage. Moreover, the microbes in feces mainly represent the luminal microbiota of the sigmoid colon, while the composition of the intestinal microbiota differs both along the gastrointestinal tract and between the lumen and the mucosa (76). For more accurate information on the population elsewhere in the intestine, samples must be taken by endoscopy or during surgery.

Second Stage of Gut Microbiota Characterization: Molecular Biology

The second stage in the study of gut microbiota was the application of molecular biology methods. The focus was initially on more accurate identification of cultured microbes from fecal samples. The development of genetic methods such as ribotyping, pulsed field gel electrophoresis, plasmid profiles, specific primers and probes for PCR and nucleic acid hybridization, 16S rRNA sequencing and sequence comparison have improved the ability to identify these culturable components. Recent

refinements in molecular techniques have made it possible to identify and quantify the intestinal microbiota without prior culturing. Specific PCR primers and probes can be designed based on the variable regions of the 16S rRNA molecule to detect certain species and groups of bacteria. These methods have also been applied to gut mucosal and biopsy material in order to characterize the differences between mucosal and fecal microbiota and elucidate aspects of microbe-host interactions (77–80). These molecular tools have resulted in more detailed characterization of the composition and the concentration of microbes in feces and in intestinal mucosa and have uncovered a large number of previously uncharacterized genera and species accounting for more than 50% of the overall diversity of intestinal microbiota.

In a study by Kalliomäki et al., the composition of the gut microbiota was assessed with regard to the risk of atopy in infants by two culture-independent methods (81). At 3 weeks of age, the bacterial cellular fatty acid profiles assessed by gas-liquid chromatography were different in infants who later developed atopy compared to those who did not. Moreover, using quantitative fluorescence in situ hybridization, infants developing atopy harbored more clostridia and tended to have less bifidobacteria in their stools than infants not developing atopy. These observations suggest that changes in gut microbiota composition precede the development of atopy and underscore the role of the indigenous microbiota in the hygiene hypothesis. The need for more detailed characterization of the gut microbiota in understanding its role in atopic disease is suggested by the observation that bifidobacteria from the feces of infants with atopic disease induce a distinct pattern of cytokine production compared to that stimulated by the bifidobacteria isolated from healthy infants (82). Interestingly, there are data indicating that infants with atopic disease harbor an adult-like pattern of bifidobacteria, whereas in healthy infants, the predominant species is *Bifidobacterium bifidum* (83).

Third Stage of Gut Microbiota Characterization: Understanding the Function of Intestinal Microbiota

Genomic data on *Bifidobacterium longum* and *Bacteroides thetaiotaomicron*, both important members of the human intestinal microbiota, provide information on how specific bacteria are adapted to the development of the gut by specific genes which enable them to use intestinal mucins and breast milk oligosaccharides as nutrients (84). Microbial genomic research also evaluates the effects of adhesive mechanisms of specific strains at different sites of the gut mucosa (85–88). Incorporating such information with host gene expression data will enable us to understand the role of microbial transfer, succession, microbe-to-microbe and host-microbe interactions. The new information demonstrates that the vast

community of indigenous microbes colonizing the human gut shape our development and biology (84).

THE HOST-MICROBE CROSSTALK

The advances in elucidating the interaction within the intestinal microbiota and between bacteria and mucosal innate and adaptive immune systems are the basis for understanding the homeostatic, disease-free state of the host. These advances also necessitate a shift of emphasis in the hygiene hypothesis of atopic disease.

The innate immune system may be decisive in determining the type of adaptive immune responses elicited against microbial antigens (89). The innate immune system is able to distinguish microbial antigens from the myriad of environmental antigens through molecules such as Toll-like receptors (TLRs) and CD14, which recognize conserved pathogen-associated molecular patterns, including unmethylated CpG motifs characteristic of bacterial DNA and the bacterial endotoxin lipopolysaccharide (LPS) (90). Recognition of bacterial CpG motifs by TLR9 induces Th1 type immune responsiveness (91–93). LPS, a component of the outer cell membrane of gram-negative bacteria, is a powerful inducer of the innate immune system via TLR2 and TLR4 (94–96), both expressed on human intestinal epithelial cells (97). CD14 appears to function as an essential co-receptor for LPS (98). In addition to LPS, CD14 also recognizes peptidoglycan, a universal cell wall component of all bacteria (99). TLR2 stimulation by LPS leads to production of the anti-inflammatory cytokine IL-10 and Th1 inducing IL-12 by human monocytes (100). Low levels of LPS signaling through TLR4 are necessary to induce atopic Th2 responses in a murine model, whereas high levels of LPS result in Th1 responses (101,102). Systemic administration of LPS before sensitization, on the other hand, inhibits both systemic and local Th2 responses without directly enhancing Th1 responsiveness in mice (103). Interestingly, LPS has been observed to induce TGF- β production through CD14 and TLR2 in human colon cancer cell lines (64).

Microbial activation of the immune system through TLRs is mediated by induction of the transcription factor NF- κ B pathway (90,94). Non-pathogenic enteric microbes, however, can elicit an immunosuppressive effect on intestinal epithelial cells by inhibiting the same pathway (104), presumably one of the mechanisms by which tolerance to the indigenous microbiota is maintained. Interestingly, *Lactobacillus rhamnosus* ATCC 53103 (*Lactobacillus* GG), a commensal strain with probiotic properties, has been observed to activate the NF- κ B pathway in a manner similar to *Streptococcus pyogenes*, a virulent pathogen (105), indicating that some members of the intestinal microbiota provide the intestinal immune system with immunomodulatory signals resembling in some respects those from infectious agents.

It has recently been suggested that atopic disease might be the result of insufficient activation of the NF- κ B pathway required for the generation of regulatory lymphocytes characteristic of the intestinal immune system (56). The tolerogenic effects of gut microbiota may be partially mediated by generation of tolerogenic Th3 and Tr1 cells. For example, *Lactobacillus paracasei* has been reported to inhibit secretion of both Th1 and Th2 cytokines while inducing the development of a population of regulatory T cells producing TGF- β and IL-10 *in vitro* (106). Furthermore, IL-10 deficient mice develop a gut barrier dysfunction that leads to Th1 type inflammation against indigenous gut microbiota (107). Interestingly, these mice harbor decreased numbers of colonic lactobacilli in the neonatal period, and normalizing the level of lactobacilli prevents the development of intestinal inflammation (108).

Different strains of lactobacilli induce distinct and even opposing dendritic cells responses with regard to their Th1/Th2-driving capacity (109), a phenomenon at least partially determined by TLRs (110). In parallel, the cytokine production patterns induced by intestinal bifidobacteria have been observed to be strain-specific (82). Thus, the type of contact and the type of microorganisms involved appear to determine responses by the innate immune system and in a strain-specific manner modulate the development of the immune responder phenotype.

Recent epidemiological data suggest an inverse relationship between environmental exposure to endotoxin and atopic sensitization and atopic disease (111). Early endotoxin exposure may be one of the reasons for the observation that individuals living in farm environments (112,113) or owning pets (114) might be less affected by atopic disease. An important link between these epidemiological observations and experimental data comes from a report indicating that blood cells from farmers' children express higher amounts of CD14 and TLR2 than those from non-farmers' children (115).

The importance of the intestinal microbiota in achieving tolerance is beautifully demonstrated in a paper by Sudo et al. (116). Animals kept in germ-free conditions exhibit impaired development of the intestinal immune system resulting in defective oral tolerance and atopic type immune responsiveness. Reconstitution of the intestinal microbiota with bifidobacteria restores the susceptibility to oral tolerance induction, but only when reconstitution is completed neonatally. Furthermore, lactobacilli have also been observed to suppress atopic immune responsiveness in both animals (117) and allergic humans (118).

Components of the human intestinal microbiota or organisms entering the intestine may have harmful or beneficial effects on human health. Abundant evidence implies that specific strains of the healthy gut microbiota exhibit powerful anti-pathogenic and anti-inflammatory capabilities and are consequently involved with enhanced colonization resistance in the intestine. The ex-

perimental demonstrations of beneficial microbial immunomodulation have been considered potentially clinically useful and have provoked interest in clinical research on the use of probiotics defined recently as specific live or inactivated microbial cultures that have documented targets in reducing human disease or in their nutritional management (119). Indeed, probiotics have shown potential in prevention and treatment of human atopic disease. Administration of *Lactobacillus* GG before birth and during breastfeeding has been shown to protect the infant from the development of atopic disease (120) with an effect lasting as much as 4 years (121). In infants with atopic eczema, enteral administration of *Lactobacillus* GG and *Bifidobacterium lactis* Bb-12 was associated with significant improvement of the skin and a decrease in systemic markers of inflammation (122). Furthermore, in infants with cow's milk allergy and atopic eczema, *Lactobacillus* GG has been shown to alleviate both the symptoms and intestinal inflammation associated with the disorder (123). On the other hand, *Lactobacillus* GG had no beneficial impact on adults with birch-pollen allergy (124). This discrepancy is perhaps explained by the suggestion that childhood food allergy may represent a primary failure to establish tolerance rather than a loss of previously acquired tolerance (56) characteristic of adult allergies.

EXTENDING THE HYGIENE HYPOTHESIS OF ALLERGY TO AUTOIMMUNE AND INFECTIOUS DISEASES: A UNIFYING HYPOTHESIS

At birth, the infant's mucosal and systemic immune responses, although not completely naïve, are considerably immature (125,126). The neonatal innate immune system is impaired with regard to granulocyte phagocytosis and chemotaxis, NK cell cytotoxicity, and complement function (127). Neonatal T cells have diminished capacity to produce cytokines, diminished cytotoxicity and less ability to promote B cell differentiation. B cell responses are characterized by limited immunoglobulin production. IgG responses to polysaccharide antigens are limited but normal to most protein antigens. The levels of IgA, IgM, and IgE are low in newborns and IgM produced is of low affinity (125,128). Serum IgG antibodies are mainly of maternal origin (125) and, thus, are at concentrations similar to those of the mother. The immaturity of the immune system renders the infant prone to infection and, combined with the relative permeability of the mucosal barrier, at an increased risk of allergic sensitization.

Initial contact with environmental antigens may be decisive in determining the type of immune responsiveness (Th1, Th2 or tolerance) elicited by the mucosal immune system and reflected systemically. Failure to establish a sound tolerogenic, disease-free state in the intestine and other mucosal surfaces in early infancy may

thus increase the risk of loss of tolerance and the development of chronic inflammatory disease. It may be further speculated that as a result of the universal Th2 skewed immunity of the newborn, the development of clinical atopic disease takes place in infancy, whereas Th1 type autoimmune diseases usually become manifest after the first year of life when Th1 responses are thought to predominate.

An extended hygiene hypothesis is emerging which suggests that the epidemic of atopic and inflammatory autoimmune disease in the developed world and the continuing challenge of infectious disease are the result of altered living conditions and improved hygiene which are causing drastic changes in factors affecting the initial establishment of the indigenous intestinal microbiota. The modern infant may lack critical interactions with bacterial strains through TLRs and CD14 which promote the development of an anti-inflammatory, tolerogenic immune environment maintained by mediators such as TGF- β and IL-10 (Fig. 2). Hence, the initial compositional development of the gut microbiota may be considered a key determinant in the development and maintenance of normal gut barrier functions and a disease-free state of the host. In affecting the development of gut-associated lymphoid tissue at an early age, the gut microbiota direct the regulation of systemic and local immune responsiveness, including responses to antigens derived from microorganisms and food, to a tolerogenic, anti-inflammatory tone whilst simultaneously maintaining the capacity for appropriate inflammatory responses.

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