

Intestinal microbial diversity in infancy and allergy risk at school age

Markus Johannes Ege, MD *Munich, Germany*

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Our perception of human disease as a result of genetic and environmental influences is currently being integrated in a metagenomic perspective. The metagenome comprises the human genome and the genome of the colonizing microbes, the microbiome, which outnumbers the human genome by a factor of 150.¹ This complex view is imbedded in a novel understanding of the coevolution of humankind and the microbiome.^{2,3} In this context microbial exposure is not only perceived as a risk factor for human health but also as a potential protective factor against certain pathologies, such as autoimmunity or allergy. The complexity of the microbiome has also shifted the focus from specific microbial exposures toward the composition, diversity, and uniqueness of a subject's microbiome. As a consequence, the hygiene hypothesis emerges in a new light, which might reconcile its evident inconsistencies.

This paradigm shift is fostered by novel techniques that offer the comprehensive assessment of microbial exposure on a molecular level, and therefore also nonviable and nonculturable microbes are covered. The main principle consists in the amplification of specific microbial DNA fragments and a subsequent differentiation of the underlying nucleotide sequences. Hence the targeted DNA fragment needs to be distinct from human DNA, specific for the respective microbial domain, and highly variable among the microbial taxa within a domain. For bacteria, a DNA fragment encoding the 16S subunit of the ribosomal RNA (16S rRNA) can be used because it is unique to bacteria and varies even between genera and species. The differentiation can be achieved by using gel electrophoresis techniques, such as denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis, or single-strand conformation polymorphisms.⁴ The electrophoretic separation might be replaced by direct parallel sequencing with high-throughput techniques, which will soon become affordable at an epidemiologic scale.

In this issue of the *Journal*, Bisgaard et al⁵ present data on the bacterial flora in fecal samples in infancy and its relation to the manifestation of atopic disorders at school age in the Copenhagen Prospective Study on Asthma in Childhood (COPSAC). This carefully followed birth cohort includes 411 children born to asthmatic mothers, of whom 253 to 301 were included in the present

From University Children's Hospital Munich.

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Reprint requests: Markus Johannes Ege, MD, Dr. von Haunersche Kinderklinik, Lindwurmstrasse 4, 80337 Munich, Germany. E-mail: markus.ege@med.uni-muenchen.de.

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analyses. With the use of DGGE, they found an inverse association of bacterial diversity with sensitization, blood eosinophil counts, and allergic rhinitis. In contrast, no single DGGE band or combination thereof were related to any clinical outcome. For asthma or atopic eczema, no association with bacterial diversity emerged. Furthermore, detection of specific IgE was positively associated with staphylococcaceae detected in neonatal fecal cultures. The latter finding might have been a chance finding because the association is inconsistent for further atopy-related outcomes, such as skin prick test responses or allergic rhinitis. Moreover, the association is effectively not significant given the many comparisons performed. However, the inverse association of atopy and band richness (ie, bacterial diversity) at 2 different time points is fairly robust and consistent across all atopy-related outcomes.

The failure to detect associations with specific bacteria can be interpreted in 2 ways. First, it simply reflects the original purpose of the techniques (ie, quantification of microbial diversity and uniqueness rather than detection of specific organisms). This is particularly true for DGGE, which does not detect rare organisms represented by less than 1% of the total DNA.⁴ In general, DGGE and the other fingerprinting techniques are susceptible to incomplete DNA extraction and selective amplification of 16S rRNA fragments.⁶ The latter might be caused by primers that are not entirely universal because their design is based on prior knowledge acquired with the use of a circumscribed number of known microorganisms.⁴ Furthermore, the number of bands might lead to an overestimation of the number of microbial taxa because some taxa might be represented by multiple bands resulting from different operons.⁴ In turn, partial sequence identity between different microbial taxa might result in an underestimation of their number.⁴ Finally, incomplete standardization of different individual gels might increase the statistical noise.⁶ In short, microbial diversity is a more robust characteristic than any specific microbial taxon.

A second interpretation of the missing associations of specific bacteria with the atopic health outcomes might even emphasize the role of bacterial diversity for atopy. Great diversity might thus reflect a well-balanced colonization, which might prevent overgrowth of potentially harmful bacteria.^{7,8} Alternatively, microbial diversity might just be a proxy for additive effects of several microbial exposures. Microbes are primarily recognized by the innate immune system. Because the number of its pattern recognition receptors is restricted and its signaling pathways are redundant, the effects of microbial diversity should be limited in itself.⁸

In contrast to the aforementioned markers of atopy, for asthma, no relation to bacterial diversity was found in the COPSAC study. Superficially, this might contradict a recent publication on 2 cross-sectional studies in which bacterial and fungal diversity were inversely associated with asthma.⁸ However, those studies

were performed in children from rural regions with a low asthma and atopy risk profile, and the source of microbial exposure was environmental dust. Those microbes might not only be ingested and affect the intestinal mucosa; rather, they are inhaled and might interact directly with the bronchial respiratory mucosa, the genuine site of asthma. As opposed to the present analysis, atopic sensitization was not related to microbial diversity in those studies, possibly because a strong protective effect of other farm exposures might have prevailed.⁸

The main conclusion drawn by Bisgaard et al⁵ is that an imbalance in the intestinal microbiome influences the development of allergic disease. This inference is challenged by 2 questions: Are fecal samples representative of intestinal microbial colonization? Is the composition of the intestinal microbiome cause or consequence of the host's immune condition?

First, intestinal bacteria reside in 2 distinct compartments: the intestinal mucosa and the intestinal lumen. Mucosal bacteria interact with the host much more intensively because of their privileged condition, close spatial proximity, and decelerated passage. Because mucosal microbes constitute only a minority of the intestinal microbiome, the question arises how well fecal samples represent the colonization of the intestinal mucosa. Unfortunately, studies comparing the 2 sampling sources in healthy subjects are scarce. A case series study revealed that differences between fecal and mucosal samples accounted for most of the variability not attributed to interindividual differences.⁹ A study comparing various detection methods showed a similar incongruence between fecal and mucosal samples.¹⁰ In summary, fecal samples probably reflect intestinal mucosal colonization rather insufficiently.¹¹ The fact, however, that Bisgaard et al⁵ did find a consistent inverse association of atopy with bacterial diversity despite using an imperfect sample source actually supports the relevance and robustness of the association. Yet for future studies targeting specific intestinal bacteria, a dilemma emerges between the need for mucosal samples and the ethical constraints of invasive sampling in epidemiologic studies.

The second question addresses the interaction of the microbiome and the host's immune system, ultimately reflecting their coevolution. There is evidence that the microbiome is actively

selected by the host.¹¹ Otherwise, the microbiome affects, for example, T-cell differentiation and maturation.³ After all, the interaction between host and microbiome might even be influenced by the outer environment of the host. Then the microbial composition might only be an intermediate step in a complex causal network.

In summary, the publication by Bisgaard et al⁵ is among the first to demonstrate an inverse association of microbial diversity and atopy by using a novel comprehensive assessment of microbial exposure in an epidemiologic setting. This pioneering work might be at the threshold of a number of exciting discoveries.

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