

EDITORIAL

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Microbes and man: an evolving story in asthma

This editorial discusses the findings of the paper in this issue by Hales et al. [9]

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The notion that microbes can influence the development of atopy and the course of asthma is not new. In 1932, Cooke proposed a role for bacteria in initiating and sustaining asthma, and this subject remains an area of intense debate today [1]. Bacteria may impact allergic disease through an array of immune pathways that are as divergent as bacteria themselves. In considering the mode of exposure, these can be broadly classified as occurring through (1) environmental bacteria; (2) non-pathogenic microbes that constitute the human microflora; and (3) pathogenic bacteria that infect the host.

If we first consider how environmental exposure may impact allergic disease, perhaps the most frequently cited example is the report of decreased atopy among children exposed to high levels of endotoxin living in rural farming areas in Europe [2]. The implication was that an environment rich in microbes, was protective. Based on subsequent studies of animal models of asthma, the assumption has been that inhalation of environmental pathogen-associated molecular patterns such as lipopolysaccharide, activate dendritic cells to prime Th1 responses. This process could, in theory, inhibit the generation or persistence of Th2 responses directed against allergens in early life.

In contrast to this scenario, the microorganisms that live inside and on humans (known as the microbiota) constitute a distinct source of bacteria that could also influence allergic disease. Considerable attention has been focused on how microbial colonization of the gut in the neonatal period relates to allergic status [3]. While the gastrointestinal tract of the newborn baby is sterile, it is rapidly colonized by microorganisms within the first week of life. Commensal microbes present in the gut may play a key role in priming the immune system in early life by 'educating' discrete T cell types and thus, influencing the

development of Th2-driven allergic disease. Differences in the composition of intestinal microflora have been reported among infants in geographically disparate regions with a different prevalence of allergy, as well as between allergic and non-allergic children [3]. One interpretation of these findings is that increased colonization with specific bacterial species (e.g. *Bifidobacteria* and *Lactobacilli*) is protective; however, this theory remains to be proven. What is clear is that the relationship between the microbiota and humans constitutes a highly complex and dynamic interaction.

Infections with diverse pathogenic microbes such as fungi, bacteria, and viruses that colonize incongruent anatomical sites have been linked to the development of asthma. For example, chronic toe and nail infections with *Trichophyton* spp. have been associated with late-onset asthma in adults who exhibit immediate hypersensitivity skin tests and IgE responses to this dermatophyte fungus [4, 5]. Similarly, colonization of the hypopharyngeal region by respiratory pathogens including *Haemophilus influenzae*, *Streptococcus pneumoniae*, and *Moraxella catarrhalis*, has been linked to childhood asthma [6]. A key question is: does infection lead to asthma or does asthma lead to infection, or are these two options inextricably linked? It has been proposed that bacterial colonization reflects a defective innate immune response that promotes the development of asthma [7]. This could culminate in a diminished Th1 response to bacteria in susceptible individuals. An alternative, though not a mutually exclusive viewpoint, is that microbial products secreted by colonizing bacteria actively promote asthma through 'pro-allergic' pathways. Approaching this from a different angle, bacterial colonization may simply reflect an epiphenomenon associated with the asthma phenotype and as such, it may not play a direct role in disease pathogenesis.

If we are to understand these arguments, we must consider immune events at the molecular level. Asthma is a complex disease that has been associated with numerous genes and the list will no doubt grow longer now that genome-wide association studies are feasible. Several

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studies have sought to link genetic elements of the innate immune response to asthma. The impetus for such studies is the premise that perturbation of innate signalling pathways could disrupt Th1-mediated responses, thereby predisposing to the development of atopy. Associations between asthma and single nucleotide polymorphisms in a variety of signalling components of the innate immune system have been reported. These include CD14, toll-like receptor 2 (TLR2), and IRAK4, all of which play a central role in pathways activated by bacterial ligands. Viral infections are a common trigger of asthma exacerbations and these may play a role in the development of asthma in early life. A genetic basis for the severity of infection with respiratory syncytial virus (RSV) has recently been proposed [8]. This is notable because there is evidence to support the view that more severe infections with RSV contribute to the risk of recurrent wheeze and asthma in early childhood. Moving forward, the challenge will be to establish how such genetic factors impact molecular events in humans that, in turn, influence the development of atopy or asthma.

An important aspect to consider is that the immune response to bacterial antigens on mucosal surfaces may be altered in subjects predisposed to atopy, not only by genetic influences but also by co-exposure to allergens. Thus, allergens may induce an inflammatory cytokine milieu in the asthmatic lung that modulates the immune response to microbial products at this site. The Gram-negative bacterium, *H. influenzae*, causes diseases in both the upper and the lower respiratory tract. In this issue of *Clinical and Experimental Allergy*, Hales and colleagues provide evidence for the induction of IgE responses to this bacterium in children with established asthma who are sensitized to house dust mite (HDM) [9]. The authors' findings build upon previous work that examined differences in antibody responses to *H. influenzae* between allergic and non-allergic subjects [10]. That study provided evidence of an allergic serum antibody profile to a conserved outer membrane protein of different non-typeable *H. influenzae* strains (P6) in HDM-allergic subjects. Among 35 children from a prospective birth cohort, 25% who were allergic to mite allergen at 5 years of age also had IgG4 antibodies directed against P6. By contrast, IgG4 antibodies were not detectable among non-allergic children at this age. Interestingly, the development of anti-P6 IgG1 antibodies appeared to be delayed in allergic children. In the same study, specific IgE antibodies were also detected in a subset of allergic adults. Because the IgG4 isotype is Th2 dependent, those findings were consistent with the induction of Th2 responses to P6 among a proportion of subjects sensitized to inhalant allergens. It was noted that, because the IgG4 titres were comparable among allergic children who had been vaccinated with a Hib conjugate to those in adults who were not vaccinated, the anti-P6 response measured was likely directed against

untypeable isolates of *H. influenzae*. Collectively, those findings solidified earlier work that demonstrated IgE antibody binding to cells from respiratory pathogens in solid-phase assays using serum samples from asthmatic subjects [11, 12].

A key issue that remains to be resolved is whether infection with pathogenic bacteria during a critical developmental window in early life is sufficient to induce asthma. If we first consider the report by Bisgaard and colleagues regarding the association between colonization with respiratory pathogens and childhood asthma [6], one possibility is that bacterial infection in early life promotes allergic disease; however, whether this arises from bystander influences driven by Th2 responses to other allergens, or else, occurs *de novo* remains to be elucidated. Looking beyond the initiation phase, we also need to consider how anti-bacterial Th2 responses may contribute to the evolution of asthma, and possibly asthma exacerbations. The prevailing view is that atypical bacteria play a minor role in asthma exacerbations, secondary to viruses. However, the findings reported in this issue of the Journal do support a role for the induction or enhancement of Th2 responses to bacteria in patients with established disease. Antibody responses to *H. influenzae* P6 were examined during convalescence from an acute episode of asthma in 53 HDM-allergic children. The major finding was an increase in anti-P6 IgE antibodies that was detected within weeks of the acute episode. Surprisingly, this change was associated with a decrease in IgE antibody to HDM allergens in conjunction with decreased total IgE titres. This seemingly paradoxical finding suggests that asthma exacerbations may trigger IgE to bacterial antigens. Moreover, up-regulation of IgE production in response to bacteria appears to be governed independently of total IgE or IgE antibodies to HDM allergen. This is striking given that anti-bacterial IgE titres reached levels comparable to those for HDM allergens. It should be stressed that patients received oral steroids as part of their treatment regimen, although for only a short period of time. While glucocorticoids may exert a differential effect on total IgE vs. allergen-specific IgE antibodies, the changes observed in the present study do not fit with those reported in the literature, suggesting that oral steroids played a small role. The authors also addressed the relevance of viral infection in their study, finding that increases in anti-P6 IgE antibodies and similar trends in IgE responses occurred irrespective of detectable virus infection. Thus, while viral infections may trigger asthma exacerbations, they do not appear to be linked to the propensity to develop anti-bacterial IgE. Considered collectively, these findings provide convincing evidence that anti-bacterial IgE responses are up-regulated by asthma exacerbations.

Work by this group highlights the dynamic nature of anti-bacterial IgE and IgG responses during the course

of asthma and the scant literature on the kinetics of antibody responses in infancy and early childhood. While certain aspects are clear, such as the sequential IgE response to food allergens followed by inhalant allergens during the 'atopic march', little is known about how anti-bacterial IgE responses mesh with this serologic background. We have recently reported anti-microbial IgE responses in adult patients with atopic dermatitis [13]. In those studies, anti-microbial IgE antibody levels were generally low, restricted to patients with high total IgE and correlated with total IgE titres, as well as the sum of specific IgE antibodies to a broad panel of inhalant and food allergens. This pattern is clearly quite distinct from that reported by Hales and colleagues in asthmatic children, which appear to reflect a highly dynamic process.

The evolving concept is that bacteria provide a source of allergens important not only for initiating IgE responses in early life, but also for up-regulating these responses during the course of disease. At first glance, this view may seem diametrically opposed to the hygiene hypothesis. However, to suggest that all exposures to bacteria are either good or bad for asthma would be disingenuous. Evoking the basic tenets of immunology, the nature of the immune response to bacteria will depend on the host, the type of microbe, and the dose and route of exposure. Thus, while high-dose environmental exposure to non-pathogenic microbes may be protective, low-dose exposure may not. Similarly, inhalation of bacterial components derived from non-pathogenic bacteria in the environment may be quite different from infection of the respiratory mucosa with pathogenic microbes. In relation to this point, the anatomical niche occupied by respiratory pathogens is likely pivotal to induction of Th2 responses. Human respiratory epithelial cells produce pro-inflammatory cytokines upon activation with non-typeable *H. influenzae* [14]. Moreover, production of the Th2-promoting cytokine, TSLP, by epithelial cells is induced by toll-like receptor ligation [15]. Such immune events may create a microenvironment conducive to the induction of Th2 responses that drive IgE production to bacteria. In addition to the induction of IgE responses, *H. influenzae* may further promote Th2 responses by activating eosinophils directly through β -glucan receptors [16]. Thus, during an asthma exacerbation when eosinophilic infiltrates in the respiratory tract are augmented, *H. influenzae* could perpetuate Th2 responses at this site.

It is clear that we have much to learn regarding how anti-bacterial responses orchestrate allergic disease. This will be essential to determining the rationale for intervening during early life. Looking to the future, there are many new exciting research initiatives on the horizon that could advance our knowledge of the microbial world and its interactions with humans. One such example is the Human Microbiome Project, which aims to sequence microbial genomes with a view to

defining the microbiota associated with health and disease in humans [17]. Such high throughput approaches may aid in elucidating the factors predisposing to infection and allergy and in designing new treatments to control or avert asthma in targeted populations.

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