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Review

Microbiota in Allergy and Asthma and the Emerging Relationship with the Gut Microbiome

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Asthma and atopy, classically associated with hyper-activation of the T helper 2 (Th2) arm of adaptive immunity, are among the most common chronic illnesses worldwide. Emerging evidence relates atopy and asthma to the composition and function of the human microbiome, the collection of microbes that reside in and on and interact with the human body. The ability to interrogate microbial ecology of the human host is due in large part to recent technological developments that permit identification of microbes and their products using culture-independent molecular detection techniques. In this review we explore the roles of respiratory, gut, and environmental microbiomes in asthma and allergic disease development, manifestation, and attenuation. Though still a relatively nascent field of research, evidence to date suggests that the airway and/or gut microbiome may represent fertile targets for prevention or management of allergic asthma and other diseases in which adaptive immune dysfunction is a prominent feature.

The Human Microbiome

The field of microbiology owes its genesis to observations made by Antonie van Leeuwenhoek, a Dutch microscope lens maker who, in 1676, first described microscopic "animalcules" in dental plaque (Porter, 1976). For much of the ensuing history of the field, research has largely focused on culture-based studies of individual microbial species, with an emphasis on understanding the basis of microbial pathogenesis. However, over the past 30 years, the sub-specialty of microbial ecology has driven development of molecular methods for microbial detection that obviate the necessity for microbial culture. These efforts have revealed the existence of a vast diversity of microbes that inhabit natural systems and demonstrated that these organisms rarely exist in isolation, but instead occur in multi-species, multi-functional communities.

Pioneered by Dr. Carl Woese, the use of molecular techniques to classify microbes without recourse to laboratory culture-based approaches led to the subsequent development of the 16S rRNA gene as a widely used bacterial biomarker for profiling bacteria present in a mixed-species community. This gene, which is exclusive to and ubiquitous among bacteria (Fox et al., 1977; Winker and Woese, 1991; Woese, 1987), is a useful molecular target for bacterial identification due to the presence of regions of sequence that are highly conserved across the majority of known bacteria. These conserved sequences flank hyper-variable regions, reflective of varying evolutionary rates across bacterial species. The conserved 16S rRNA sequence regions permit design of PCR primers and amplification of the gene (or a portion thereof) from the majority of known bacterial species. The amplified hyper-variable region is typically sequenced to permit classification of amplicons into discrete taxonomic groups, generating a fingerprint of bacterial phylotypes within a given community. Application of ecological statistics and theory to these profiles allows for analyses and interpretation of the composition of microbial communities. Distinct biomarkers, e.g., the internal transcribed spacer region 2 (ITS2) of the fungal rRNA gene, are used for mycological community profiling, which is performed in a similar manner. More recent advances and declining costs associated with high-throughput sequencing platforms have permitted more expansive microbial biomarker sequencing efforts, leading to identification of novel microbial species, expansion of the known microbial tree of life, and an enhanced appreciation of the diversity of microbes that inhabit natural systems. This increase in sequence capacity has also led to the development of complementary microbiome analyses tools, including shotgun sequencing approaches to determine the functional genes encoded or expressed by a microbial community (metagenomics or metatranscriptomics, respectively; Figure 1). Parallel advances in mass spectroscopy platforms have led to improvements in methods for detecting microbial products, including the dominant proteins (metaproteomics) or metabolites (metabolomics) collectively produced by microbial members within a community (Figure 1). As the field continues to rapidly evolve, efforts are becoming more focused on integration of these approaches to produce a more comprehensive view of microbial community composition and function.

Though microbial ecology research has traditionally focused on terrestrial and aquatic ecosystems, more recently, predominantly biomarker-based microbial profiling techniques have been applied to the study of the human microbial ecosystem. The results have led to a more comprehensive view of the healthy human body as a series of ecosystems, each with its own particular microbial composition, when considered at the broadest (phylum) level of classification. It has been estimated that microbial cells outnumber human cells by approximately 10-fold (Savage, 1977) and that healthy humans possess 500–1,000 distinct bacterial phylotypes (Claesson et al., 2009; Frank et al., 2007). Though much research has focused on bacterial communities

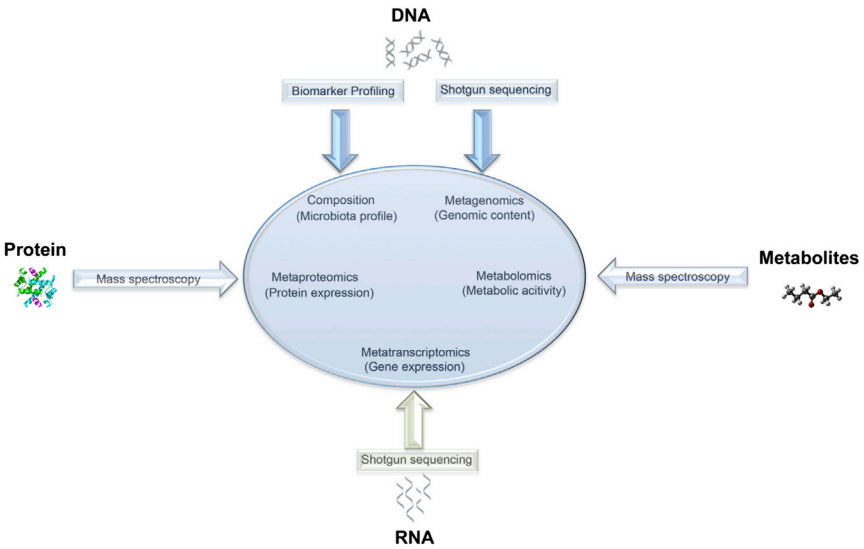

Figure 1. Molecular Tools for Interrogating Microbiome Composition and Function
DNA, RNA, protein, and metabolite fractions of the samples may be interrogated using next-generation sequencing and mass spectroscopy platforms to assess microbiome composition and function.

in the human host, fungi, viruses, and archaea have also been detected in these assemblages. The genetic capacity of such a diverse microbial ecosystem is immense; the pan-genome of the healthy human microbiome is estimated to encode approximately 100 times the number of genes found in the human genome. Though the genomic capacity of the healthy gut microbiota, where much research has focused to date, is predominantly encoded by bacteria, fungal and archaeal genes are also present in this consortium, though their functional contribution to host health status is as yet largely undefined (Qin et al., 2010).

Microbial members of the human super-organism contribute critical functions to their host, including enzymic digestion of complex carbohydrates; gastrointestinal microbes digest fermentable fiber to produce short-chain fatty acids (SCFAs; Roediger 1980), an essential energy source for the epithelial cell lining of the gut (Kaneko et al., 1994; Flint et al., 2008). Production of SCFAs also acidifies the local gastrointestinal microenvironment, making it less hospitable for colonization or overgrowth of pathogenic species such as *Escherichia coli* and *Salmonella* spp. (Cherrington et al., 1991). Commensal microbes also engage in competitive colonization, which provides further protection against pathogen overgrowth. The gut microbiome also biosynthesizes essential vitamins and hormones (Yatsunenko et al., 2012), as well as a range of anti-inflammatory compounds (Herbst et al., 2011; Mazmanian et al., 2008; Round and Mazmanian, 2010; Sokol et al., 2008). Our reliance on microbial function is evident from the critical role microbes play in mammalian development; germ-free mice are both immunologically and physiologically aberrant, a phenotype that can only be rescued through introduction of commensal bacteria (Atarashi et al., 2011; Smith et al., 2007). Beyond early-life development, evidence has emerged that the microbiome influences the tone of host immune response, particularly that of the adaptive arm. Specific *Clostridium* species belonging to clade IV or XIVA as well as members of the *Bacteroides* exhibit the capacity to induce T regulatory (Treg) cells, which are essential for immune tolerance and abrogation of chronic inflammatory or autoimmune disease (Atarashi et al., 2011; Ochoa-Repáraz et al., 2010). In addition, murine studies have demonstrated the capacity of specific species in the gut microbiome to induce discrete arms of the adaptive immune response. Black 6 mice whose ileum is overtly colonized by segmented filamentous bacteria (SFB) exhibit robust proliferation of IL-17- and IL-22-expressing T helper 17 (Th17) cells (Ivanov et al., 2009). This phenotype could be conferred to Th17 deficient mice by co-housing with SFB colonized mice or by transfer of fecal material from SFB mono-colonized animals. SFB, an unculturable member of the *Clostridiales*, was identified as the species responsible for induction of Th17 cell proliferation through culture-independent microbiome profiling (Ivanov et al., 2009). However, perhaps more importantly, these studies demonstrate a direct role for microbiome members in the promotion of distinct adaptive immune responses associated with protection against or development of a range of chronic inflammatory and autoimmune diseases. These pertinent observations in mice raise the possibility that diseases associated with overactive arms of adaptive immunity may owe their genesis to microbiome dysbiosis and related dysfunction. It also raises the possibility that diseases currently associated with "inappropriate immune activation" may in fact represent perfectly appropriate host immune responses to specific pathogenic microbiome compositions and their related activities that promote immune activation.

The Asthmatic Airway Microbiome

Asthma is estimated to affect approximately 300 million individuals worldwide, incurs significant health care expenditure (Accordini et al., 2013; Barnett and Nurmagambetov, 2011), and is one of the most common chronic diseases. Given increases in disease prevalence over the last several decades, it is predicted that the number of individuals affected worldwide will increase by 100 million people by 2025 (Masoli et al., 2004). In the United States, the risk of developing asthma is highest for children during the period between birth and four years of age (Jackson et al., 2014); disease prevalence is also higher among women, families below the poverty line, and people of multiple races compared to other groups (Akinbami et al., 2009). Though risk alleles have been associated with asthma development (reviewed in Ober and Yao, 2011), the rapid increase in prevalence over the recent decades, particularly among children, points to environmental factors playing a key role in disease development (Cookson and Moffatt, 1997). Atopic sensitization (allergy), characterized by elevated levels of total and allergen-specific IgE in the serum and typically by positive skin-prick tests to at least one of a panel of common allergens, is considered the strongest risk factor for