Clinical reviews in allergy and immunology

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Microbial influence on tolerance and opportunities for intervention with prebiotics/probiotics and bacterial lysates

Petra Ina Pfefferle, PhD, DrPH,[a,b] Susan L. Prescott, MD, PhD,[b,c] and Matthias Kopp, MD[d] *Marburg and Lübeck, Germany, and Perth, Australia*

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Activity Objectives

1. To discuss why interventions aimed at achieving a more favorable microbiome are likely to have multisystem benefits.
2. To discuss the challenges in defining optimal colonization patterns.
3. To discuss the evidence that the early environment and early nutritional patterns are major determinants in initiating a long-lasting microbial colonization pattern.

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Epidemiologic studies indicate that microbes and microbial components are associated with protection against chronic inflammatory disease. Consequently, a plethora of clinical approaches have been used to investigate the benefits of a range of microbial products on inflammatory conditions in human trials. Centered particularly on the use of prebiotics, probiotic bacteria, and bacterial lysates in early life, this review provides an overview on clinical approaches aimed at reducing the global burden of allergic disease through primary prevention. Microbial interventions beginning before birth and in early infancy are discussed in the context of underlying mechanisms of oral tolerance and the establishment of gut colonization as a critical early homeostatic influence. We explore both the findings and challenges faced in existing studies with a view toward improving future clinical studies of the application of microbial compounds for the prevention of allergic disease and other inflammatory diseases. (J Allergy Clin Immunol 2013;131:1453-63.)

Key words: *Microbiome, probiotics, prebiotics, allergy, prevention, immune tolerance, bacterial lysates, allergic disease, Developmental Origins of Health and Disease*

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On the basis of Robert Koch's perception of human health being influenced by the host, potential pathogenic agents, and the environment, the concept of the "health or epidemiologic triad"[1] evolved: human subjects were seen within a homeostatic ecosystem affected by environmental and host-determined factors. With the (re)-discovery of the human microbiome, the contrast between

From [a]the Institute of Laboratory Medicine and Pathobiochemistry, Molecular Diagnostics, Philipps University Marburg, Marburg; [b]the University of Gießen and Marburg Lung Center (UGMLC), Marburg; [c]the School of Paediatrics and Child Health, University of Western Australia and Princess Margaret Hospital for Children, Perth; and [d]the Department of Pediatric Allergy and Pulmonology, Children's Hospital, University Lübeck.

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Corresponding author: Petra Ina Pfefferle, PhD, DrPH, Institute of Laboratory Medicine and Pathobiochemistry, Molecular Diagnostics Biomedical Research Centre, Philipps University Marburg, Hans-Meerweinstr. 2, 35043 Marburg, Germany. E-mail: pfefferl@med.uni-marburg.de.

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Abbreviations used
ARTI: Acute respiratory tract infection
BL: Bacterial lysate
FOS: Fructo-oligosaccharide
GOS: Galacto-oligosaccharide
NCD: Noncommunicable disease
PNG: Papua New Guinea
RR: Relative risk

host and environment resolved into an integral view of human subjects themselves being an entire ecosystem.[2,3] The microbiome (ie, the entirety of microorganisms colonizing epithelial surfaces) seems to be highly diverse between subjects but also between habitats at different body sites. Although ultimately acquired from the environment, the composition of this internal ecosystem is distinct from that of the biosphere.[4] We are only starting to understand these interactions between human subjects and microbes; nevertheless, the importance of a well-balanced microbial environment for human health is evident.

In parallel, our view of the immune system has moved from the mere defense against pathogenic or hazardous agents to an interface between host and environment, actively organizing the interactions with all sorts of microorganism, from pathogens over harmless commensal agents to helpful symbionts. As a key function of the immune system, tolerance developed through coevolutionary phylogenetic adaptation of human populations to their microbial environment.[5] From the dawn of mankind, the biosphere incessantly stimulated the human immune system. The development of affluent lifestyles and postmodern habitats has led to skewed microbial diversity and disturbance of the human-microbial balance only within the last century, with health consequences subsumed under the hygiene hypothesis.[6] Altered microbial exposure in early life predisposes populations in industrialized countries and increasingly in developing regions of the globe to disease development.[7] In particular, chronic inflammatory diseases driven by immune dysregulation, such as allergies, autoimmune diseases, and other noncommunicable diseases (NCDs), have become a major health problem. As a preventive strategy, early supplementation of infants with microbial compounds was proposed for priming the developing immune system toward more tolerance.[8]

In many cultures nutrients fermented by microorganisms are a traditional part of the everyday diet. Originally used to preserve food, fermented nutrients were also recognized to provide health benefits.[9] Metchnikoff[10] initially postulated that dairy products protect the gut from toxic bacteria and thereby prolong life. Medical application of viable bacteria was first reported for *Escherichia coli* Nissle 1917, a gut bacterium that was isolated from a soldier who safely survived a strong epidemic of diarrhea during World War I.[11] Despite these early approaches, the related term probiotic was introduced only in the 1960s. Favoring pharmaceutical approaches, the therapeutic and preventive potential of live microorganisms was vastly neglected. This paradigm was changed by the increasing success of the hygiene hypothesis and recent insights into functional aspects of the microbiome.[12] In the example of allergy and asthma, preventive strategies are now changing from avoidance of allergens to the establishment of tolerance.[13]

PRENATAL STRATEGIES TO MODIFY MATERNAL COLONIZATION PATTERNS

There is now little doubt that the foundations for immune tolerance are established during fetal life and that factors in the maternal environment during this period can have a significant influence. Importantly, the modern environmental lifestyle changes associated with failing tolerance and immune disease are also implicated in the increase of many other chronic inflammatory NCDs. Allergy is arguably the earliest and most common manifestation of the increasing propensity for inflammation and highlights the specific vulnerability of the immune system to environmental changes. Although the focus here is primarily on changing microbial exposure as a modifiable risk factor, this cannot realistically be examined in isolation. This is integrally determined by changes in behavior, lifestyle, and nutritional patterns,[14] and therefore prevention strategies will ultimately need to be considered in this wider context. Similarly, although the focus of these discussions might center around allergic disease, it should be recognized that similar environmental risk factors for many other modern NCDs could require common solutions.

Until recently, the dominant focus of early microbial exposure was in the immediate postnatal period. Even intervention strategies, such as probiotics, commenced in the final stages of pregnancy were generally aimed at modifying initial postnatal colonization patterns. Although the fetus resides in the relatively "sterile" uterine environment, it is becoming clearer that maternal microbial exposures and colonization patterns throughout pregnancy can also have an influence on the developing fetal immune system and many other developing systems. Animal models have clearly demonstrated how maternal exposure to both pathogenic[15] and nonpathogenic[16] microbial products in pregnancy can prevent allergic outcomes in the progeny. In human subjects the now well-known allergy-protective effects of animal exposure in Alpine farmhouses has provided a natural model to examine the effects of early microbial exposure.[17] Within this setting, high prenatal microbial exposure has an independent protective effect on subsequent allergic outcomes.[18] This has been associated with differences in innate immunity,[4] as well as increased numbers and function of cord blood regulatory T cells.[19] Investigating this further in an animal model, prenatal administration of a cowshed-derived bacterium, *Acinetobacter lwoffii* F78, can prevent the development of an asthmatic phenotype in progeny. Furthermore, this effect is IFN-γ dependent and appears to be mediated through epigenetic mechanisms.[20] The asthma-preventive effect has also been associated with modification of Toll-like receptor expression in the placenta[21] and establishes a direct relationship between maternal bacterial exposures, functional maternal Toll-like receptor signaling, and asthma protection in the progeny. Human studies also provide preliminary evidence that prenatal microbial exposure is associated with epigenetic variations (decreased methylation) of the forkhead box protein 3 regulatory gene *(FOXP3)*, again in the farming environment.[19] This is consistent with observations in other populations that subsequently nonallergic children are likely to have higher placental forkhead box protein 3 expression.[22] Extensive differences in both innate[23] and adaptive[23,24] immune function at birth in children who go on to have allergy also point to the importance of *in utero* exposures.

Studies of neonates born in areas of the developing world with very high microbial burden, such as Papua New Guinea (PNG), also show extensive differences in neonatal immune function