

A fascinating look at the world with a new microscope

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In the mid-17th century in Delft, Antony Leeuwenhoek was the first to invent the microscope.¹ He was enormously skilled in making lenses and mounted them on frames that would eventually become the first microscope, allowing a new look at tiny little things no one had seen before. Leeuwenhoek put everything he could possibly get hold of under his lenses and discovered a new form of life that no longer remained invisible to human eyes. This was the beginning of microbiology.

In those days, the observations were exciting by themselves and prompted religious debates because it was unclear how microbes spawn.¹ Could the microscope allow men to watch God's creation *ex nihilo*? This hope had to be abandoned once the reproduction of microbes was better understood.

However, the golden age of microbiology was to come when Pasteur and Koch first demonstrated that microbes were the cause of human disease. By isolating microbes from sick animals and inoculating these into healthy animals, they proved that individual microbes caused the very same disease as in the animals from which they had been taken. Many individual pathogens were discovered, and new methods of prevention and treatment were found: hygiene, vaccinations, and antimicrobial medications. These seminal advances have saved millions of lives, dramatically decreased infant mortality, and increased life expectancy.

Recently, a new type of microscope has been developed that works without lenses. The pioneering advances in genomics have enabled us to isolate, amplify, and sequence DNA in large quantities. Microbial DNA can specifically be targeted and, through DNA microbes, can be identified directly without the cumbersome need for culture. With this new technique, about 100 times more microbes can be detected in specimens such as tissues and other body habitats, as well as in environmental samples. A whole new world becomes visible that had been unsearchable before. This series is dedicated to the emerging concepts and insights of research into the microbiome and its relevance for disease, in particular asthma and allergies.

As Leeuwenhoek did many centuries ago, the first step in research is to describe what lies in front of our new eyes. Ursell et al² illustrate in the first fascinating article of this series the enormous richness and diversity of the microbiota in key body sites within and among persons. The human body harbors 10 to 100

trillion microbes that greatly outnumber the human cells. In healthy persons a huge variety of microbial compositions exists, depending on the sampled body site, ethnicities, geographic locale, and other environmental influences. Interestingly, the diversity between persons over time is higher than within persons over time, suggesting that subjects might carry individual microbiota.³ The characterization of the healthy microbiome is critical to the understanding of the deviations from normality, which can be linked to human disease.

The coevolution of the human host with trillions of microbes is a mesmerizing notion that clearly necessitates broader approaches than the causal unidirectional relationship between pathogen and disease. As Hörmannspurger et al⁴ point out in the second intriguing article of this series, the human body can be considered a metaorganism that is made up of our own eukaryotic cells and the outnumbering cells of the microbiota. This coevolution ensures a peaceful relationship in most subjects. A complex network of host-microbe interactions warrants tolerance of commensals, as well as protection against deleterious infections. However, there is more to the microbiome: bacteria residing in the gut help digestion and have a role in vitamin metabolism.⁵ The intestinal microbiota can also be seen as a gatekeeper of immunity. It is known that in the absence of a normal microbiome, numerous immune responses do not develop adequately. As one out of many other abnormalities, germ-free mice have an exaggerated T_H2 response, increased airway hyperresponsiveness, and airway inflammation after sensitization and challenge with ovalbumin compared with mice maintained under standard specific pathogen-free conditions.⁶ The intestinal microbiota can also effect the mucosal barrier function and thereby contribute to oral tolerance.

Because of the relative ease of accessibility, the human intestinal microbiota assessed in stool samples has been studied more than the airway microbiota in relation to allergic diseases. One of the many unresolved questions relates to the source of intestinal material needed for such studies. Is the gut mucosa the site of action, and if so, do fecal samples adequately reflect microbiota at mucosal surfaces? Thus far, translation of scientific deliberations into prevention strategies has failed. Hörmannspurger et al⁴ carefully discuss the inconsistent findings from the various intervention studies using either prebiotics or probiotics for the prevention of atopic eczema and other allergic outcomes. The disappointment should encourage us to apply more ecologic approaches and to better understand not just the composition but also the function of such microbial communities.

The microbiome on mucosal surfaces can be regarded as the interface between the human body and the environment. Moreover, microbes residing in the environment might shape an exposed subject's immune responses and thereby his or her risk of asthma and allergies. There is substantial evidence now that such exposures can exert a protective effect, particularly in childhood, as discussed in the last article of this series. Heederik and von Mutius⁷ report the evidence that proxies of exposure, such as endotoxin derived from gram-negative bacteria but also culture-based and DNA-based measures of microbial exposures in the environment, are inversely associated with these outcomes.

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Recently, the diversity of microbial exposures was suggested to be a key component of the protective effect. Such a concept of diversity is rather challenging when aiming at refining methods of exposure assessment for future studies. In turn, it might better grasp the nature of microbial exposures, which will always relate to microbial communities and the shift in such communities rather than to single microorganisms.

The challenges associated with this “new microscope” are tantalizing. The statistical analyses of the enormous datasets emerging from deep sequencing are to be further developed because microbiome data are complex high-dimensional datasets. These data require appropriately tailored analytic approaches to enable the necessary group comparisons (healthy vs diseased and exposed vs nonexposed). Results must be interpreted with caution: the presence of certain microorganisms on mucosal surfaces does not necessarily imply that these microbes are the pathogens causing the disease, as in conventional microbiology. Rather, the host’s disease, such as inflammation at mucosal surfaces with subsequently altered nutrient supply, might serve as a niche for colonization with certain microbial communities. Only prospective studies investigating the microbiome before and after the onset of disease in conjunction with other environmental exposures,

such as diet, will allow a better understanding of this hen-egg question. The “new microscope” is such an exciting new instrument to make the invisible world of microbes approachable. In the future, we will thrive to better understand what the benefit is of such an enormous microbial “organ” that we carry with us.

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