

THE ROLE OF THE INTESTINAL MICROBIOTA IN THE AETIOLOGY OF ALLERGIC DISEASES

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SUMMARY

The prevalence of allergic diseases, such as eczema, food allergy, hay fever and allergic asthma has been increasing in the past decades, predominantly in the Western world and particularly amongst children. An altered normal intestinal colonization pattern in infancy, which fails to induce immunological tolerance, could be partly responsible for this increase. The majority of epidemiological studies have indeed shown that the microbiota of allergic children differs from that of healthy children. Furthermore, the few prospective studies indicated that differences in the intestinal microbiota actually preceded the manifestation of allergic diseases, which strengthens the evidence for a causal relationship. Yet, results on which microbes might be involved in the aetiology of allergic diseases are inconsistent between studies and therefore no specific harmful or protective microbes can at present be identified. Furthermore, some studies indicate that low diversity and/or strain turnover rather than specific microbes may contribute to the development of allergic diseases.

The development of allergic diseases depends not only on environmental factors, like microbial stimulation, but also on genetic factors and it is likely to be an interaction of these which determines the allergic status of an individual. It is therefore most likely that the effects of certain microbes on allergy development differ according to the genetic susceptibility of an individual. To examine the influence of such host-microbial interactions there is a need for studies consisting of large populations in which both faecal samples for microbial analyses as well as blood or buccal swabs will be collected for genotyping. More knowledge on host-microbial interactions in allergic diseases could lead us to new concepts, such as the intervention with specific treatments to modulate the gut microbiota of infants at risk.

THE MICROBIOTA HYPOTHESIS IN ALLERGIC DISEASES

Atopic (or allergic) diseases, such as eczema, food allergies, hay fever and allergic asthma, are chronic inflammatory disorders caused by aberrant immune responses against common “innocuous” environmental antigens (allergens) in susceptible individuals (Romagnani, 2006). An enhanced T helper (Th)2 immune response and the elaboration of cytokines such as inter-

leukin (IL)-4, IL-13 and IL-5 contribute to the induction of these diseases (Ngoc et al., 2005).

The prevalence of allergic diseases has been increasing worldwide in the past decades, predominantly in the Western world and particularly amongst children (Nakagomi et al., 1994). Because this increase occurred much faster than the genetic constitution of any population can possibly shift (Nowak et al., 2004), it is generally believed that environmental changes associated with “western” lifestyles are responsible for the allergic epidemic.

Twenty years ago, David Strachan introduced the “hygiene hypothesis”, which states that reduced exposure to infections during childhood result in aberrant immune responses to innocuous antigens later in life (Strachan, 1989, 2000). This hypothesis was based upon Strachan’s observations that infants with higher number of siblings were at decreased risk for developing allergy. Although sibship size (Karmaus and Botezan, 2002; Strachan, 2000), and other indirect markers of microbial exposure such as rural and farm living (especially contact with livestock) (von Mutius, 2002) were consistently shown to be associated with a decreased risk of developing allergic diseases, studies on the association between bacterial and viral infections and allergy were less consistent (Björkstén, 2004; Flohr et al., 2005).

In 1998 Agnes Wold introduced an alternative interpretation of this hypothesis by suggesting that rather than a decrease in viral or bacterial infections, an altered normal intestinal colonization pattern in infancy, which fails to induce immunological tolerance, could be responsible for the increase in allergies (Wold, 1998).

The gut microbiota is indeed a key source of microbial driven immune regulation and tolerance induction in early life. Animals bred in a germ-free environment show low densities of lymphoid cells in the gut mucosa, and the specialized follicle structures are small, additionally circulating immunoglobulin levels are low. Immediately after exposure to microbes, the number of mucosal lymphocytes expands, germinal centres are formed and immunoglobulin-producing cells appear rapidly in follicles and in the lamina propria and there is a significant increase in serum immunoglobulin levels (Butler et al., 2000; Falk et al., 1998). Furthermore animal studies have shown that it is difficult to achieve oral tolerance in germ-free animals (Sudo et al., 1997) and that administration of lipopolysaccharides (a constituent of the outer membrane of Gram-negative bacteria) together with food antigens increases the tolerizing effect of feeding (Kim and Ohsawa, 1995). It seems therefore plausible that the gut microbiota composition (due to e.g. increased antibiotic use, changed diet) is involved in the pathogenesis of allergic diseases.

IMMUNOLOGICAL FRAMEWORK

The initial immunological explanation for the hygiene hypothesis was a lack of microbial antigen-induced immune deviation from the Th2 cytokine profile to a Th1 type profile, resulting in the

development of enhanced Th2 cell responses to allergens (Baker, 2006; Matricardi and Bonini, 2000; Romagnani, 2004). However, this explanation did not take into account that the

prevalence of Th1-associated diseases, such as Crohn's disease, type 1 diabetes and multiple sclerosis, were also increasing and that chronic parasitic worm (helminth) infections which induce strong Th-2 responses and high IgE levels are not associated with an increased risk of allergy (Yazdanbakhsh et al., 2002).

An alternative interpretation conceives anti-inflammatory immune responses to be of fundamental importance in the development of mucosal and systemic tolerance (Rautava et al., 2005). These immunosuppressive mechanisms are orchestrated by regulatory T cell classes (Treg cells) that control (largely via the production of IL-10 and/or TGF- β) both Th1 and Th2 responses and hence the development of both atopic and autoimmune diseases (Rautava et al., 2005; Rook and Brunet, 2005a). Indeed the importance of a delicate balance between allergen-specific Treg cells and allergen specific Th2 cells in healthy and allergic immune responses to common environmental allergens was demonstrated in a study conducted by Akdis and colleagues (Akdis et al., 2004). Furthermore, a study on duodenal biopsies of healthy infants and infants with multiple food allergy, showed that the dominant mucosal abnormality was not Th2 deviation but impaired generation of TGF- β producing Treg cells (Perez-Machado et al., 2003).

Relatively harmless organisms, including bifidobacteria, lactobacilli, but also helminths and saprophytic mycobacteria, may skew immune responses towards immunoregulation by inducing

Treg cells, rather than eliciting a proinflammatory immune response.

For example, *Lactobacillus paracasei* has been reported to inhibit the secretion of both Th1 and Th2 cytokines, while inducing the development of a population of CD4(+) T cells producing TGF- β and IL-10, reminiscent of previously described subsets of regulatory cells implicated in oral tolerance and gut homeostasis (von der Weid et al., 2001).

Lactobacillus reuteri and *Lactobacillus casei* have been shown to prime monocyte-derived DCs to drive the development of IL-10 producing Treg cells, through binding the C-type lectin DC-specific intracellular adhesion molecule 3-grabbing non-integrin (DC-SIGN) (Smits et al., 2005). The Bifidobacterium genomic DNA has been reported to induce the secretion of IL-10 by PBMCs from healthy donors *in vitro* (Lammers et al., 2003).

The "microbiota hypothesis" proposes that the loss of exposure to these harmless microorganisms in the westernized environment might explain the increase in immunedysregulatory disorders (Guarner, 2006; Rook and Brunet, 2005b). The epidemiological findings and the experimental evidence available so far suggest that both the reduced immune suppression by Treg cells and the lack of immune deviation from a Th2 to Th1 profile are involved (Romagnani, 2004). Furthermore, the impact of the gut microbiota on the development of IgA antibody responses, which contribute in pathogen and allergen exclusion in the gut lumen, may also be involved (Rautava et al., 2004).

GUT MICROBIOTA AND ATOPIC DISEASES

Bengt Björkstén and his research group instigated the epidemiological research on the role of the intestinal microbiota

in the aetiology of allergic diseases. Björkstén's group showed that the intestinal microbiota of healthy children

living in a country with a low prevalence of allergy (Estonia) differed from the microbiota of healthy children living in a country with a high prevalence of allergic diseases (Sweden). Lactobacilli and eubacteria were more prevalent in Estonian children, while *C. difficile* was more prevalent in Swedes (Sepp et al., 1997). Subsequently, this research group conducted a case-control study in which they showed that lactobacilli were less prevalent in allergic children compared to healthy children both in Sweden and Estonia (Björkstén et al., 1999). The disadvantage of case-control studies is their cross-sectional design, which makes it impossible to find out if differences in the intestinal microbiota actually preceded the development of allergic diseases. Therefore, Björkstén and co-workers additionally performed a birth cohort study including 44 newborns with a high risk of developing allergy (positive family history of allergy). Faecal samples were collected at the children's age 1 week and 1,3,6 and 12 months and analyzed using traditional bacteriological culture techniques and biochemical identification (Björkstén et al., 2001). The prevalence of bifidobacteria was consistently lower throughout the first year of life in children who developed allergic symptoms and sensitization, indicating that differences in intestinal microbiota actually precede the development of allergic manifestations. In the subsequent 5 years several, mostly cases-control, studies were published. Almost all these studies reported differences in the intestinal microbiota between allergic and non-allergic subjects. However, as mentioned before, case-control studies are limited by their cross-sectional design. In 2007 results from 2 large birth cohort studies, our own KOALA study and the ALLERGYFLORA project, were published.

The KOALA Birth Cohort Study

The KOALA Birth Cohort Study was the first large-scale prospective study in which the role of the gut microbiota in the aetiology of allergic disorders has been investigated.

Within the KOALA study 2834 pregnant women were recruited at 34 weeks of gestation. Beginning halfway during this recruitment of the cohort, faecal samples of infants (n=1176) at the age of 1 month were collected. During pregnancy and early childhood data on perinatal determinants of the child's health as well as on hygiene, infections, nutrition, child rearing, other life style characteristics and on allergic symptoms was collected for all members of the cohort by repeated questionnaires. A large number of children of whom faeces had been collected were also visited at home by a trained nurse at the age of 1 and 2 years for the collection of a blood sample and a clinical diagnosis of atopic dermatitis (Kummeling et al., 2005).

This study enabled us, not only to examine the association between the gut microbiota and allergic diseases, but also to examine the external factors influencing the composition of the intestinal microbiota.

Using quantitative real-time PCR, we found that infant feeding had a major effect on the gut microbiota of 1-month-old infants. Breastfed infants were less often colonized with bacteria other than bifidobacteria compared to formula-fed infants. Mode and place of delivery also appeared to be of great importance. Children born by C-section were less often colonized by *Bacteroides* spp., whereas they were significantly more often colonized by *Clostridium difficile*. Also hospitalization after birth resulted in an increased risk of becoming colonized by *C. difficile*, demonstrating the hospital environment as an important source of this bacte-

rium. As expected, children who received oral antibiotics in their first month of life had a strong reduction in obligate anaerobes (Penders et al., 2006a).

We also found strong support for the role of the gut microbiota composition in the development of atopic manifestations. The prevalence and counts of faecal *Escherichia coli* at the age of 1 month was significantly higher in infants who subsequently developed eczema within the first two years of life. Colonization with *Clostridium difficile* was positively associated with the subsequent development of eczema, recurrent wheeze and allergic sensitization at age 2 years (Penders et al., 2006b; Penders et al., 2007). Interestingly, two studies examining *Clostridium difficile*-specific immunoglobulin G also identified this bacterium as a risk factor for atopic diseases (Linneberg et al., 2003; Woodcock et al., 2002). Furthermore, a study on faecal short chain fatty acid-profiles showed that allergic children had higher levels of the rarely detected i-caproic acid, which has been associated with the presence of *Clostridium difficile* (Bottcher et al., 2000).

ALLERGYFLORA

Another large-scale prospective birth cohort examining the hypothesis that allergic sensitization and atopic eczema/dermatitis are influenced by the infant gut microbiota is the ALLERGYFLORA-project (Adlerberth et al., 2007). In Göteborg, London and Rome 324 children were recruited perinatally. A rectal sample was collected at age 3 days, whereas faecal samples were collected at 7, 14, 28 days and 2, 6 and 12 months and analyzed by traditional culture techniques. At the age of 18 months, the presence of atopic dermatitis was assessed as well as serum specific IgE to common food-allergens. This study reported a comparable

“negative” impact of birth by C-section with delayed *E. coli* and Bacteroides colonization and increased colonization by Clostridium species as was found in our KOALA-study.

However, neither atopic eczema nor food-specific IgE by age 18 months were associated with time of acquisition of any particular bacterial group. In a second publication from this project, the microbial diversity of faecal samples collected at the age of 1 week was assessed by terminal restriction fragment length polymorphism (T-RFLP) and temperature gradient gel electrophoreses (TTGE) (Wang et al., 2008). It was demonstrated that the microbial diversity in early faecal microbiota was reduced in infants who subsequently developed atopic dermatitis.

An overview of the studies conducted in the past decade on the association between the intestinal microbiota and allergy is given in Table 1. Although most of the studies, conducted so far, showed an association between the intestinal microbiota composition and allergic symptoms and/or sensitization, results are far from being consistent and no specific harmful or protective microbes can be identified yet.

It is unlikely that publication bias and false positive findings due to multiple comparisons in several studies can completely explain the high percentage of studies that report an association between the intestinal microbiota and allergies.

So why do the majority of studies report an association, but are results far from consistent? It is likely that this relates to the large differences in methodological aspects between studies. Current studies are very difficult to compare due to differences in study design, differences in the bacteria under study and the techniques used to identify them and probably most important differences in the age at which

Table 1: Overview of studies on the association between the intestinal microbiota composition and allergy

Study	Allergic outcome	Study population	Design	Main results for allergic compared to non-atopic subjects
Björkstén et al., 1999	AD & SPT+	27 cases & 35 controls (aged 2 yr.)	Case-control	Lower prevalence of lactobacilli
Björkstén et al., 2001	AD/SPT +	44 newborns (until age 2 yr.)	Birth cohort	Lower prevalence of bifidobacteria
Kirjavainen et al., 2001	AD	27 cases & 10 controls (aged 5-13 mo.)	Case-control	No differences in concentrations of specific genera
Ouwehand et al., 2001	AD & SPT+	7 cases & 6 controls (aged 2-7 mo.)	Case-control	Higher prevalence of <i>Bifidobacterium adolescentis</i> Lower prevalence of <i>Bifidobacterium bifidum</i>
Kalliomaki et al., 2001	SPT+	76 newborns (until age 1 yr.)	Birth cohort	Lower bifidobacteria : clostridia ratio (at age 3 weeks)
Watanabe et al., 2003	AD	30 cases & 68 controls (minors)	Case-control	Higher prevalence of <i>S. aureus</i> Lower counts of bifidobacteria
Matsumoto et al., 2004	Severe AD	11 cases & 14 controls (adults)	Case-control	Lower total counts and total anaerobes. Higher proportion of enterobacteriaceae Higher content of sIgA
Murray et al., 2005	Recurrent wheeze & SPT+	33 cases & 33 controls (aged 4 yr.)	Case-control	No differences between cases & controls in lactobacillus and bifidobacterial colonization
Sepp et al., 2005	AD, asthma or allergic rhinitis	19 cases & 19 controls (aged 5 yr.)	Case-control	Lower prevalence and proportion of bifidobacteria Higher proportion of clostridia
Mah et al., 2006	AD	21 cases & 28 controls (aged 3 yr.)	Case-control	Higher counts of LAB Lower counts of bifidobacteria and clostridia.
Penders et al., 2007	Eczema, spIgE or recurrent wheeze	957 newborns (until age 2 yr.)	Birth cohort	Higher prevalence & counts of <i>E. coli</i> in infants who subsequently developed eczema Higher prevalence of <i>C. difficile</i> in infants who subsequently developed eczema, recurrent wheeze and/or became sensitized
Penders et al., 2006b	Eczema & spIgE	26 cases & 52 controls (aged 1 yr.)	Nested case-control *	Higher prevalence of <i>E. coli</i> No differences in total bacterial profiles No difference in bifidobacterial counts & species composition
Adlerberth et al., 2007	AD, sp IgE	324 newborns (until age 18 mo.)	Birth cohort	No differences in time of acquisition of any bacterial group

AD: Atopic dermatitis; SPT: Skin Prick Test; spIgE: specific serum IgE to one or more allergens; FISH: Fluorescence *in situ* hybridisation.

* (Nested) case-control studies with a prospective design (faecal samples collected prior to the onset of allergic symptoms and sensitization).

Table 1 (continued): Overview of studies on the association between the intestinal microbiota composition and allergy

Study	Allergic outcome	Study population	Design	Main results for allergic compared to non-atopic subjects
Songjinda et al., 2007	AD, asthma, food allergy	8 cases & 7 controls (aged 2 yr.)	Nested case-control*	Higher population of Bacteroidaceae
Stsepetova et al., 2007)	AD, asthma, allergic rhinitis	20 cases & 20 controls (aged 5 yr.)	Case-control	Lower bacterial diversity Higher prevalence of <i>Bifidobacterium adolescentis</i> Lower prevalence of <i>B. catenulatum/pseudocatenulatum</i>
Gore et al., 2008	Eczema	37 cases & 34 controls (aged 3-6 months)	Case-control	No difference in total bacterial profiles Higher prevalence of <i>Bifidobacterium pseudocatenulatum</i>
Wang et al., 2008	AD	15 cases & 20 controls	Nested case-control*	Lower bacterial diversity
Verhulst et al., 2008	wheeze	154 newborns (until age 12 mo.)	Birth cohort	Lower clostridial concentrations Higher total anaerobes
Sjögren et al., 2009)	Symptoms (AD, asthma or allergic rhinitis) & SPT+	47 newborns (until age 5 yr.)	Birth cohort	Lower prevalence of lactobacilli group I Lower prevalence of <i>C. difficile</i> Lower prevalence of <i>B. adolescentis</i>

AD: Atopic dermatitis; SPT: Skin Prick Test; spIgE: specific serum IgE to one or more allergens; FISH: Fluorescence *in situ* hybridisation.

* (Nested) case-control studies with a prospective design (faecal samples collected prior to the onset of allergic symptoms and sensitization).

the intestinal microbiota is being studied.

The timing of exposure to environmental factors is essential to promote beneficial or harmful effects regarding the development of allergic diseases. It has been suggested that the most important “window of opportunity” for immune education seems to be in early life, when the maturation of the immune system is not yet completed and is still building up immune tolerance against food and microbial antigens (Björkstén, 1999; Strachan, 1989, 2000). Based upon this critical window period, it thus seems unlikely that perturbations in the intestinal microbiota beyond infancy may still have an effect on the aetiology of allergic diseases. It is more likely that these differences reflect disturbances in the gut microbiota already present in early life or reflect perturbations caused by the allergic disease that had manifested already (reverse causation).

Birth cohort studies quantifying the intestinal microbiota in early life and relating this to allergic manifestations later on in childhood are therefore the most powerful studies.

Another question that has not been answered yet is the differential effect of

different faecal *Lactobacillus* species in the development of allergic diseases. The quantification of the gut microbiota has mainly relied on the quantification of bacteria at the genus level, such as *Lactobacillus* spp. Most studies found no association between the presence and quantity of total lactobacilli and allergy. It is, however, well known that different species of lactobacilli induce distinct and even opposing immune-responses (Christensen et al., 2002). Thus it is of special importance to unravel the potential species-specific effects of lactobacilli in the aetiology of allergic disorders, since it will gain more insight into the candidate species proficient as probiotics in the treatment or prevention of these disorders.

New upcoming birth cohort studies using molecular techniques to study the intestinal microbiota and assessing a broad range of allergic outcomes and immune parameters will probably gain more insight into the role of the gut microbiota in the aetiology of allergic diseases. Furthermore, the use of more recently introduced techniques such as microarrays and pyrosequencing within such studies will result in a far more detailed examination of the intestinal microbiota composition.

CANDIDATE GENES

The development of allergic diseases, including asthma, depends, however, not only on environmental factors (like microbial stimulation), but also on genetic factors and it is likely to be an interaction of these, particularly in early life, which determines the allergic status of a person (Koppelman, 2006, Postma et al., 2005). It is most likely that the effects of certain microbes on the development of allergy and asthma therefore differ according to the genetic susceptibility of an individual. The

separate research on genetic (genetic epidemiology) and environmental influences (environmental epidemiology), as in most studies so far, has considerably hindered the understanding of the role of these influences in determining complex diseases like asthma and allergy. To get a better understanding of the biological importance of genetic and environmental factors, upcoming studies should include the interaction between these factors in relation to the disease phenotype.

Microbes are recognized by the innate immune system using pattern recognition receptors (PRRs). Interestingly Single Nucleotide Polymorphisms (SNPs) in PRR genes have been associated with allergy and asthma (Eder et al., 2004; Fageras Böttcher et al., 2004; Koppelman et al., 2001; Yang et al., 2006). Polymorphisms in these PRR encoding genes can alter the immune responsiveness of the host to microbial agents and may indicate the development of aberrant immune responses that are associated with immune-mediated diseases such as allergic diseases. Examples of PRRs are *CD14*, the toll-like receptors (TLRs), the NOD-like receptors (NLRs) and several C-type lectins.

CD14 is, together with *TLR-4*, involved in the recognition and signal transduction of bacterial endotoxin, a major component of the bacterial cell wall of Gram-negative bacteria. *CD14* does not have a transmembrane receptor domain, but contributes to the affinity of the interaction between the microbial products and TLRs. *TLR4* more selectively forms the receptor for endotoxin. Downstream effects of *CD14/TLR4* receptor activation include the release of cytokines, such as IL-10 and IL12, and the activation of regulatory T cells (Vercelli, 2003a). The *CD14* gene has several SNPs, the most important one being *CD14/-159* C-T SNP (also called *CD14/-260*) localized in the promoter region. This SNP affects the transcription rate of the *CD14* gene (Koppelman, 2006, LeVan et al., 2001). Genetic associations of this -159 C-T polymorphism with markers of allergy have been shown in several studies (Koppelman et al., 2001). However, in some studies, the *CD14/-159* C allele was associated with allergic phenotypes, whereas in other studies the T allele was. Finally other populations reported no association between the

CD14 genotype and allergy (Kedda et al., 2005). Vercelli explained these apparent contradictory results by proposing the “endotoxin switch” (Vercelli, 2003b). Different levels of (i.e. high or low) endotoxin exposure would trigger different host responses, resulting in either Th1 or Th2 type responses. The *CD14* genotype may shift this endotoxin response curve, highlighting the importance of studying the combined effect of endotoxin exposure and *CD14* genotype.

Two SNPs, A896G (Asp299Gly) and C1196T (Thr399Ile), in the *TLR4* gene have been associated with LPS hyporesponsiveness in primary human epithelial cells and alveolar macrophages *in vitro*, and with airway hyporesponsiveness to inhaled endotoxin *in vivo* (Yang et al., 2006). In a study among Swedish school children, the Asp299Gly polymorphism was associated with a 4-fold higher prevalence of asthma (Fageras Böttcher et al., 2004).

Polymorphisms in the *TLR2* gene have also been associated with the frequency of allergies and asthma (Eder et al., 2004). *TLR2* recognizes bacterial lipopeptides and lipoteichoic acid, which are abundantly found in cell walls of Gram-positive bacteria. Studies using murine models on the interaction between *TLR2* stimulation and allergy have provided contradictory data. Initial studies using murine models of allergic asthma reported that *TLR2* ligands administered during the sensitization period led to enhancement of Th2-mediated allergic inflammation. Other studies suggested that *TLR2* stimulation inhibits Th2-type responses and allergic airway inflammation. In a recent study stimulation of blood mononuclear cells of allergic individuals with *TLR2* ligands inhibited Th2 responses (Taylor et al., 2006).

The gene encoding the C-type lectin, dendritic cell-specific ICAM-3-

grabbing nonintegrin (*DC-SIGN*), is another candidate gene. Different species of lactobacilli have, for example, shown to induce distinct and even opposing dendritic cell (DC) responses with regard to their Th1/Th2/Treg-driving capacity. Recently it has been shown that certain, but not all, lactobacillus species induce IL-10-producing regulatory T cells *in vitro* by modulat-

ing dendritic cell function by targeting *DC-SIGN* (Smits et al., 2005). This could explain how certain lactobacillus species might exert beneficial effects in the treatment of allergic disorders.

Other candidate PRR encoding genes that have been suggested to be associated with asthma and allergy are the Mannose-binding lectin gene, *TLR6* and *TLR10* (Yang et al., 2006).

HOST-MICROBIAL INTERACTIONS

TLR and *CD14* gene association studies with asthma and allergy are suggestive but have not been fully replicated. One of the most likely explanations for differences between studies is difference in the level of microbial exposures interacting with the PRRs.

Support for the concept that the interactions between these innate immunity genes and microbes play a central role in the pathogenesis of asthma and allergies is derived from the few studies in which both (markers of) microbial exposures and SNPs in genes that encode for proteins that interact with these exposures were assessed. For example, in two independent populations, a SNP in toll-like (TLR) 4 (Asp299Gly) that disrupts *TLR-4*-mediated LPS signalling was associated with a lower prevalence of bronchial responsiveness and allergy, respectively, but only in subjects heavily exposed to endotoxin (Eder et al., 2004; Werner et al., 2003). Several studies have reported that the association between a functional SNP in the promoter

region of *CD14* (CD14/-260C-T) and total serum IgE levels is modified by exposure to microbial products/endotoxin (Simpson et al., 2006).

Genetic variants within *NOD1/CARD4* also appear to be important determinants of allergy susceptibility. Recently, the Allergy and Endotoxin (ALEX) study reported that SNPs in *NOD1/CARD4*, an intracellular PRR that interacts with muropeptides found in common Gram-negative bacteria, modify the protective effect of farming (Eder et al., 2006).

So far, however, no gene-environment studies have been performed with respect to the interaction between candidate genes and the most important source of microbial stimulation, the gut microbiota.

More knowledge of host-microbial interactions in asthma and allergy could lead us to new concepts, such as the intervention with specific treatments to modulate the gut microbiota of infants at risk.

CONCLUSION

Most studies show an association between the intestinal microbiota composition in early infancy and allergic diseases. Moreover, the few prospective

studies have shown that differences in the gut microbiota composition precede the development of allergic symptoms, which strengthens the evidence for a

causal relationship. However, no specific microbes or perturbations in the intestinal microbiota can be identified yet. Different microbes have been linked to allergies within the different studies. Furthermore, some studies indicate that a low diversity and/or strain turnover rather than specific microbes may contribute to the development of allergic diseases.

To get more insight into the role of the gut microbiota in the development of allergic diseases, more large-scale prospective cohort studies are necessary. Especially, birth cohorts in whom faecal samples will be collected at regular time-points during the first year of life will probably add to our current knowledge.

In addition, allergic diseases are complex diseases caused by the interplay of both genetic and environmental factors. The separate research on ge-

netic (genetic epidemiology) and environmental influences (environmental epidemiology), as in most studies so far, has considerably hindered our understanding of the role of these influences in these diseases. So far, no studies have examined the interaction between host factors, such as genetic variations in PRR encoding genes, and the most abundant source of microbial stimulation, the intestinal microbiota.

To examine the influence of such host-microbial-interactions there is a need for studies consisting of large population in which both faecal samples as well as blood or buccal swabs will be collected for genotyping. More knowledge on host-microbial interactions in asthma and allergy could lead us to new concepts, such as the intervention with specific treatments to modulate the gut microbiota of infants at risk.

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